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INFLUENCE OF CONCANAVALIN A ON PROTEIN SYNTHESIS AND PROTEIN RELEASE IN BHK 21 CELLS

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Summary

Addition of concanavalin A to baby-hamster kidney cells (strain BHK 21/C13) grown in Eagle's minimal essential medium devoid of serum but supplemented with insulin as growth-promoting factor, caused a marked reduction of the total protein content of the cells: as early as 1 h after treatment, the amount of protein decreased to about 60—70% of the values found in untreated cultures.

Pulse-labelling experiments performed with [^{3}H]leucine demonstrated that the uptake and incorporation of the labelled amino acid was not affected by the lectin up to 6 h after treatment. Pulse-chase experiments gave no evidence for an enhanced degradation of proteins.

Examination of the supernatants of concanavalin A-treated cultures as well as their controls, pre-labelled with [^{3}H]fucose and [^{14}C]leucine revealed that the amount of membrane-derived glycoproteins which were shed into the culture medium was considerably higher in concanavalin A-treated cultures.

However, the bulk of protein which accounts for the difference between lectin-treated and untreated cultures consists of intracellular material which was released during the cell harvest procedure. The loss of protein was prevented by α-methyl-D-mannoside (10^{-2} M).

Scanning electron microscopy of concanavalin A-treated cells showed a change from the smooth surface of the fibroblastic cells to a retracted one as early as 30 min after addition of the lectin. The surface of the altered cells was characterized by the presence of numerous bleb-like protuberances.

The viability of the cells was not affected by concanavalin A-treatment during the course of the experiments.

Experiments performed with variable concentrations of insulin excluded the possibility that the observed effects might be due to a competition between the lectin and the hormone.

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Introduction

Concanavalin A is widely used to study the blast transformation of lymphocytes [1]. In these cells, concanavalin A stimulates protein, RNA and DNA synthesis, and increases the turnover of concanavalin A-binding membrane receptors [2]. In fetal cells, the biosynthesis of DNA and RNA as well as cell division is stimulated by the lectin [3]. On the other hand, there are reports that concanavalin A inhibits the growth of transformed cells [4] and prevents infection, synthesis and release of infective virions from lytically-infected cells [5].

While the effect of concanavalin A on the various cell systems and functions has been extensively studied, the mechanism of action of concanavalin A on cellular metabolism is not yet understood. Since it is reasonable to investigate basic effects in a simplified defined system to avoid contamination by various membrane receptor-bound serum constituents, we investigated the action of concanavalin A on the protein metabolism of normal BHK 21 cells. The results thus obtained may serve to understand events in more complicate systems.

Results are presented of experiments performed with BHK 21 fibroblasts grown in Eagle's minimal essential medium without serum but supplemented with insulin in order to investigate the action of concanavalin A on protein synthesis, glycoprotein excretion and morphology of the cells.

Materials and Methods

(1) Chemicals and isotopes

Concanavalin A (solution in 4 M NaCl) was obtained from Boehringer, Mannheim, G.F.R.; α-methyl-D-mannoside from Roth, Karlsruhe, G.F.R.; bovine insulin from Hoechst AG., Frankfurt, G.F.R.; [^{14}C]leucine (specific activity 56.0 mCi/mM), [^{3}H]leucine (specific activity 51.0 mCi/mM) and [^{3}H]-fucose (specific activity 18.5 mCi/mM) from the Radiochemical Centre, Amersham, U.K. All other compounds obtained commercially were of reagent grade.

(2) Experimental procedure

An established line of hamster kidney fibroblasts (strain BHK 21/C13) was used throughout this work. To investigate uptake and incorporation of [^{3}H]-leucine, $1.5 \cdot 10^6$ cells were suspended in 10 ml of Eagle's minimal essential medium containing 5% calf serum, penicillin (200 units/ml), streptomycin (200 μg/ml), 6.0 mM *N*-tris-(hydroxymethyl)methylglycine (Tricine), 6.0 mM $NaHCO_3$, and were seeded in 100-mm plastic petri dishes. Cultures were incubated at 37°C under a humidified atmosphere. 6 h after seeding, the medium was discarded, the cultures were washed twice with Hanks' balanced salt solution [6] and were further incubated in Eagle's minimal essential medium devoid of serum but supplemented with insulin (80 munits/ml) as growth promoting factor [7]. After incubation for 18 h, 5 μg/ml of concanavalin A were added, and the cells were pulse-labelled at the times indicated.

