Concanavalin A added to intact cells at 37° caused rapid and reversible inactivation of a soluble enzyme, tyrosine aminotransferase, in two lines of rat hepatoma tissue culture cells grown in monolayer culture. This temperature-dependent process was independent of de novo protein and RNA synthesis and independent of increased uptake of Ca^{2+} and Mg^{2+} or glucose. The inactivation could be reversed by adding α-methyl-d-mannopyranoside a competing sugar for concanavalin A binding. Other lectins known to bind to different sugars did not bring about the inactivation of tyrosine aminotransferase. Addition of concanavalin A did not result in the inactivation of another soluble enzyme, lactate dehydrogenase. The maintenance of tyrosine aminotransferase in an inactive form after the binding of concanavalin A to the cells required the continued presence of concanavalin A. This effect of concanavalin A could not be mimicked either by dibutylryl cyclic adenosine or guanosine monophosphoroc acid. Incubation of cell extracts with concanavalin A did not result in inactivation nor did mixing of extracts from concanavalin A-treated cells with extracts from untreated cells. On the basis of these results we conclude that the following are the essential requirements for concanavalin A to bring about the inactivation of tyrosine aminotransferase: (a) the binding of native concanavalin A to the cells; (b) integrity of certain structural elements of the cells.

Receptors on the cell surface play an important role in the communication of the cell with its environment. One class of compounds widely used to study such interactions is the plant lectins, which bind to specific sugar moieties of surface glycoproteins (1). For example, the jack bean lectin, concanavalin A, which binds to α-f-d-mannopyranosyl residues (2), has been shown to evoke a number of profound cellular alterations. Peripheral lymphocytes treated with Con A are transformed

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1 The abbreviations and trivial name used are: Con A, concanavalin A; CAMP, cyclic adenosine 3':5'-monophosphate; cGMP, cyclic guanosine 3':5'-monophosphate; dexamethasone, 1,4-pregnadiene-9-fluoro-16α-methyl-11β,17α,21-triol-3,20-dione; PBS, phosphate-buffered saline containing 0.138 M NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.47 mM KH_2PO_4, pH 7.4. into cells actively engaged in DNA synthesis (3, 4). Con A also evokes increased Ca^{2+} uptake in mouse T lymphocytes (5), an effect mediated by cyclic nucleotides. However, the relation between Con A-induced Ca^{2+} ion uptake and DNA synthesis is not understood. Other cellular effects of Con A binding include agglutination of growing cells or protease-treated density-inhibited cells (6, 7) and, at sufficiently high doses, cell killing (8, 9). No general model is available to explain the mechanisms by which surface binding of the lectins leads to these effects. In this report we present data describing a new cellular effect of Con A. Binding of the lectin to either of two lines of rat hepatoma cells in tissue culture resulted in a rapid and reversible inactivation of the intracellular enzyme, tyrosine aminotransferase (EC 2.6.1.5). This enzyme, found in the soluble fraction of cell extracts, is induced in both lines by glucocorticosteroid hormones (10, 11) and insulin (12, 13), and in one of them (FU-5-5) by analogs of cyclic adenosine monophosphate (14, 15). The reversible inactivation of tyrosine aminotransferase soon after Con A treatment occurred within minutes, contrasting with the normal half-time of several hours for decay of the enzyme. The effect seems to be relatively specific, since a second corticosteroid-inducible aminotransferase, alanine aminotransferase (EC 2.6.1.2), was only slightly affected.

**Experimental Procedures**

**Cells and Culture Conditions**—Cells used in this study were the clonal line FU-5-5 isolated from Reuber hepatoma H35 (10), kindly supplied by Dr. Mary Weiss, and HTC-H1 (10), a line of HTC cells lacking hypoxanthine, guanine phosphoribosyltransferase (EC 2.4.2.8). The cells were grown in Improved Minimum Essential Medium (IMEM-ZO) (17) supplemented with 10% fetal calf serum (North American Biologicals). The cells were grown at 37° in a water-jacketed incubator maintained with 5% CO_2, 95% air, humidified atmosphere.

