Insulin Receptors in Zajdela Rat Ascites Hepatoma Cells and Their Sensitivity to Certain Enzymes and Lectins

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ABSTRACT

The insulin receptors in two strains of Zajdela ascites hepatoma cells have been studied. Specific binding of insulin was investigated at 4, 15, and 22°C, and the amount of insulin bound at steady state was proportional to the cell concentration. The optimum pH was 7.8.

Scatchard analysis of the insulin binding exhibited curvilinear plots. The number of insulin receptors was markedly reduced in Zajdela D and H cells as compared to normal hepatocytes. Furthermore, the estimated number of sites was much lower in Zajdela D hepatoma, the most transplanted tumor, compared to Zajdela H hepatoma. The dissociation constants were not modified by the transformation. Dissociation experiments have revealed enhancement of the dissociation rate of bound 125I-labeled insulin by native insulin.

Trypsin and papain digestion of Zajdela cells led to a marked inhibition of insulin binding. After digestion with glycosidases, sialidase, and/or galactosidase, the insulin binding was not decreased.

The specific binding of insulin to cells was diminished after pretreatment of Zajdela cells with concanavalin A. Scatchard analysis of insulin binding indicated that low concentrations of the lectin inhibited especially high-affinity sites. Preincubation of the cells with wheat germ agglutinin or the lectin Sophora japonica resulted in a biphasic effect on insulin binding.

Thus, the Zajdela D and H ascites hepatoma cells present a decreased number of specific insulin receptors compared to normal cells, this number being much lower in the most transplanted tumor.

INTRODUCTION

A great number of studies have shown that many of the altered properties of transformed cells involve modifications in cell surface functions. The cell surface is implicated in a variety of physiological properties that are modified in malignant cells. In particular, specific modifications in surface proteins and glycoproteins, dynamic changes in lectin receptors, and enhancement of the cellular transport of glucose have been described (25, 33). These differences in glucose metabolism and carbohydrate composition may reflect alterations in insulin responsiveness as has been shown in some tumor cells (2, 10, 15, 23).

We have chosen to examine the first step in insulin action, i.e., the interaction of the hormone with its specific receptors. These sites are localized on the cell surface of target cells (6, 20, 21) and in particular on hepatocytes (18), which are cells where insulin plays an important metabolic role. Thus, we have investigated the presence of specific insulin receptors in transformed hepatocytes. We have then studied whether their properties or their interactions with surface glycoproteins were different from those of normal cells.

For this purpose we have chosen 2 strains of Zajdela ascites hepatomas transplanted with different schedules and sensitive to some insulin activations. We have characterized specific insulin receptors on their surface that show properties similar to those of normal hepatocytes. The effect on insulin binding of different enzymes or lectins that modify surface glycoproteins was then investigated.

MATERIALS AND METHODS

Transplantation and Preparation of Zajdela Ascites Hepatoma Cells. Zajdela ascites hepatoma cells were transplanted into female albino Wistar rats weighing 180 to 250 g by i.p. injection of 0.3 ml of ascitic fluid.

For the experiments the cells were harvested after 5 to 6 days. Tumor cells were freed from contaminating erythrocytes by a wash in 0.2% NaCl solution. Cells were then washed in 0.9% NaCl solution, resuspended in Krebs-Ringer phosphate buffer (pH 7.8) [NaCl solution, (118 mM): KCl (5 mM): CaCl2 (1.3 mM): MgSO4 (1.2 mM): KH2PO4 (1.2 mM)] containing 1.5% (w/v) bovine serum albumin. Cells were counted, and viability was verified by the trypan blue exclusion test. Preparations used in the experiments had greater than 90% viability.

Two strains of Zajdela ascites hepatoma cells were chosen. Strain D was originally derived from parenchymal liver cells after chronic administration of dimethylaminoazobenzene. In culture these cells were still sensitive to some insulin actions (activation of glycogenetic enzymes) (J. P. Beck, personal communication). Strain H originated from the same tumor but was kept frozen for 10 years instead of being transplanted.

Indeed, strain H secreted $\alpha$-fetoprotein, this property being lost by strain D, which only synthesized the protein (Ref. 37; F. Zajdela, personal communication).