(3) Processing of concanavalin A-treated and control cultures

The cultures were washed with ice-cold saline (0.15 M NaCl) and harvested by scraping. Following centrifugation at 2000 × *g* for 10 min, the cell pellets were lysed in 1.0 M KOH. The solutions were neutralized with 1.0 M HCl, and DNA and proteins were then precipitated with ice-cold trichloroacetic acid in a final concentration of 5%. Aliquots of the supernatants were used to estimate trichloroacetic acid soluble radioactivity.

(4) Determination of trichloroacetic acid insoluble radioactivity

The trichloroacetic acid-precipitated pellets were washed twice with ice-cold 5% trichloroacetic acid and then dissolved in 0.5 M KOH. Aliquots of the solutions were counted in 10 ml of Bray's solution in a Packard Tricarb scintillation spectrometer.

(5) Chemical determinations

Determination of the protein content was carried out according to the method of Lowry et al. [8]. DNA was measured by the method of Burton [9].

(6) Preparation of excreted material and microsomal fractions

$5 \cdot 10^7$ cells were suspended in 100 ml of Eagle's minimal essential medium supplemented with 10% calf serum, seeded in roller bottles (volume, 1.7 l), and incubated for 24 h. The medium was then discarded and 50 ml of fresh medium without leucine were added. The cultures were labelled for 24 h with 0.5 μCi/ml of [^{14}C]leucine and with 2.0 μCi/ml of [^{3}H]fucose or [^{3}H]glucosamine, respectively. After labelling, the cells were thoroughly rinsed with Hanks' solution and then further incubated for 5 h in 50 ml of minimal essential medium without serum but supplemented with insulin, containing the ten-fold concentration of unlabelled leucine. After the chase period, 50 ml of normal minimal essential medium containing insulin and 25 μg/ml of concanavalin A were added. The cultures were then incubated for 5 h. Control cultures were treated identically except for the addition of concanavalin A.

(a) Preparation of excreted material. The culture fluids were collected and spun at 100 000 × *g* for 1 h in a Beckman Spinco Model L centrifuge to sediment cellular debris. The supernatants thus obtained were concentrated by lyophilisation and extensively dialyzed against distilled water to remove salts, phenol red and non-incorporated radioactivity. The soluble protein was precipitated by addition of the 10-fold volume of ice-cold acetone and pelleted at 2000 × *g* for 10 min. The pellets were solubilized in a defined volume of 1% aqueous sodium dodecyl sulfate (SDS) solution. Aliquots of this solution were used to determine protein content and incorporated radioactivity. After addition of 1% of 2-mercaptoethanol, the solution was boiled for 2 min, and samples containing 200 μg of protein were subjected to polyacrylamide-gel electrophoresis.

(b) Preparation of microsomal fractions. Microsomal fractions were prepared according to the method of Kamat and Wallach [10] as modified for BHK 21 cells and described in detail by Gahmberg et al. [11]. Briefly, the cells were scraped off the glass and washed 3 times with phosphate-buffered saline (10 min, 2000 × *g*). The pellets were combined with the cellular debris of the

100 000 × *g* run as mentioned above, and were resuspended and disrupted using the nitrogen cavitation method. Cellular organelles were separated by centrifugation at 30 000 × *g*. The supernates of this run were subjected to a 100 000 × *g* run for 90 min. The microsomal fractions thus obtained were solubilized in 1% SDS and further treated as described above.

(7) Polyacrylamide-gel electrophoresis

This was performed according to the method of Davis and Ornstein [12]. The gels consisted of 8 cm 7% acrylamide with a 0.5 cm stacking gel in glass tubes (inside diameter, 5 mm). Electrophoresis was carried out with a constant current of 3 mA per gel. The gels were fractionated in 2-mm slices with a Gilson fractionator and solubilized in 30% H_2O_2 at 70°C for 24 h before counting.

(8) Estimation of cell number

The cultures were treated with 10 ml of phosphate-buffered saline without Ca^{2+} and Mg^{2+}, containing 0.2% trypsin and 0.3% EDTA. Aliquots of the suspension were counted with the aid of a Neubauer haemocytometer. The determination of the relation of DNA content to cell number revealed that 10 μg of DNA correspond to $1.0 \cdot 10^6$ cells. A comparison of the DNA content of concanavalin A-treated and untreated cultures revealed no difference in the total amount of DNA throughout the duration of the experiments. Thus, the DNA content was used as a standard and reference for the other determinations used in this work.

(9) Scanning electron microscopy

Cell cultures grown on glass sides were washed with Hanks' solution and fixed in freshly distilled glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at room temperature. After 90—120 min, the cells were washed in the same buffer followed by critical point drying (Frigen II). The specimens were mounted on aluminium supports and exposed for 24 h to OsO_4 fumes. These preparations were covered with a thin layer of gold by means of evaporation. Finally, they were examined in the Cambridge SEM Mark 2 A at 20—30 kV, at a tilt angle of 55°, and the data were recorded on film.