**Chemicals**—Con A was obtained from Sigma Chemical Co. A stock solution of 20 mg/ml of Con A was prepared in NaCl-saturated PBS (phosphate-buffered saline containing 0.138 M NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.47 mM KH_2PO_4, pH 7.4). The stock solution was sterilized by Millipore membrane filtration, stored at 5°, and diluted with PBS when needed. Cycloheximide and α-methyl-d-mannopyranoside were obtained from Sigma Chemical Co. N-acetylg卢cosamine, N-acetylgalactosamine, and actinomycin D were obtained from Calbiochem. Dexamethasone was a gift from Merck, Sharp and Dohme (PHAcet)-1-anhydride (specific activity 500 mCi/mmol) was obtained from Amersham/Searle. (PHAcet)-Con A was prepared according to the method of Miller and Great (18). Succinyl-Con A was prepared according to the method of Gunther et al. (19).
Inactivation of Tyrosine Aminotransferase by Concanavalin A

Preparation of Samples for Enzyme and Protein Assays—After different treatments, monolayers of cells grown on 35-mm tissue culture dishes (Falcon plastics) were washed once with 2 ml of PBS at 5°C. The cells were scraped from the dishes with a plastic policeman, suspended in cold PBS, and centrifuged at 800 rpm for 3 min in a Sorvall RC-3 centrifuge. For tyrosine aminotransferase assay, the cell pellet was suspended in 0.5 ml of 0.1 M potassium phosphate buffer, pH 7.6; 0.2 mM pyridoxal phosphate; and 0.5 mM α-ketoglutarate. The cell suspension was lysed by sonication for 10 s in a Bronwill Biosonik probe sonicator. An aliquot of the crude lysate was assayed according to the method of Diamondstone (20) using bovine serum albumin as a standard. The specific activity of tyrosine aminotransferase is expressed as the amount which produces 10⁻⁹ mol of p-hydroxyphenylpyruvic acid in 1 min/mg of protein. For the aniline aminotransferase and lactic dehydrogenase (EC 1.1.1.27), the cell pellet was suspended in 0.5 ml of 0.1 M potassium phosphate buffer, pH 7.5, and the cells were lysed by 3 cycles of freezing and thawing in baths of dry ice/acetone and water. The suspension was centrifuged at 20,000 g for 15 min in the Sorvall RC-2B centrifuge. The supernatant fluid was assayed for aniline aminotransferase according to the method of Segal and Matsuzawa (23). For estimation of lactic dehydrogenase activity, 50 µl of the extract were added to a cuvette, with a light path of 10 mm, containing 0.1 mM phosphate buffer (pH 7.5), 7.6 mM sodium pyruvate, and 0.2 mM NADH in a final volume of 3.0 ml; the shift in absorbance at 340 nm as the reaction proceeded was measured spectrophotometrically. The specific activities of these two enzymes are expressed as milliunits/mg of protein. One unit of aniline aminotransferase was taken to be the amount of enzyme catalyzing the formation of 1 mol of pyruvate/min at 37°C. For lactic dehydrogenase, 1 unit has been defined as the amount of enzyme which converts 1 mol of substrate in 1 min at 25°C.

Binding of [3H]Acetyl-Con A to FU-5-5 at 5°C and 37°C—FU-5-5 cells grown in 35-mm dishes were kept in PBS with (0.9 mM) Ca²⁺ and (0.99 mM) Mg²⁺ either at 5°C (over ice) or at 37°C (0.9 ml of PBS to each dish). To each dish 0.1 ml of 0.5 M α-methyl-D-mannopyranoside solution was added and the dishes were maintained at their respective temperatures for 10 min. Another set of dishes which served as the un竞争 control received 0.1 ml of PBS. At the end of 10 min incubation with the competing sugar or PBS, 50 µl of [3H]Acetyl-Con A (0.6 mg/mg, 3700 cpm/µg) were added to each dish. Cells were harvested at different times after exposure to Con A by aspirating the PBS, washing the monolayer of cells three times with cold PBS, scraping, and washing once more with cold PBS by centrifugation. The cell pellet was dissolved in 0.3 ml of 1 N NaOH and an aliquot of the solution was counted in 10 ml of Aquasol (New England Nuclear). The amount of Con A bound/mg of total cellular protein was converted into amount bound per 10⁶ cells by using the conversion factor determined empirically of 10⁶ cells = 260 µg of protein. The difference in the amount of Con A bound with and without α-methyl-D-mannopyranoside was taken as the specific binding of Con A. The binding of Con A to FU-5-5 cells in a suspension was carried out in an identical manner, except that Con A was added to the cell suspension and cells were washed four times in PBS by centrifugation.

