THE EFFECTS OF CON A ON CELL SURFACE
SHEDDING IN CELL CULTURES

THOMAS C. DOETSCHMAN

Biological Sciences Group, Genetics and Cell Biology Section, U-125,
University of Connecticut, Storrs, CT. 06268 U.S.A.

SUMMARY

A cell surface immobilizing concentration of Con A inhibits the shedding of [3H]fucose-
containing glycoproteins from the surface of chick embryonic leg and breast muscle cell
cultures and cultures of the rat skeletal muscle cell line L6. The Con A-induced inhibition of
shedding is much less in the mouse fibroblast cell line 3T3. In all 4 cell types the lectin inhibits
the shedding of some fucosyl-glycoproteins more than others, especially those of a lipid-
containing fraction which is excluded in Biogel A-5m chromatography. This differential
nature of the Con A effect is not changed by the cytoskeletal disrupters cytochalasin B or
colchicine. Con A causes an increase in the amount of trypsin-sensitive surface fucosyl-
glycoprotein in the cell surface and appears to decrease the overall amount of cell surface
degradation suggesting that the inhibition of shedding caused by Con A is not due to an
increase in internalization and degradation. The data suggest that some shedding may occur at
specific cell surface sites to which surface materials must laterally migrate.

INTRODUCTION

The effects of Con A on cell surface shedding in cell cultures

The mechanisms involved in cell surface shedding are poorly understood. Cell
surface proteolytic activity is one of the mechanisms by which shedding can occur
(Kapeller, Gal-Oz, Grover & Doljanski, 1973; Doljanski & Kapeller, 1976; Hynes,
can also occur by the release of plasma membrane vesicles as suggested by some
studies (Peterson & Rubin, 1969; Vitetta & Uhr, 1972; Nowotny et al. 1974; Doetsch-
man, 1980a) and demonstrated by others (Stanbridge & Weiss, 1978). Cell surface
caps (Karnovsky, Unanue & Leventhal, 1972; Leonard, 1973; Stanbridge & Weiss,
1978; Nicolson, 1979) can be the site of shedding, and shedding may also occur at
coated endocytotic pits (Brown, Yeh & Holley, 1979). With the exception of proteo-
yisis most of these processes would require that the molecules to be shed have some
lateral mobility within the plasma membrane. Consequently, agents known to interfere
with cell surface mobility would be useful in probing the mechanisms of cell surface
shedding.

At high concentrations Con A loses its mitogenic properties and inhibits the
patching and capping of many cell surface receptors (Yahara & Edelman, 1975).

• Present address: ETH-Zürich, Institut für Zellbiologie, Hönggerberg, CH-8093 Zürich,
Switzerland.
Con A has been shown to decrease the lateral diffusion of Con A receptors (Schlessinger et al. 1976) and of other receptors as well (Schlessinger et al. 1977).

Because of the wide range of its effects on cell surface mobility, Con A was used in this study to investigate the relationship between shedding and cell surface mobility. Con A is shown to have an inhibitory effect on shedding and this does not appear to be the result of increased internalization and degradation of surface molecules. Since the modulation of surface mobilities by Con A can be partially reversed in some instances by colchicine (Yahara & Edelman, 1975; Schlessinger et al. 1977), and patching and capping of cell surface receptors can be inhibited by cytochalasin B (Edelman, 1976), these drugs were also used simultaneously with Con A and were found to have little effect on the Con A inhibition of shedding. The results suggest that at least some surface glycoproteins are shed in the form of membanous vesicles at specific cell surface sites.

MATERIALS AND METHODS

Cells were mechanically dissociated from leg or breast muscles of 11-day chick embryos as previously described (Tepperman, 1972; Tepperman, Morris, Essien & Heywood, 1975). The rat muscle cell line L6 was a gift from Dr David Yaffe (Weizmann Institute, Israel). All muscle cells were cultured on Falcon plastic dishes coated with 3% gelatin (Difco). The culture medium was prepared as previously described (Herrmann, Havarranis & Doetschman, 1975), except that no HEPES-TE buffer was used for the L6 cells since it is toxic to them. The medium for the 3T3 cells consisted of 0.2% NaHCO3-buffered DME nutrient mixture, 10% foetal calf serum (Gibco), 200 units/ml penicillin, and 200 μg/ml streptomycin. The 3T3 cells were at subconfluent densities throughout the experimental periods.

The cultures were labelled with L-[6-3H]fucose (13.4 Ci/mmol, New England Nuclear) with the amounts indicated in the figure legends and the footnotes to the tables. Before addition to the cultures the labelled fucose was concentrated to dryness and redissolved in 1/20 volume 95% ethanol in order to keep the ethanol concentration of the medium at a low level. The label was removed by rinsing the cultures 4 times with nutrient mixture. The medium released material was then collected in nutrient mixture with 50 μg/ml unlabelled fucose. During the release period Con A (Miles, 3 times crystallized, lyophilized), cytochalasin B (Sigma, Lot. No. C 6267), and colchicine (Sigma) were added as described in the figure legends and footnotes to the tables. At the end of the release period phenylmethyl sulphonyl fluoride (Sigma) was added to all medium released fractions to a final concentration of 16 μg/ml in order to inhibit proteolytic activity. Cell debris was removed by centrifugation at 900 g for 10 min. The released fraction was dialysed extensively against distilled water, analysed for radioactive content, concentrated to dryness in a Speed Vac Concentrator (Savant), and dissolved and boiled in electrophoresis sample buffer containing 2-3% SDS and 5% β-mercaptoethanol for 3 min. The samples were electrophoresed according to Laemmli (1970) on slab gels with a 3-step acrylamide gradient: 3/8 at 12.5%, 3/8 at 10%, and 1/4 at 7.5% with a 3% acrylamide stacking gel. Gels were stained for protein (Heywood & Kennedy, 1979) and then photographed. Fluorograms of the gels were prepared as described previously (Bonnor & Laskey, 1974; Laskey & Mills, 1975).
RESULTS