Preparation of Isolated Hepatocytes. Cell dissociation was conducted in a manner that retains functional integrity of hepatocytes as shown by le Cam et al. (28). This method conserves insulin receptors (28) but may remove some other surface components (45). Isolated hepatocytes were prepared from female albino Wistar rats by the method of Seglen (39) modified by le Cam et al. (28). Isolated cells...
were then washed twice with the collagenase-free buffer of Seglen. Cells were resuspended in Krebs-Ringer phosphate buffer containing 1.5% albumin and counted, and their viability was controlled.

**Insulin Binding.** 125I-labeled insulin (100 to 200 mCi/mg) was purchased from the Radiochemical Centre (Amersham, Buckinghamshire, England), and its purity was checked by the radioimmunoassay test and native porcine insulin from Schwarz/Mann (Orangeburg, N. Y.). Binding experiments were performed at 4, 15, and 22°. Various concentrations of 125I-labeled insulin (0.006 to 0.6 nM) were added to the cells in a total volume of 0.5 ml of Krebs-Ringer phosphate buffer:1.5% albumin (pH 7.6). Each tube contained 0.8 to 2.2 x 10^6 cells for Zajdela D and H cells or 0.1 to 0.3 x 10^6 cells for hepatocytes.

First, optimum times and pH of incubation were determined. Each assay was performed in quadruple in plastic tubes to minimize adsorption of insulin. Control tubes for nonspecific binding were assayed in duplicate. They contained native insulin in 16 µM added 30 min before 125I-labeled insulin.

Incubation was terminated by adding 3 ml of ice-cold Krebs-Ringer phosphate buffer (pH 7.6):0.1% albumin. Tubes were centrifuged for 3 min at 2000 rpm and washed twice with the same buffer. The cell pellet was collected and counted in an Intertechnique γ counter.

The nonspecific binding determined for each assay did not generally exceed 10% and was subtracted from the total 125I-labeled insulin binding.

All experiments were repeated at least 3 times.

Degradation of the unbound insulin was monitored by TCA precipitation (18) and rebinding to fresh cells. The binding activity of [125I]insulin remaining at the end of the experiments conducted at 4° for 18 hr, 15° for 3 hr, and 22° for 1 hr was determined. Four hundred µl of the supernatant containing the [125I]insulin obtained after centrifugation of the tubes for 3 min at 2000 rpm were mixed with 100 µl of Krebs-Ringer phosphate buffer containing the same number of cells as that used in the first incubation.

After 1 hr at 22°, cell-bound radioactivity was determined as described previously.

**Enzymic Digestions.** The activities of the enzymes used were verified with standard substrates in the buffer and under the conditions used for cell digestion. Digestions with Clostridium perfringens neuraminidase (1 to 20 µg/ml) (Worthington Biochemical Corp., Freehold, N. J.) and β-galactosidase (1 to 100 µg/ml) (Sigma Chemical Co., St. Louis, Mo.; Grade III) were performed for 15 min at 37° in Krebs-Ringer phosphate buffer (pH 7.6):1.5% albumin. Digestions with trypsin (1 to 100 µg/ml) (Miles Laboratories, Inc., Elkhart, Ind.) and papain (1 to 100 µg/ml) (Worthington) were performed in the same conditions.

At the end of incubation, cells were centrifuged and resuspended in fresh buffer. Cell viability was controlled for each experiment by trypan blue exclusion test, and the insulin-binding experiments were performed only if viability was more than 90%.

**Effect of Lectins.** Con A (Worthington Biochemical Corp.; twice crystallized), plant lectin Sophora japonica [prepared as described by Font et al. (17)] and WGA (Miles Laboratories, Inc.) were used.

Cells were incubated with lectins at various concentrations (0 to 100 µg/10^6 cells) at 22° for 1 hr. 125I-labeled insulin was then added in presence of lectins, and incubation was performed as described previously. The viability determined at the end of the incubation with insulin was not decreased. Nonspecific binding and insulin degradation were not increased as compared to experiments without lectins.

**Isolation of Surface Glycopeptides.** Cells were digested in nonlytic conditions with papain (Worthington Biochemical Corp.; 19 units/mg) that removes surface glycopeptides (46). Cells were incubated with enzyme (1 mg/g of cells) in 5 ml of buffer (0.9% NaCl solution for Zajdela D and H cells:Krebs-Ringer phosphate buffer (pH 7.6), 1.5% albumin for hepatocytes).

Digestion was performed for 1 hr at 37° for Zajdela D and H cells and 40 min at 4° for hepatocytes with gentle agitation to liberate glycopeptides without destroying the cells.