RESULTS

Effect of Con A on Tyrosine Aminotransferase—When FU-5-5 cells with tyrosine aminotransferase preinduced by overnight exposure to 1 µM dexamethasone were added to a Con A-treated coverslip (24), we noted an immediate drop in the level of the enzyme. This observation prompted us to study the effect of Con A on tyrosine aminotransferase. The effect of increasing concentrations of Con A on the induced level is shown in Fig. 1. In growth medium, above 25 µg/ml of Con A, the level of the enzyme decreased with increasing concentration of Con A and the effect was maximum at 200 µg/ml of Con A, no further effect being seen up to 500 µg/ml (data not included). However, when Con A was added after replacing the growth medium with PBS containing Ca²⁺ and Mg²⁺, the maximum effect of Con A was seen at a much lower concentration (Fig. 1). This could be due to the competition by serum glycoproteins and glucose for the binding of Con A to the cell surface. Hence, in the later experiments on the kinetics of inactivation of tyrosine aminotransferase by Con A, a constant concentration of 200 µg/ml of Con A was used to ensure maximal inactivation. The kinetics of the inactivation of tyrosine aminotransferase by Con A, shown in Fig. 2, is very rapid with a half-life of about 3 min. Similar rapid inactivation of induced tyrosine aminotransferase was observed in HTC-H1 cells (data not shown). It is highly unlikely that such a rapid inactivation would depend on de novo protein or RNA synthesis. Nevertheless, to test this possibility, cycloheximide at 100 µg/ml (a dose which pilot experiments showed to block amino acid incorporation by 95%) was added to the cells 10 min before the addition of Con A. As can be seen from Fig. 2, the inactivation was unaffected. The identical result was obtained when high concentrations of cycloheximide were added to the cells 10 min after the addition of Con A. Before the addition of Con A, tyrosine aminotransferase specific activity by Con A at 37°C with and without cycloheximide. FU-5-5 cells preinduced overnight in HTC-H1 cells were used to determine the specific activity of con A and enzyme assay was carried out as described under "Experimental Procedure." Con A was added to cells either in growth medium (●) or PBS with Ca²⁺ and Mg²⁺ (○—○).

Fig. 1. Effect of increasing concentrations of Con A on the induced level of tyrosine aminotransferase specific activity in FU-5-5 cells. FU-5-5 cells grown in 35-mm dishes were preinduced overnight (about 16 h) with 1 µM dexamethasone. Different concentrations of Con A were added to the induced cells incubated at 37°C. The cells were harvested after 30 min of treatment with Con A and enzyme assay was carried out as described under "Experimental Procedure." Con A was added to cells either in growth medium (●) or PBS with Ca²⁺ and Mg²⁺ (○—○).

Fig. 2. Kinetics of inactivation of induced level of tyrosine aminotransferase specific activity by Con A at 37°C with and without cycloheximide. FU-5-5 cells preinduced overnight with 1 µM dexamethasone in 35-mm dishes were treated with 200 µg/ml of Con A. At different times after the addition of Con A, the cells were harvested after washing the cells once with 2 ml of cold PBS. Tyrosine aminotransferase assay was done as described (●—●). To study the effect of cycloheximide on the inactivation brought about by Con A, cycloheximide at a concentration of 100 µg/ml was added to the induced cells 10 min before the addition of Con A. The rest of the procedure was the same as described before (○—○).
the experiment was carried out with a dose of actinomycin D (1 μg/ml) capable of blocking RNA synthesis by 90%, instead of cycloheximide (data not shown). The experiments above were performed on induced cells. To see whether the lectin was acting through some mechanism related to the prior treatment of the cells with steroid, Con A was added to uninduced cells. There was a rapid decrease in the basal activity of the enzyme (Fig. 3). Con A is effective only in its native tetravalent form in bringing about the inactivation of tyrosine aminotransferase in FU-5-5 cells, for the divalent succinylated Con A did not do so in these cells (Table I). Certainly blocking protein synthesis per se does not cause rapid inactivation. Both cycloheximide and puromycin have been used to block protein synthesis in cultured hepatoma cells, after which tyrosine aminotransferase has shown a t1/2 of decay of several hours (25). Furthermore, cycloheximide interfered neither with the Con A inactivation nor with the α-methyl-D-mannopyranoside reactivation of the enzyme (Figs. 2 and 9).