Results

(1) Effect of concanavalin A on the protein content of the cells

Addition of 5 μg/ml of concanavalin A to BHK 21 cells grown in Eagle's minimal essential medium devoid of serum caused a marked reduction of the total protein content of the cells. 3 h after treatment, the protein content decreased to about 60% of that of the controls (Fig. 1). This decrease occurred very rapidly. Results of other experiments demonstrated that the reduction of the protein content was accomplished as early as 1 h after application of concanavalin A (see Table II).

In contrast, the DNA content of concanavalin A-treated- and control cultures was the same over the time period investigated (data not shown). The number of cells and their viability was not influenced by the lectin. This is in

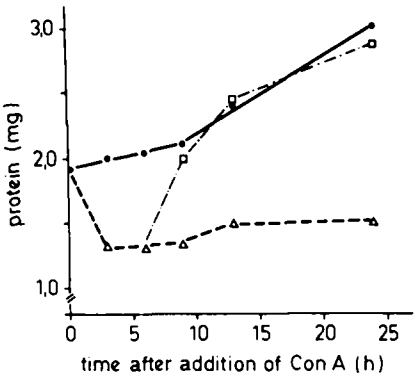

Fig. 1. Effect of concanavalin A (Con A) on the protein content of BHK cells and the reversal of the effect by α-methyl-D-mannoside. Cell cultures were treated with concanavalin A (5 μg/ml) 24 h after seeding. Prevention of the protein loss was performed by addition of 10^{-2} M α-methyl-D-mannoside 6 h after lectin treatment. The cells were harvested at the times indicated, and the protein content of the samples was determined as described under Materials and Methods. Each point represents the average of three determinations. ●———●, Controls; △- - - - -△, cell cultures treated with concanavalin A; □- · · · -□, cells incubated with concanavalin A for 6 h, after which α-methyl-D-mannoside was added.

agreement with results of Aubery and coworkers [18] who were unable to demonstrate either leakage of labelled chromium from the cells or staining of the cells with trypan blue even when concanavalin A was used in concentrations up to 100 μg/ml.

The effect of the lectin on the protein content was completely reversible by addition of 10^{-2} M α-methyl-D-mannoside, the specific inhibitor of concanavalin A (Fig. 1). This finding suggests that no cell damage or loss of viability occurred.

(2) Effect of concanavalin A on the uptake and incorporation of [^{3}H]leucine

Pulse-labelling experiments performed with [^{3}H]leucine revealed that neither

TABLE I

UPTAKE AND INCORPORATION OF [^{3}H]LEUCINE IN CONCANAVALIN A-TREATED AND CONTROL CULTURES

Cell cultures were pulse-labelled with [^{3}H]leucine (0.4 μCi/ml) for 1 h and then harvested at the times indicated. Trichloroacetic acid-soluble- and incorporated radioactivity were determined as described under Materials and Methods.

Time after addition of concanavalin A	Addition of concanavalin A (5 μg/ml)	[^{3}H]Leucine acid soluble (cpm)	% of controls	[^{3}H]Leucine acid insoluble (cpm)	% of controls
0	—	1982		1125	
3	—	2180		1255	
	+	2136	98	1260	100
6	—	2187		1290	
	+	2121	97	1285	99
9	—	2545		1325	
	+	1297	51	1258	95
13	—	2640		1385	
	+	1267	48	1155	83

TABLE II

EFFECT OF CONCANAVALIN A ON CELLS PRE-LABELLED WITH [^{3}H]LEUCINE: DETERMINATION OF ACID-SOLUBLE- AND INCORPORATED RADIOACTIVITY

Cells were labelled for 15 h with [^{3}H]leucine (0.4 μCi/ml). The medium was then discarded and the cultures were thoroughly rinsed with Hanks' solution. Minimal essential medium containing the ten-fold concentration of cold leucine was added. After 2 h incubation the chase medium was replaced by normal medium without serum but supplemented with insulin and concanavalin A (5 μg/ml). Cell cultures were harvested at the times indicated. Radioactivity and protein content of each sample were determined as described and related to DNA.