At the end of the incubation period, cell viability was verified, and the cells were centrifuged. The supernatant was collected and centrifuged at 15,000 rpm for 10 min. Three volumes of the latter supernatant were mixed with 1 volume of 20% TCA at 4° overnight. The supernatant recovered by centrifugation was precipitated by ethanol (3:1, v/v) in the presence of saturated sodium acetate. The precipitate was dried and dissolved in distilled water.

Proteins were determined by the method of Lowry et al. (29) with bovine serum albumin as a standard.

The determination of total hexoses was directly performed by a modified version of the sulfuric orcinol method (30) or by the aniline method (31).

Hexosamine content was estimated after hydrolysis with 4 M HCl for 4 hr at 100° by the colorimetric method of Elson and Morgan (19). Separation and identification of hexosamines was performed by an amino acid analyzer; sialic acid content was determined by the colorimetric technique of Warren (43) after hydrolysis (0.01 M H2SO4; 80°; 2 hr). Uronic acids were evaluated by the method of Bitter and Muir (1).

**RESULTS**

**Characteristics of Insulin Binding to Zajdela Ascites Hepatoma Cells**

The time course of binding was studied at 3 different temperatures (Chart 1). At 22° steady state was reached within 1 hr, and a plateau was maintained for 1 hr. At 15° steady state of binding was achieved after 3 hr. At 4° specific binding reached a plateau at 8 hr and remained constant for 16 hr. On the basis of these results, 1-, 3-, and 16- to 22-hr incubations were used when experiments were conducted at 22, 15, and 4°, respectively.

As shown in Chart 1, the reaction was more rapid at 22°, but the level of steady state binding was higher at 4 and 15° than at 22°.

At steady state the amount of 125I-labeled insulin bound was proportional to the number of cells present.
Chart 1. Association of insulin with Zajdela ascites hepatoma cells (strain D) as a function of temperature. Each sample contained $^{125}$I-labeled insulin (0.067 nM) and 1 to $2 \times 10^6$ cells. Incubation was performed at $\Delta, 4^\circ$; $\square, 15^\circ$; $\triangledown, 0.22^\circ$.

The optimum pH of insulin binding was 7.6 as shown in Chart 2, and binding showed a marked decrease at lower and higher pH's. The nonspecific binding was between 5 and 10% of the total binding for each value.

Degradation of insulin by cells was monitored by TCA precipitation and rebinding to fresh cells. As indicated in Table 1, there was only a slight degradation (less than 5%) of $^{125}$I-labeled insulin, and Con A did not increase this degradation. Similar results were obtained with Zajdela H cells and hepatocytes.

Comparative Analysis of Insulin Binding

The Scatchard analysis of insulin binding was performed at 4 and 15°C (Chart 3a) and 22°C (Chart 3b). Preliminary studies had shown that at steady state there was no difference in insulin binding when the same cells were incubated either at 4 or 15°C.

In all cases Scatchard plots were curvilinear and could be resolved into 2 linear components. These results were in favor of the presence of 2 classes of binding sites. Site-site interactions of the negative cooperative type seemed less probable. In the first hypothesis dissociation constants and the numbers of specific sites were evaluated (Table 2) after

Table 1

<table>
<thead>
<tr>
<th>Conditions of incubation</th>
<th>Rebinding to cells of unbound insulin (%)</th>
<th>Precipitation by TCA of unbound insulin (%)</th>
</tr>
</thead>
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<tr>
<td>22°C, 1 hr</td>
<td>94</td>
<td>87</td>
</tr>
<tr>
<td>4°C, 18 hr</td>
<td>93</td>
<td>86</td>
</tr>
<tr>
<td>4°C, 18 hr, with Con A</td>
<td>96</td>
<td>87</td>
</tr>
</tbody>
</table>

$^{a}$ $^{125}$I-labeled insulin prior incubation was 90% precipitated by TCA.

Chart 2. Effect of pH on the specific insulin binding to Zajdela ascites hepatoma cells (strain D). $^{125}$I-labeled insulin (0.067 nM) was incubated at 4°C with 1 to $2 \times 10^6$ cells for 18 hr. The pH was adjusted as indicated.