The inactivation seems specific for Con A, since neither wheat germ nor soybean agglutinins caused any change in intracellular tyrosine aminotransferase levels. The addition of Con A to FU-5-5 cells did not result in any decrease in the level of lactic dehydrogenase and not more than a 30% decrease in alanine aminotransferase as shown in Table II. Thus the effect seems lectin-specific, and probably enzyme-specific as well.

Many of the effects caused by Con A seem to be temperature dependent (26-28). For example, it has been shown that cells bind Con A at 0°C without agglutination (7). However, when the temperature was raised to 22°C after preincubating the cells with Con A at 0°C, the cells did agglutinate (29). We therefore undertook to study the temperature dependence of the Con A effect on tyrosine aminotransferase. When Con A was added at 5°C to preinduced FU-5-5 cells, the process of inactivation was not blocked but was slowed. Thus, complete inactivation which took only 10 min at 37°C took nearly 3 h at 5°C (Fig. 4). To see whether the rapid decay of the enzyme would return at higher temperatures, the preinduced FU-5-5 cells were incubated with Con A at 5°C, washed, and then shifted to

![Table I](http://www.jbc.org/)

**Table I**

**Effect of Con A and succinyl-Con A on induced level of tyrosine aminotransferase in FU-5-5 cells**

FU-5-5 cells grown in 35-mm dishes were induced with 1 μM dexamethasone for 48 h. Control dishes received only PBS. The cells were then treated either with 0.2 ml of 1:10 dilution of Con A stock solution bringing the final concentration of Con A to 200 μg/ml or 0.2 ml of 1:10 dilution of NaCl-saturated PBS. After 1 h the cells were harvested and alanine aminotransferase and lactic dehydrogenase assays were carried out as described under “Experimental Procedures.”

| Treatment | Enzyme specific activity |
|-----------|-------------------------|
|           | Alanine aminotransferase | Lactic dehydrogenase |
|           | milliunits/mg protein   | milliunits/mg protein |
| Uninduced cells + NaCl-saturated PBS | 116 | 84 |
| Induced cells + NaCl-saturated PBS | 256 | 76 |
| Uninduced cells + Con A | 95 | 68 |
| Induced cells + Con A | 183 | 92 |

![Table II](http://www.jbc.org/)

**Table II**

**Effect of Con A on alanine aminotransferase and lactic dehydrogenase**

FU-5-5 cells grown in 35-mm dishes were treated with 1 μM dexamethasone for 48 h. Control dishes received only PBS. The cells were then treated either with 0.2 ml of 1:10 dilution of Con A stock solution bringing the final concentration of Con A to 200 μg/ml or 0.2 ml of 1:10 dilution of NaCl-saturated PBS. After 1 h the cells were harvested and alanine aminotransferase and lactic dehydrogenase assays were carried out as described under “Experimental Procedures.”

![Figure 3](http://www.jbc.org/)

**Figure 3.** Effect of Con A on the basal level activity of tyrosine aminotransferase specific activity. The experimental conditions were the same as described in Fig. 2 except that the cells were not treated with dexamethasone.

![Figure 4](http://www.jbc.org/)

**Figure 4.** Inactivation of induced level of tyrosine aminotransferase specific activity by Con A at 5°C. FU-5-5 cells were preinduced as described previously. The medium was then replaced by cold (5°C) Improved Minimum Essential Medium buffered with 50 mM tricine containing 1 μM dexamethasone and the dishes were kept over ice. The dishes were then treated with either 200 μg/ml of Con A in 0.2 ml of PBS (○ - ○) or 0.2 ml of PBS alone (● - ●). Cells were harvested at different times for enzyme assay.
37° in the absence of Con A. The result of such an experiment is shown in Fig. 5. As expected there was a slow fall of tyrosine aminotransferase with Con A treatment at the low temperature, then a rapid fall after the temperature increase. The enzyme activity decreased to about 50% of the level present at the time of the temperature shift but did not reach as low a level as when Con A was present at 37° continuously. The decreased enzyme level persisted no more than 15 min after which it returned to the preinduced level within 1 h. This experiment suggests that the continued presence of Con A is required for the maintenance of decreased levels of enzyme. This happened even in the case of the cells treated with Con A at 37° and then replaced in growth medium without Con A as shown in Fig. 6. The temperature shift experiment also shows that binding of Con A and inactivation of tyrosine aminotransferase can be dissociated to some extent, and that the overall process is temperature-dependent.