Time after addition of concanavalin A (h)	Addition of concanavalin A (5 μg/ml)	Total protein in cells (mg)	% of controls	[^{3}H]Leucine Trichloroacetic acid-insoluble (cpm)	% of controls	[^{3}H]Leucine Trichloroacetic acid-soluble (cpm)	% of controls
0	—	2500		12 320		80	
1	—	2560		11 970		83	
	+	1600	62	7 302	61	60	73
3	—	2860		10 547		53	
	+	1680	59	6 328	60	45	75
5	—	3480		10 760		39	
	+	1980	57	5 866	54	29	76

TABLE III

COMPARISON OF PROTEIN CONTENT AND [^{14}C]LEUCINE LABEL IN EXCRETED FRACTIONS, ENTIRE CELLS AND MICROSOMAL FRACTIONS

Cell cultures prelabelled with [^{14}C]leucine (0.5 μCi/ml) were treated with concanavalin A (25 μg/ml) for 5 h. The different fractions were prepared as described under Materials and Methods. Protein content and radioactivity of each fraction were determined and the values thus obtained were related to 100 μg of DNA.

	Excreted fractions				Entire cells				Microsomal fractions			
	Total protein (μg)	% of control	Total [^{14}C]leucine (cpm × 10^{-4})	% of control	Total protein (μg)	% of control	Total [^{14}C]leucine (cpm × 10^{-4})	% of control	Total protein (μg)	% of control	Total [^{14}C]leucine (cpm × 10^{-4})	% of control
Control	300		11.15		4160		213.73		421		21.59	
Concanavalin A-treated	679	226.3	25.17	225.7	3100	74.5	159.56	74.7	212	50.3	10.91	50.5

the uptake of the amino acid into the trichloroacetic acid-soluble pool, nor the incorporation into precipitable material was inhibited by the lectin up to 6 h after treatment (Table I). These results exclude the possibility that the decrease of the total protein content might be caused by an inhibition of the protein synthesis.

A significant reduction of both the uptake and the incorporation as well of [^{3}H]leucine occurred late after concanavalin A treatment. This may be a result of a gradual, progressive inhibition of overall metabolic processes.

(3) Effect of concanavalin A on cells pre-labelled with [^{3}H]leucine

The loss of proteins observed after concanavalin A treatment might also be explained by the assumption that an increased degradation within the cell takes place. Pulse chase-experiments, as shown in Table II, were performed to study the validity of this assumption. As can be seen from Table II, the total amount of protein in concanavalin A-treated cells decreased, as already shown. This decrease is also reflected by the fact that the values of trichloroacetic acid-insoluble radioactivity decreased in parallel. Interestingly, the pool of soluble radioactivity did not increase after treatment. These results indicate that no intracellular degradation of incorporated radioactivity does occur.

(4) Examination of the supernatants of concanavalin A-treated cells and comparison of the protein content in excreted fractions, entire cells and microsomal fractions

The results of the preceding experiments led to the assumption that concanavalin A may cause or enhance the release of undegraded polypeptides. To further substantiate this conclusion, we determined the amount of proteins which are shed into the supernatant fluids of lectin-treated and untreated control cultures.

As can be seen from Table III, the supernatants (excreted fractions) derived from concanavalin A-treated cultures contained more than twice as much protein than those of untreated cultures. This finding indicates that the excretion of proteins is indeed enhanced by the lectin. Determination of [^{14}C]-leucine content confirmed this finding. Moreover, in comparing the rates of reduction in the protein content of entire cells to that of microsomal fractions it was noted that, using entire cells, the protein content decreased to about 75% of that of the controls, while in microsomal fractions it decreased to about 50%. These results indicate that the microsomal fraction, known to consist mainly of the outer membrane of the cell, is predominantly affected by concanavalin A. The following data further support this concept. The content of [^{3}H]fucose, a carbohydrate which is mainly associated with the outer cell membrane [13,14], was found to be strongly reduced in the microsomal fraction of concanavalin A-treated cells (40% of that of the controls). In contrast, the amount of label found in the excreted material was twice as high compared to untreated cultures. Moreover, the total amount of [^{3}H]fucose in lectin-treated- and untreated cultures was identical. This finding excluded a possible loss of label by degradation or during the processing of the different fractions. Similar results were obtained using [^{3}H]glucosamine as a label of glycoproteins.

The amount of protein shed into the supernatants, however, was too small to

account for the loss of 30—40% as shown above. We therefore had to consider the possibility that concanavalin A-binding to the plasma membrane might affect the stability of the membrane and thus render it more sensitive to the cell harvest procedure. Therefore, we checked the cell harvest supernatants for intracellular proteins. The results obtained show that indeed a considerable loss of intracellular proteins occurs on concanavalin A-treated cultures. While in untreated cultures about 30% of the total protein is released from the cells during the various steps of the cell harvest procedure, we found in concanavalin A-treated cultures an increased protein release of up to 60% of the total amount. These data confirm the assumption that binding of the lectin to the plasma membrane renders the latter more sensitive to the cell harvest procedure and leads to an increased loss of cytoplasmic proteins.