Chart 3. Scatchard analysis of the specific insulin binding to Zajdela ascites hepatoma cells (strains D and H) and to hepatocytes. In a, insulin binding assay was performed in 0.5 ml at 4°C for 18 hr or at 15°C for 3 hr with hepatocytes (0.1 to $2 \times 10^6$ cells); $\Delta$, Zajdela H cells (1 to $2 \times 10^6$ cells). $\square$, Zajdela D cells (1 to $2 \times 10^6$ cells). In b, the insulin binding assay was performed in 0.5 ml at 22°C for 1 hr with hepatocytes (0.1 to $2 \times 10^6$ cells); $\Delta$, Zajdela H cells (1 to $2 \times 10^6$ cells). All data were corrected for nonspecific binding. Bars, mean ± S.E. for samples done at least in triplicate. The resolution of this experimental curve into high and low affinity, independent linear components yields the intrinsic binding plots shown (---). Determination of high-order site was conducted with hormone concentration in the range of 0.006 to 0.03 nM insulin, while data in the range of 0.17 to 0.87 nM insulin were analyzed in determination of the low order by calculation of regression lines. Inset, extrapolation of low-affinity plots for high hormone concentrations.
Table 2

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Cell type</th>
<th>First-class sites</th>
<th>Second-class sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$K_d$ (x10^{-11})</td>
<td>Sites/cell</td>
</tr>
<tr>
<td>4 and 15°</td>
<td>Hepatocytes</td>
<td>2.6</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>Zajdela H cells</td>
<td>1.9</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>Zajdela D cells</td>
<td>2.5</td>
<td>360</td>
</tr>
<tr>
<td>22°</td>
<td>Hepatocytes</td>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>Zajdela D cells</td>
<td>2.1</td>
<td>130</td>
</tr>
</tbody>
</table>

We observed that the dissociation constants were similar regardless of the cell type or the temperature (no statistical significant differences in slopes by comparison of regression coefficients). However, the estimated number of insulin receptors per cell was markedly decreased when the incubation was performed at 22° instead of 4 or 15°. The comparison of the different cell types used revealed that the number of binding sites was reduced in transformed cells as compared to normal hepatocytes. Furthermore, the most transplanted hepatoma cells, D, contained less binding sites than did Zajdela H cells.

Dissociation of Bound Insulin

Experiments were conducted as described by De Meyts et al. (11, 13) to examine the time course of dissociation of specifically bound insulin in the presence or absence of unlabeled insulin.

Experiments were conducted at 15 and 7° from steady state. The tubes were centrifuged, and the supernatant was discarded. The cell pellet (100 µl) was resuspended in 5 ml of Krebs-Ringer buffer:0.1% albumin in presence or absence of unlabeled insulin, 1 µg/ml. At different times cells were washed as described previously, and the radioactivity was counted.

The results shown in Chart 4 indicate that the dissociation rate of [125I]-labeled insulin from cells after dilution from steady state was increased in the presence of insulin compared to that after dilution alone.

This effect was observed only with specifically bound insulin molecules since no differences in dissociation were obtained for nonspecific insulin binding (Chart 4, inset).

Effect of Enzymic Digestion of Zajdela Cells on Insulin Binding

Proteolytic Enzymes

Trypsin. Trypsin was used in nonlytic conditions. The specific insulin binding to cells was largely decreased after digestion with trypsin and was about 10% of the total fixation when cells were treated with 9 µg of enzyme per 10^8 cells (Chart 5).

Papain. When digestion was performed with papain, which liberated other surface glycopeptides, similar results were obtained (Chart 5).
for Zajdela D cells and in Chart 7b for Zajdela H cells. The dissociation constants and the numbers of sites (Table 3) were estimated by determination of regression lines with insulin concentration ranges indicated in Chart 3.

For both Zajdela D and H cells, we observed that low concentrations of Con A resulted in a reduction of 30% in the number of first-class sites. The number of second-class sites was decreased to a lesser degree. When high concentrations of lectin were used, a marked reduction was observed for all sites. In no cases were the dissociation constants affected.

Thus, Con A at low concentration inhibits especially high-affinity class sites.

Glycosidases

When Zajdela D and H cells were digested with neuraminidase:β-galactosidase, there was no modification in insulin specific binding even with concentrations of 1 μg of neuraminidase per 10⁶ cells, which liberated 0.9 μg of sialic acid. When the 2 enzymes were used together, a slight decrease in insulin binding was observed.