The delayed effect of Con A on the inactivation of tyrosine aminotransferase at 37° could be due to a difference in the binding of Con A to FU-5-5 cells at 37° and 5°. This possibility was investigated by studying the cell binding of radiolabeled Con A. The specific binding of [3H]acetyl-Con A to FU-5-5 cells at the two temperatures is shown in Fig. 7. The binding increases with time both at 5° and 37° for up to 60 min at least. However, the specific binding is considerably higher (3- to 4-fold) at 37° than at 5°. This difference in binding at these temperatures offers one possible explanation for the delayed effect of Con A at 37°.

When Con A was added to a suspension of preinduced FU-5-5 cells prepared either by scraping or trypsinization, rather than to the undisturbed monolayer, there was little fall in the activity of tyrosine aminotransferase (Table III). The data there also show that Con A did not produce any inactivation of the enzyme in preinduced HTC-Hl cells grown in suspension culture. These experiments suggest that for Con A to bring about its full effect on the inactivation, the cells have to remain attached to the growth surface. However, the specific binding of labeled Con A to either scraped or trypsinized FU-5-5 cells was not significantly different from the binding observed in FU-5-5 cells that were still attached (Table IV); thus the binding of Con A to the cells alone is not sufficient for bringing about inactivation in these cells. The addition of colchicine (100 µm) or cytochalasin B (10 µg/ml) simply did not mimic the effect of Con A, nor did these compounds block the effect of Con A when added together with it (data not shown), suggesting that the effect of Con A does not involve microtubules and microfilaments.
To be sure that the Con A-provoked inactivation of tyrosine aminotransferase was occurring intracellularly, and not during sonication of Con A-treated cells, a histochemical assay for enzyme activity by the method of Thompson and Tomkins (30) was applied to cells in situ. Fig. 8 shows that the Con A-treated cells possessed very little stainable enzyme compared to untreated cells. Thus, the effect of Con A in bringing about inactivation seems to be an in vivo effect and not an artifact caused during the handling of the cells subsequent to the treatment with Con A.

Externally added cAMP or cGMP, their analogs, or theophylline did not mimic the effect of Con A in bringing about the inactivation of tyrosine aminotransferase in FU-5-5 cells, nor did adding these compounds with the lectin alter the usual inactivation (Table V). This suggests that the interaction of Con A with the cell does not inactivate the enzyme via changes in the intracellular level of either cAMP or cGMP. To test the possibility that the inactivation could be due to the release of some unknown diffusible inhibitor, an extract prepared from Con A-treated FU-5-5 cells was mixed with an extract of induced FU-5-5 cells. As can be seen from Table VI, this did not result in any inactivation. A similar result was obtained when the cell extract from Con A-treated cells was prepared 2 min after the addition of Con A. Further, when cells treated with Con A for 2 min were mixed with untreated induced cells and then sonicated together immediately after mixing, enzyme activity in the mixed cells' extract was simply additive (data not shown). The effect of the addition of Con A to the cell extract prepared from induced FU-5-5 cells is shown in Table VII; the inactivation of tyrosine aminotransferase brought about by Con A is not due to the direct interaction of Con A with the enzyme.

Reversibility of Tyrosine Aminotransferase Inactivation Caused by Con A—It is known that Con A interacts with polysaccharides that contain $\alpha$-$\beta$-glucopyranosyl, $\alpha$-$\beta$-mannopyranosyl, or $\alpha$-$\beta$-glucosaminyl residues (2). If the inactivation of tyrosine aminotransferase brought about by adding Con A to preinduced FU-5-5 cells is also due to its binding to $\alpha$-$\beta$-glucopyranosyl or $\alpha$-$\beta$-mannopyranosyl residues on the cell surface, then this effect should be reversed by adding the competing sugar. In fact, this was found to be the case. When

![Fig. 8](http://www.jbc.org/)