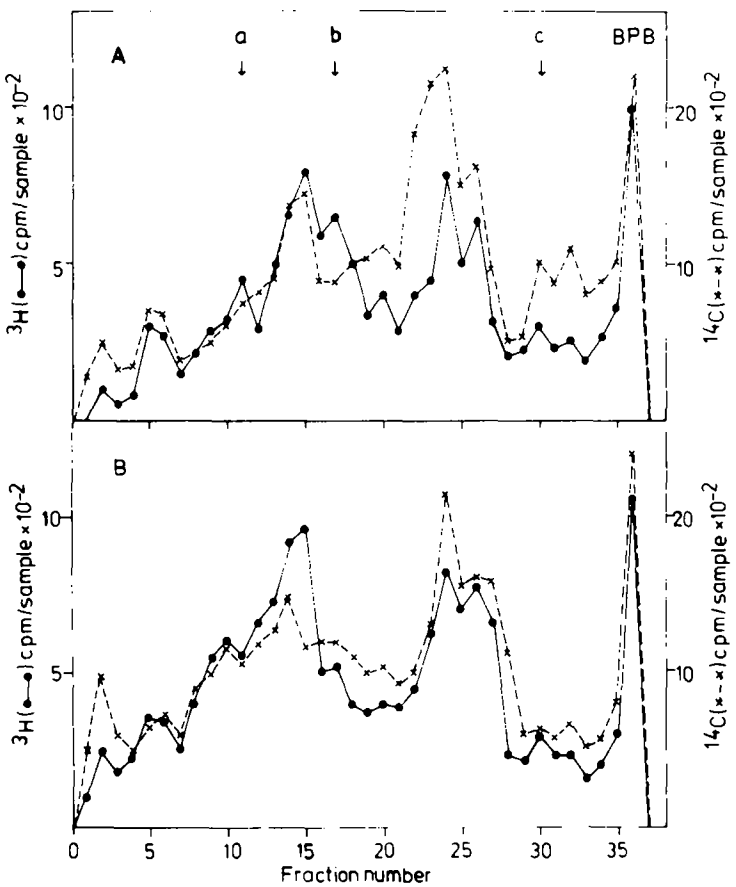

Fig. 2. Polyacrylamide-gel electrophoresis profiles of microsomal fractions from normal (A) and concanavalin A-treated cells (B). The experimental procedure was carried out as described under Materials and Methods. Cell cultures were labelled with [^{3}H]fucose (2.0 μCi/ml) and [^{14}C]leucine (0.5 μCi/ml) for 24 h before concanavalin A-treatment. Gels were run from left to right. BPB, bromophenol blue. Solid line, [^{3}H]fucose; dashed line, [^{14}C]leucine. Molecular weight markers: a, β-galactosidase (130 000); b, phosphorylase *a* (94 000); c, aldolase (40 000).

(5) Examination of microsomal fractions and excreted material by polyacrylamide-gel electrophoresis

The data presented in the last paragraph suggest that concanavalin A preferentially affects the outer cell membrane and leads to an enhanced release