Effect of Various Lectins on Insulin Binding by Zajdela Cells

The experiments of insulin binding in the presence of lectins were conducted at 4°. At this temperature no agglutination of the cells was observed that would have impeded the binding of hormone.

Action of Con A

Chart 6 reveals that the lectin markedly inhibited insulin binding to Zajdela D and H cells. Maximum inhibition was of 50% of the total binding.

The Scatchard analysis of insulin binding in the presence of various concentrations of Con A is presented in Chart 7a for Zajdela D cells and in Chart 7b for Zajdela H cells. The dissociation constants and the numbers of sites (Table 3) were estimated by determination of regression lines with insulin concentration ranges indicated in Chart 3.

For both Zajdela D and H cells, we observed that low concentrations of Con A resulted in a reduction of 30% in the number of first-class sites. The number of second-class sites was decreased to a lesser degree. When high concentrations of lectin were used, a marked reduction was observed for all sites. In no cases were the dissociation constants affected.

Thus, Con A at low concentration inhibits especially high-affinity class sites.
Table 3

Effect of Con A on estimated dissociation constants and numbers of specific insulin binding sites analyzed according to the procedure of Scatchard in Zajdela ascites hepatoma cells

The results were corrected according to Klotz and Hunston (27) for 2 classes of sites. Experimental details are given in Chart 8 and in "Materials and Methods."

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Concentration of Con A (µg/10⁶ cells)</th>
<th>Kᵣ (x10⁻¹¹ M)</th>
<th>Sites per cell</th>
<th>% inhibition</th>
<th>Kᵣ (x10⁻⁹ M)</th>
<th>Sites per cell</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zajdela D cells</td>
<td>0</td>
<td>2.3</td>
<td>360</td>
<td>0</td>
<td>3.4</td>
<td>10,100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20-25</td>
<td>2.3</td>
<td>250</td>
<td>31</td>
<td>3.9</td>
<td>9,800</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>50-80</td>
<td>2.0</td>
<td>170</td>
<td>51</td>
<td>3.9</td>
<td>5,800</td>
<td>43</td>
</tr>
<tr>
<td>Zajdela H cells</td>
<td>0</td>
<td>1.9</td>
<td>500</td>
<td>0</td>
<td>3.9</td>
<td>13,500</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15-25</td>
<td>1.8</td>
<td>340</td>
<td>32</td>
<td>4.0</td>
<td>11,700</td>
<td>14</td>
</tr>
</tbody>
</table>

**Action of Lectin *S. Japonica***

This lectin, which binds specifically to galactose, caused a biphasic effect on insulin binding to Zajdela D and H cells (Chart 8).

**Action of WGA**

This lectin had also a biphasic effect on insulin binding to Zajdela D cells; low concentrations (0 to 2 µg/10⁶ cells) enhanced insulin binding (by 10% at 1 µg/10⁶ cells), higher concentration being inhibitory (at 15 µg/10⁶ cells of WGA, insulin binding was decreased by 65% as compared to control without lectin).

**Studies of the Glycopeptidic Fraction Released by Papain from Zajdela D and H Cells and from Hepatocytes**

In view of the decreased number of insulin receptors found in transformed hepatocytes, the presence of other modifications in cell surface proteins was investigated.

Thus, an increase in sialic acid content was observed in the glycopeptidic fraction liberated by mild proteolysis from transformed hepatocytes as compared to normal cells (Table 4).

**DISCUSSION**

The aim of this work was to determine whether the insulin receptors of hepatocytes are modified after malignant transformation of the cells.

For this study, 2 strains of Zajdela ascites hepatoma transplanted with different schedules were chosen. Experiments were conducted with isolated cells obtained either directly for strains D and H of Zajdela ascites hepatomas or after enzymic dissociation for hepatocytes. We were thus able to study physiological unities and avoid the problem of membrane fractionation.

For inhibition of the insulin and receptor degradation often exhibited by entire cells, most of the experiments were conducted at 15 or 4°C.

We have demonstrated here the presence of specific insulin receptors on the surface of transformed hepatocytes. Their characteristics were similar to those of isolated hepatocytes and those reported for other cells (3, 6, 20, 21, 26) as regards pH dependence; time courses at 22, 15, and 4°C; and dissociation constants.
Analysis of insulin binding data by the method of Scatchard (38) yielded curvilinear plots. Dissociation experiments conducted as described by De Meyts et al. (11, 13) indicated enhanced dissociation in presence of excess ligand.