**Fig. 8.** Histochemical staining of tyrosine aminotransferase in preinduced FU-5-5 cells with and without treatment with Con A. Preinduced FU-5-5 cells grown on glass coverslips were treated with either (a) PBS or (b) PBS containing 200 $\mu$g/ml of Con A for 30 min. The cells were then fixed by cold acetone, stained for tyrosine aminotransferase, and photographed. $\times$ 250.
Inactivation of Tyrosine Aminotransferase by Concanavalin A

**Table VI**

Effect of mixing cell extract from Con A-treated FU-5-5 cells with that from untreated cells

| Cell extract from | \(A_{m/15 \text{ min}}\) | \(A_{m/15 \text{ min}}\) |
|------------------|----------------|----------------|
| Observed         | Expected       |
| Induced cells (25 \(\mu l\)) | 0.619 | 0.619 |
| Con A-treated cells (100 \(\mu l\)) | 0.128 | 0.128 |
| Induced cells (25 \(\mu l\)) + Con A-treated cells (75 \(\mu l\)) | 0.766 | 0.718 |

**Table VII**

Effect of direct addition of Con A to crude or partially purified tyrosine aminotransferase

FU-5-5 cells preinduced overnight with 1 \(\mu M\) dexamethasone in 150-mm dishes were harvested and the cell extract was prepared by sonication after suspending the cells in 0.9 ml of sonication buffer. The cell extract was divided; to one portion increasing concentrations of Con A in NaCl-saturated PBS were added. After thorough mixing and incubation at 0° for 2 h the extracts were assayed for tyrosine aminotransferase. The tyrosine aminotransferase in a second portion of the extract was partially purified by affinity chromatography (31) and then incubated with Con A and assayed.

| Enzyme | NaCl-saturated PBS | Con A (10 mg/ml) in NaCl-saturated PBS | Tyrosine aminotransferase activity |
|--------|-------------------|---------------------------------------|-----------------------------------|
| 0.100  | 0.100  | 0.100  | 0.100  | 0.100  | 0.100  | 0.100  | 0.100  |
| 0.090  | 0.090  | 0.090  | 0.090  | 0.090  | 0.090  | 0.090  | 0.090  |
| 0.075  | 0.075  | 0.075  | 0.075  | 0.075  | 0.075  | 0.075  | 0.075  |
| 0.050  | 0.050  | 0.050  | 0.050  | 0.050  | 0.050  | 0.050  | 0.050  |
| 0.025  | 0.025  | 0.025  | 0.025  | 0.025  | 0.025  | 0.025  | 0.025  |
| 0.100  | 0.100  | 0.100  | 0.100  | 0.100  | 0.100  | 0.100  | 0.100  |

a. Crude extract

b. Partially purified

When the extract was incubated at 37° for 30 min after the addition of Con A, a similar result was obtained.

α-methyl-β-mannopyranoside was added to cells treated with Con A, the enzyme returned to induced level as rapidly as it had been inactivated by the addition of Con A, as shown in Fig. 9. This rapid reactivation was independent of de novo protein or RNA synthesis. The rapid reactivation brought about by this sugar was a specific effect, since N-acetylglucosamine and N-acetylgalactosamine, sugars known to compete for different lectins, could not reverse the effect of Con A (Table VIII). The inactivation of tyrosine aminotransferase by Con A and the reactivation of the inactivated enzyme by α-methyl-β-mannopyranoside could be repeated a number of times, without any great loss of the induced level, as shown in Fig. 10. This result suggests that when this enzyme is inactivated by Con A, it can be converted back to its active form immediately upon removing the Con A bound to the cell.

To test whether transcription and translation of the mRNA

**Table VIII**

Reversal by sugars of tyrosine aminotransferase inactivation caused by Con-A in crude or partially purified extracts

FU-5-5 cells grown in 35-mm dishes were induced overnight with 1 \(\mu M\) dexamethasone. To the induced cells Con A was added in 0.2 ml of PBS containing 1 mg/ml of cycloheximide (○) or 10 mg/ml of actinomycin D (□) so as to bring the final concentration of cycloheximide to 100 \(\mu M\) and of actinomycin D to 1 \(\mu M\), respectively, was added to three different sets of Con A-treated cells. Then 15 min after the addition of PBS or cycloheximide or actinomycin D, 0.2 ml of α-methyl-β-mannopyranoside solution in PBS was added to all the three sets of dishes, bringing the final concentration to 50 mM. Samples were removed at different times after the addition of α-methyl-β-mannopyranoside and assayed for tyrosine aminotransferase.