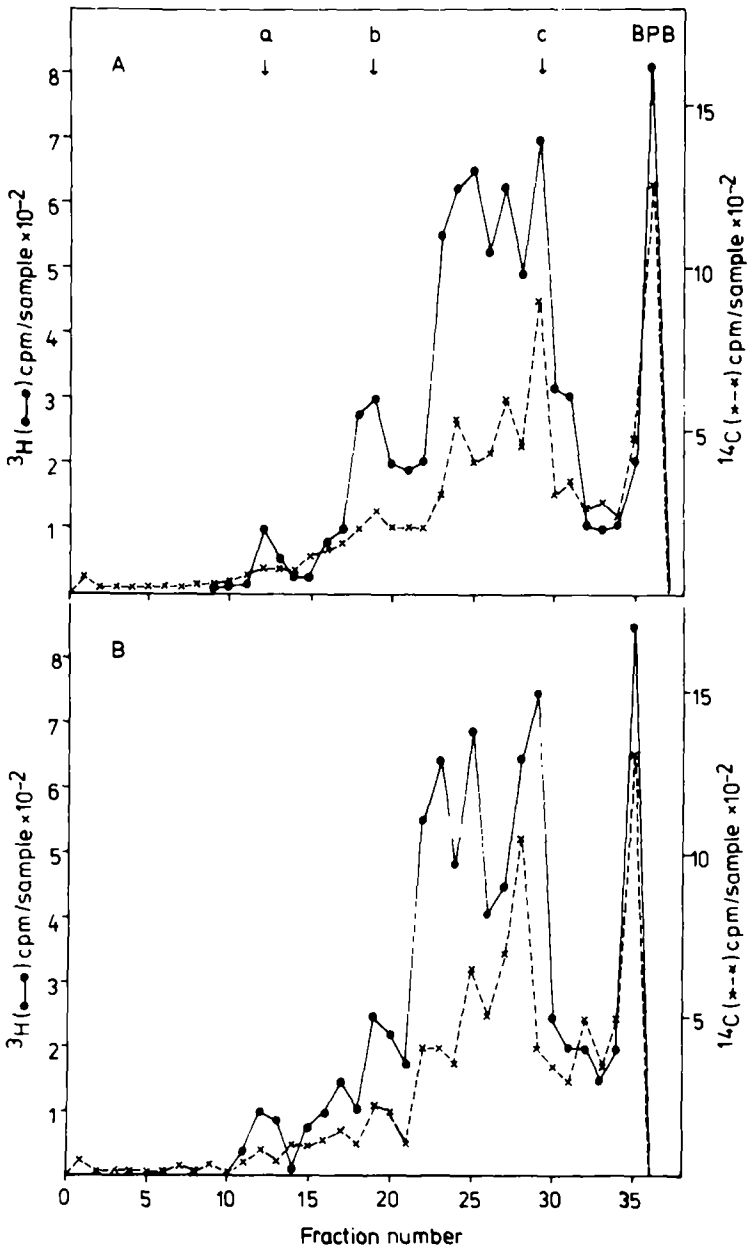

Fig. 3. Polyacrylamide-gel electrophoresis profiles of excreted material from normal (A) and concanavalin A-treated cell cultures (B). This experiment was performed as described in the legend to Fig. 2. Solid line, [^{3}H]fucose; dashed line, [^{14}C]leucine.

of membrane glycoproteins. The question arose whether treatment with concanavalin A causes an increased turnover of concanavalin A-receptors and a shedding of these receptors or of concanavalin A-receptor complexes as shown by Schmidt-Ullrich et al. [2] in concanavalin A-treated thymocytes. In order to elucidate this point, both the microsomal and the excreted fractions were subjected to polyacrylamide-gel electrophoresis. The gel profiles shown in Figs. 2 and 3 of both the fractions of concanavalin A-treated- and control cultures reveal no principal, but only minor differences following treatment, and examination of the excreted fractions does not show any accumulation of distinct membrane receptors.

(6) Microscopic examination of the concanavalin A-treated cells

Finally, microscopic methods were employed to relate the biochemical changes of the concanavalin A-treated- and untreated cells to morphological alterations. By conventional light microscopy, we detected that the fibroblast-like cells had retracted to a rounded-up shape due to the action of concanavalin A without any signs of agglutination. By scanning electron microscopy, considerable morphological alterations could be observed compared to non-treated cells (Figs. 4 and 5). In contrast to the elongated, untreated cells with a smooth surface (Fig. 4), concanavalin A-treated cells were rounded and showed the

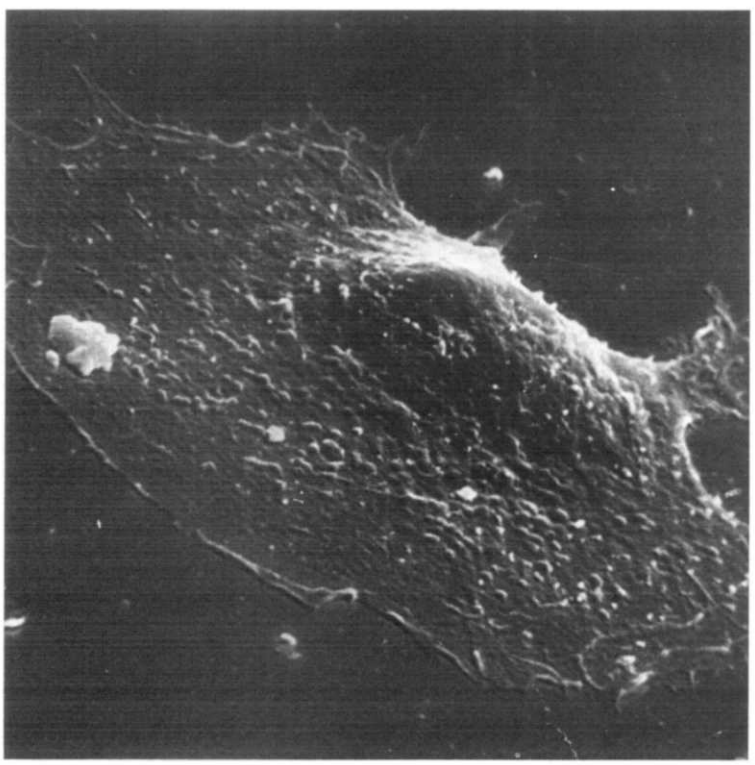

Fig. 4. A scanning electron micrograph of a BHK cell grown for 18 h in serum-depleted, insulin-supplemented minimal essential medium before fixation. X2000.