However, these results did not demonstrate negative cooperativity. In fact Pollet et al. (34) have recently shown that enhanced dissociation of bound insulin by excess ligand was seen whatever the binding site occupancy and that insulin binding kinetic data were consistent with 2 orders of binding sites. In our case the experimental curves (Scatchard plots) could be resolved into high- and low-affinity linear plots, and the number of binding sites and dissociation constants could be evaluated in each case according to Klotz and Hunston (27).

Thus the differences in insulin binding were due to a reduction in the numbers of receptors in transformed cells as compared to normal hepatocytes without changes in their affinity for insulin. This reduction was enhanced in the most transplanted hepatoma cells.

A decrease in the number of insulin receptors in transformed cells without modification of the dissociation constant has also been described for fibroblasts (42) and for mammary adenocarcinoma of the rat (24).

Furthermore, the reduced number of receptors was not related to an increase in insulin blood levels, since normal rats had an insulinema of 19 ± 2 μunits/ml, rats transplanted with Zajdela H hepatoma of 15 ± 2 μunits/ml, and those transplanted with Zajdela D hepatoma of 11 ± 2 μunits/ml.

We then studied whether the insulin binding was modified when the cells were digested with some proteases and glycosidasers or when lectins were fixed on the cells. The nonlytic digestion of Zajdela ascites hepatoma cells by proteases resulted in a marked inhibition of insulin binding as has been described for normal cells (3, 5, 20). Digestion with sialidase of Zajdela cells had no effect on the insulin binding. Similar results have been found with other cells (3, 8, 20, 35). Digestion of Zajdela cells with β-galactosidase alone or together with sialidase or the action of low concentrations of lectin S. japonica did not inhibit insulin binding. Similar experiments have been performed by Caron (3) with a different material (mouse liver plasma membranes) and resulted in an inhibition.

The effect of WGA and Con A was also investigated since these 2 lectins exhibit some insulin-like effects at very low concentrations (9). The fixation of Con A on Zajdela ascites hepatoma cells resulted in a marked inhibition of the insulin binding, low lectin concentrations being also inhibitory, as described for some normal cells (3, 7). Some authors have noted an increase in insulin binding at low concentration of lectin (12, 41), higher concentrations being inhibitory (41). For Zajdela hepatoma cells Scatchard analysis indicated that at low concentrations the Con A was an effective inhibitor of high-affinity sites. High concentrations of Con A markedly inhibited insulin binding at all hormone concentrations. This is probably related to a complete redistribution of surface glycoproteins.

The fixation of WGA on Zajdela cells resulted in an increase in insulin binding for low concentrations of lectin. Higher concentrations inhibited the fixation of the hormone. Similar results have been reported for adipocytes (7).

Thus these 2 lectins affect the insulin receptors of the transformed hepatocytes in a manner not unlike that reported for normal cells. Numerous authors have found that transformed and normal cells possess similar numbers of lectin surface receptors (14, 33). They differ in their distribution of surface glycoproteins and the mobility of lectin receptors (33, 36).

Our results on transformed hepatocytes are in good agreement with those reported for normal cells (7) concerning the receptors of Con A and WGA. These lectin receptors are distinct from but lie close to the insulin binding site.

The biphasic effect of WGA and lectin S. japonica could be related to lectin fixation to different classes of receptors, each lectin possessing heterogenous surface receptors (32).

Thus, the decreased number of insulin receptors in transformed hepatocytes indicate cell surface modifications. Other surface changes associated with malignant transformation have been reported. In particular, a modification of the distribution of glycoproteins and increased quantities of sialic acid have been noted (4, 16, 40, 44). The increased levels of sialic acid found in the glycopeptidic fraction released from Zajdela hepatoma cells are in agreement with these results. This content was higher in the most transplanted hepatoma cells. A positive correlation between sialic acid content and tumorigenesis has been found by Glick et al. (22).

Thus the 2 strains of Zajdela ascites hepatoma, D and H, present 2 classes of specific insulin receptors (first-class sites with high affinity and low capacity and second-class sites with low affinity and high capacity.

These receptors do not exhibit modifications after malignant transformation, but their number is decreased for each class as compared to normal hepatocytes.

Furthermore, the 2 strains of Zajdela ascites hepatoma transplanted with different schedules and with different surface sialic acid content also differ in their number of insulin receptors.

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