| Treatment | Enzyme specific activity |
|-----------|-------------------------|
| 1. Control | 233.4 |
| 2. 200 \(\mu M\) of Con A | 27.6 |
| 3. 200 \(\mu M\) of Con A + 50 mM α-methyl-β-mannopyranoside | 228.5 |
| 4. 200 \(\mu M\) of Con A + 50 mM N-acetylgalactosamine | 19.9 |
| 5. 200 \(\mu M\) of Con A + 50 mM N-acetylglucosamine | 25.0 |

* Control value is for the cells induced overnight with 1 \(\mu M\) dexamethasone.
Lectins and their binding to cell surfaces are known to influence many intracellular events. Con A, for instance, may have either a mitogenic or a cytotoxic effect, depending on cell type and concentration used (8, 9, 32). The mechanism by which these cell surface active compounds influence intracellular events, however, remains unknown. The experiments presented here show for the first time the effect on a soluble intracellular enzyme of the binding of Con A to the cell. In both FU-5-5 and HTC cells, practically all tyrosine aminotransferase specific activity by Con A and its reactivation by α-methyl-β-mannopyranoside, respectively. The rapidity (Fig. 2) of the inactivation of tyrosine aminotransferase by Con A suggests that this effect could be due either to the direct interaction of the enzyme with Con A, resulting in its inactivation or to the release of some signal subsequent to the binding of Con A, an event which in turn brings about the inactivation. The former alternative has been ruled out because of the lack of inactivation by the direct addition of Con A (up to 1 mg/ml) to crude or partially purified extract prepared from preinduced cells (Table VII). The inactivation could be mimicked neither by cAMP nor by cGMP (Table V). These are the two most widely studied mediators for the intracellular actions of membrane active ligands. Hence, the effect reported here seems to be due to an alternate mechanism. It is known that Con A stimulates the uptake of Ca²⁺ in T lymphocytes (5); it is possible that the inactivation of tyro-

**DISCUSSION**

The rapidity (Fig. 2) of the inactivation of tyrosine aminotransferase by Con A suggests that this effect could be due either to the direct interaction of the enzyme with Con A, resulting in its inactivation or to the release of some signal subsequent to the binding of Con A, an event which in turn brings about the inactivation. The former alternative has been ruled out because of the lack of inactivation by the direct addition of Con A (up to 1 mg/ml) to crude or partially purified extract prepared from preinduced cells (Table VII). The inactivation could be mimicked neither by cAMP nor by cGMP (Table V). These are the two most widely studied mediators for the intracellular actions of membrane active ligands. Hence, the effect reported here seems to be due to an alternate mechanism. It is known that Con A stimulates the uptake of Ca²⁺ in T lymphocytes (5); it is possible that the inactivation of tyro-

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**Fig. 10.** Repeated inactivation of tyrosine aminotransferase specific activity by Con A and its reactivation by α-methyl-β-mannopyranoside in induced FU-5-5 cells. FU-5-5 cells preinduced overnight with 1 μg dexamethasone in 35-mm dishes were treated with 200 μg/ml of Con A for 15 min at 37°C. Then, 0.2 ml of an α-methyl-β-mannopyranoside solution in PBS was added to all the dishes, to a final concentration of 50 mM. The medium was replaced 15 min later by regular growth medium containing 200 μg/ml of Con A and kept at 37°C for 15 min. They were all then treated with α-methyl-β-mannopyranoside as before for 15 min. This cycle was repeated two more times. Samples were harvested for enzyme assay at the end of each treatment of either Con A or α-methyl-β-mannopyranoside. The solid arrows and dashed arrows represent the time of addition of Con A and α-methyl-β-mannopyranoside, respectively.