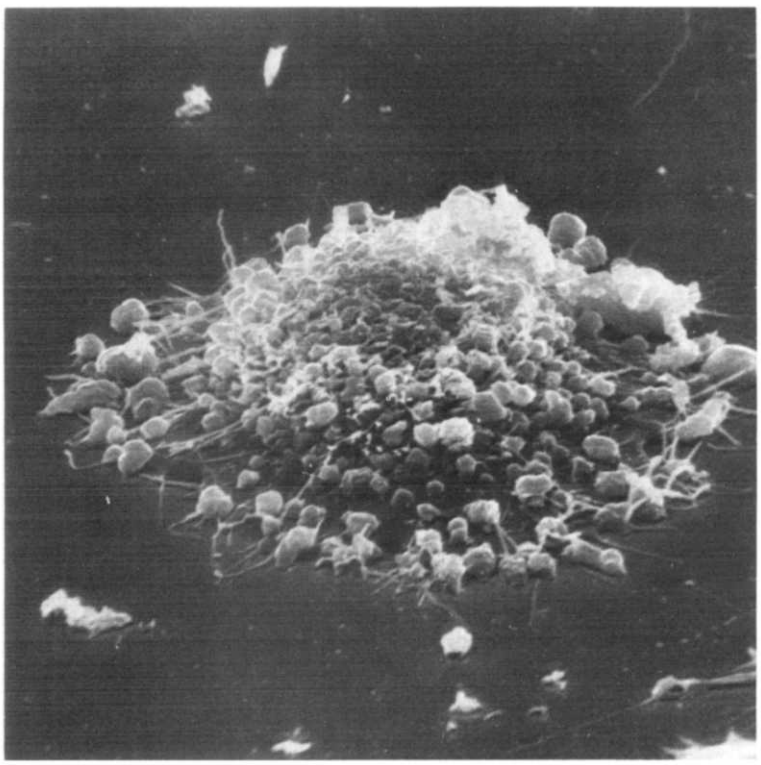

Fig. 5. A scanning electron micrograph of a BHK cell grown for 18 h in serum-depleted, insulin-supplemented minimal essential medium, 30 min after addition of 5 μg/ml of concanavalin A to the culture medium. ×2000.

appearance of bleb-like protuberances on their surfaces (Fig. 5). (Other pictures show more irregularly distributed blebs differing in size). These blebs may represent detaching parts of the cell surface membrane, which are shed into the supernatant of the cultures.

(7) Influence of insulin on the action of concanavalin A

Examination of a possible competition between the lectin and the hormone. Cuatrecasas [19] was able to show that the isolated insulin receptor could be specifically bound to and eluted from a concanavalin A-agarose column. This led to the conclusion that the lectin acts directly on the cell surface receptor of insulin. To exclude the possibility that the effects observed in our system might be due to an interference between insulin and concanavalin A, in that the lectin could lower the concentration of insulin by binding to the insulin receptors, we performed experiments in which the concentration of insulin was varied, whereas the amount of the lectin was kept constant.

The results presented in Table IV show that the observed effects occur independently of the insulin concentration used: the release of protein as well as the incorporation of [^{3}H]leucine was not influenced in the presence of insulin. We therefore conclude that the effects described here are not due to an

TABLE IV

EFFECT OF VARIABLE INSULIN CONCENTRATIONS ON PROTEIN CONTENT AND INCORPORATION OF [^{3}H]LEUCINE IN NORMAL AND CONCANAVALIN A-TREATED CELL CULTURES

Cell cultures were seeded as described under Materials and Methods. Insulin in the concentrations indicated, as well as concanavalin A, was added 18 h after seeding. Labelling with [^{3}H]leucine (0.4 μCi/ml) was performed for 4 h, starting 1 h after addition of insulin and concanavalin A.

Concentration of insulin (munits/ml)	Addition of concanavalin A (5 μg/ml)	Protein content in cells (μg)	% of controls	Acid insoluble [^{3}H]leucine (cpm)
0	—	468		4878
	+	310	66	4860
20	—	459		4729
	+	298	65	4591
40	—	457		4345
	+	311	68	4567
80	—	494		4721
	+	315	64	4713
100	—	459		4293
	+	290	63	4323
120	—	436		500
	+	292	67	546
160	—	477		528
	+	284	60	525
200	—	492		476
	+	329	67	468

interference of concanavalin A with insulin, but are caused by the lectin alone. This conclusion is supported by results obtained in experiments where no insulin was applied to stimulate cell growth.

Discussion

The results of our experiments indicate that concanavalin A causes distinct changes in the properties of the plasma membrane and the morphology of normal hamster kidney fibroblasts.

The lectin concentrations used in our experiments (5—25 μg/ml) induced an enhanced release (shedding) of membrane components. This finding is in agreement with results of Schmidt-Ullrich et al. [2] and Jones [15] who were able to demonstrate that concanavalin A binding to intact thymocytes induced an increased turnover of concanavalin A-receptors and a release of the receptor glycoproteins into the culture media. In our experimental system however, we were unable to demonstrate the release of particular receptor molecules. Examination by polyacrylamide-gel electrophoresis of microsomal fractions and of excreted material from lectin-treated- and control cultures showed only minor differences. There was no evidence for the presence of an increased amount of concanavalin A-receptors within the culture fluid.

The reason for the enhanced excretion of membrane material as induced by concanavalin A-treatment of the cells is not yet clear. A possible explanation might be the hypothesis that the cells try to get rid of the lectin by shedding parts of their membranes, since an incubation of the cells with concanavalin A

for a longer period of time leads to a progressive inhibition of the cellular metabolism, and finally causes cell death. The shedding of membrane material in concanavalin A-treated cells is perhaps comparable to the findings of Leonhard [16] who demonstrated the release of antigen/antibody conjugates from the membrane of antibody-treated hepatoma cells. The possible mechanisms for tumor cell escape from immune surveillance, which depends on cell surface antigen dynamics as reviewed by Nicolson [17], may be used to explain these findings.

The bleb-like protuberances on the surface of concanavalin A-treated cells which appear very early (30 min) after treatment may represent detaching parts of the membrane and may be a possible source of material which is released into the culture medium. This assumption is supported by the observation that the blebs disappear after addition of 10^{-2} M α-methyl-D-mannoside, the specific inhibitor of concanavalin A.

The protein loss of 30—40%, compared to the controls, which results from the cell harvest procedure of concanavalin A-treated cells gives further evidence of a strong effect of the lectin on the plasma membrane. Obviously, the stability of the membrane is changed by concanavalin A-binding in a way that intracellular proteins can more easily penetrate. Since we were not able to detect any significant differences in the DNA content of the cells, the loss of protein seems to be limited to cytoplasmic material. Experiments performed with labelled fucose, a marker for the plasma membrane, exclude any loss of membrane material during the process of cell harvest. Thus, the data suggest that concanavalin A may affect the stability of the membrane and, hence, renders the cells more sensitive to external influences, which occur during the harvest procedure. The observed protein loss can be prevented by addition of α-methyl-D-mannoside. This suggests a restoration of the original membrane properties after removal of the lectin. The uptake and incorporation of labelled leucine was not affected by concanavalin A up to 6 h after treatment. This result indicates that cell viability was not influenced within the first hours after addition of the lectin.

A reduction of the uptake of [^{3}H]leucine, followed by a decreased incorporation of the label, occurred late after lectin treatment. This may result from an inhibition of membrane-bound enzymes and/or a derangement of transport sites by concanavalin A binding to the membrane. Such an inhibition induced by concanavalin A was reported from tumor cells [21] and retina cells of chick embryos at a certain stage of development [3]. In contrast to the observations in lectin-treated lymphocytes, our experiments did not reveal any evidence for a stimulating effect of concanavalin A on BHK cells.

Experiments performed by Cuatrecasas [19] and Jacobs et al. [20] suggested a possible competition between concanavalin A and insulin for a common receptor. Since we used insulin as the growth-stimulating factor in our experiments, we had to consider the possibility that the observed effects might be caused by competition for the same receptor between concanavalin A and the hormone in such a way that the lectin is lowering the actual insulin concentration and, therefore, does disturb the action of the hormone. The experiments performed with varying insulin concentrations, however, demonstrated that the effects which were accounted to lectin action occurred in any case,

even if excessive insulin was used. These results indicate that the physiological actions of concanavalin A do not depend on the presence of insulin neither are they influenced by binding competitions.

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