**Fig. 11.** Induction of tyrosine aminotransferase specific activity in FU-5-5 cells after treatment with Con A. Fresh medium was added to uninduced FU-5-5 cells grown in 35-mm dishes containing 200 μg/ml of Con A and 1 μM dexamethasone. Samples were harvested for tyrosine aminotransferase assay as described previously at different times (up to 8 h). The medium was replaced in the rest of the dishes with fresh medium containing 50 mM α-methyl-β-mannopyranoside along with (O—O) or without (□—□) 1 μM dexamethasone. Samples were harvested for enzyme assay at different times after the addition of α-methyl-β-mannopyranoside.
sine aminotransferase brought about by Con A binding could also be mediated by changes in the permeability for different ions. The experiments, in which Con A added to cells in Ca\(^{2+}\), Mg\(^{2+}\), and glucose-free medium still caused rapid inactivation (data not shown), suggest that the inactivation is not due to altered uptake of these cations (or of glucose). So far all our attempts to demonstrate the inactivation by mixing experiments (Table VI) have been without success. Either the putative inhibitor is very highly labile, or some other mechanism is involved. Our prejudice is that some such inactivator exists, but that conditions necessary to demonstrate it in broken cell preparations have not yet been found.

The rapid activation of tyrosine aminotransferase brought about by the addition of \(\alpha\)-methyl-\(\beta\)-mannopyranoside to the Con A-treated cells (Fig. 9) suggests that for the maintenance of the enzyme in an inactive form, it is necessary to have the Con A in a bound form. Moreover, the rapid reactivation of the enzyme further suggests that the putative inhibitor must be very labile. The inactivation of tyrosine aminotransferase brought about by Con A occurred much more slowly at 5\(^\circ\) than at 37\(^\circ\) (Figs. 4 and 2) a fact which could partially be accounted for by the lower specific binding of Con A to FU-5-5 cells at 5\(^\circ\) compared to 37\(^\circ\) (Fig. 7). The membrane is rigid at low temperatures (35); this explains the agglutination of cells by Con A an event which is dependent upon the fluidity of the cell membrane. In contrast, the inactivation brought about by the binding of Con A to cell membrane was not an all or none phenomenon, for the addition of Con A to cells at 5\(^\circ\) only prolonged the inactivation period. This may be due to the lack of a stringent requirement for membrane fluidity to bring about the release of the putative inhibitor for tyrosine aminotransferase once Con A binds to the cell.

The lack of inactivation of tyrosine aminotransferase when Con A was added to cells that were in suspension (Table III) demands the assumption of a correlation between the extended state of the cells when they are attached to a substratum and the release of the putative inhibitor for the enzyme since the binding of Con A was not impaired significantly when the lectin was added to a cell suspension rather than to a monolayer culture. The lack of inactivation in suspension cells (HTC-H1) could possibly be due to a difference in the arrangement of microtubules and microfilaments in these cells as compared to cells remaining attached to a substratum. However, a mere perturbation of either the microtubules or microfilaments by the addition of colchicine or cytochalasin B was not sufficient to bring about inactivation in these cells. The observation that the reactivation of the enzyme in cells treated with Con A either at 5\(^\circ\) or 37\(^\circ\), after simply washing off excess Con A, was very slow compared to the reactivation brought about by the addition of \(\alpha\)-methyl-\(\beta\)-mannopyranoside may be due to the slow or incomplete removal of membrane-bound Con A by simple washing. These results also imply that once the excess Con A is washed off from the medium, the Con A that was responsible for the inactivation is released slowly from its original site, resulting in the slow reactivation of the enzyme.

The addition of Con A to uninduced FU-5-5 cells in the presence of dexamethasone did not interfere with the induction of tyrosine aminotransferase (Fig. 11). However, the newly made enzyme was inactivated immediately after being synthesized since the addition of \(\alpha\)-methyl-\(\beta\)-mannopyranoside to cells treated with Con A in the presence of dexamethasone resulted in bringing the newly made enzyme to an active form. This suggests that Con A did not simply act by intercellular interaction with overall macromolecular synthesis, but brought about a relatively specific effect on the enzyme.

Although the results presented in this report did not enable us to arrive at the exact mechanism of inactivation of tyrosine aminotransferase by Con A, the following steps seem essential for the effect to be seen in FU-5-5 and HTC-H1 cells: (a) binding of native Con A to the cells; (b) cells growing in undisturbed monolayers.

In conclusion, we feel that the results reported here describe a novel phenomenon in the interactions of lectins with cells, the rapid, reversible inactivation of a soluble enzyme resulting from lectin binding. The rapidity, specificity, and ease of assay of this inactivation make it attractive as a model for studying cell surface-intracellular communication systems. We are currently studying this effect of Con A in hepatoma cells using both biochemical and genetic approaches.

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