Con A inhibition of shedding

The shedding of fucosyl-glycoprotein is inhibited by Con A (Table 1). This is the case with all of the muscle cell types tested (leg, breast, and L6) and it occurs in the presence of cytochalasin B, colchicine, or both. Alone, cytochalasin B has no consistent effect on the amount shed, and colchicine always inhibits shedding to a lesser degree than Con A. The effect of Con A and colchicine are generally additive. The only exception to these results is found in 3T3 cell cultures where Con A does not inhibit shedding as much as in the muscle cells. In one experiment it did not inhibit at all. In the 3T3 cells Con A inhibits shedding less than does colchicine.

In experiment 3 the shed material was collected from 4 to 10 h after removal and chase of the label to provide a control experiment in which the released fraction is not complicated by the presence of secreted labelled material. There is evidence that L6 cells (Doetschman, 1980a) as well as other cells (Yurchenco & Atkinson, 1977; Doyle et al. 1978; Gottesman, 1978) secrete proteins and glycoproteins within the first 4 h after being synthesized. Consequently, by eliminating the presence of secreted labelled material from the released fraction, the effects of Con A, cytochalasin B, and colchicine could be compared to those found during a time when some labelled-glycoprotein was being secreted. A comparison of the results of experiments 1 and 2 with experiment 3 shows little difference. Consequently, the various effects described above are primarily on shedding and not secretion.

Differential nature of shedding inhibition

Since Biogel A-5m gel exclusion chromatography separates the shed fucosyl-glycoproteins into large (greater than $5 \times 10^8$ Daltons) lipid-containing and smaller lipid-free materials (Doetschman, 1980a) it was of interest to know if Con A inhibits the shedding of one of these fractions more than the other. This was found to be the case in leg muscle and 3T3 cell cultures. The shedding of the lipid-containing fraction was inhibited by $24 \pm 8\%$ (standard error, 4 experiments) in leg and by $32 \pm 8\%$ (6 experiments) in 3T3 cultures. The amount shed in the lipid-containing fraction is given as the percent of the total amount shed.

Evidence for the shedding inhibition and its differential nature is also found in fluorograms of the $^{3}H$-fucosylated glycoproteins that are shed into the medium (Fig. 1). Con A inhibits the shedding of some glycoproteins more than others. Those affected the most migrate slower than the albumin marker. In fact, the differential Con A effect is more evident in the 3T3 cultures where Con A has less of an inhibitory effect on the total amount of shedding than it is in the muscle cultures (Fig. 1c). This is expected because of the greater inhibition of shedding of the lipid-containing gel exclusion fraction in the 3T3 cells. The differential nature of the Con A inhibition is unaltered even if cytochalasin B and/or colchicine are present with the Con A during the release period.

In a similar experiment the differential effects of Con A were more strongly evident. The results are shown in Fig. 2. There are several intense bands in the fluorogram of
Table 1. Quantitative effects of Con A, cytochalasin B, and colchicine on the shedding of fucosyl-glycoproteins

<table>
<thead>
<tr>
<th>Additions</th>
<th>Leg Exp. 1</th>
<th>Leg Exp. 2</th>
<th>Leg Exp. 3</th>
<th>Breast Exp. 1</th>
<th>Breast Exp. 2</th>
<th>Breast Exp. 3</th>
<th>L6 Exp. 1</th>
<th>L6 Exp. 2</th>
<th>L6 Exp. 3</th>
<th>3T3 Exp. 1</th>
<th>3T3 Exp. 2</th>
<th>3T3 Exp. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
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<td>100</td>
<td>—</td>
<td>100</td>
<td>100</td>
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<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(18)</td>
<td>(106)</td>
<td></td>
<td></td>
<td>(38)</td>
<td>(134)</td>
<td>(75)</td>
<td>(18)</td>
<td>(130)</td>
<td>(145)</td>
<td>(14)</td>
<td>(113)</td>
<td>(27)</td>
</tr>
<tr>
<td>Con-A</td>
<td>67</td>
<td>66</td>
<td>—</td>
<td>45</td>
<td>55</td>
<td>54</td>
<td>56</td>
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<td>CB</td>
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<td>110</td>
<td>—</td>
<td>—</td>
<td>90</td>
<td>80</td>
<td>106</td>
<td>115</td>
<td>155</td>
<td>79</td>
<td>110</td>
<td>—</td>
</tr>
<tr>
<td>COL</td>
<td>89</td>
<td>90</td>
<td>—</td>
<td>76</td>
<td>88</td>
<td>—</td>
<td>78</td>
<td>77</td>
<td>96</td>
<td>43</td>
<td>64</td>
<td>45</td>
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<tr>
<td>Con-A &amp; CB</td>
<td>61</td>
<td>64</td>
<td>—</td>
<td>—</td>
<td>52</td>
<td>54</td>
<td>—</td>
<td>69</td>
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<td>—</td>
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<td>89</td>
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<tr>
<td>Con-A &amp; COL</td>
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<td>50</td>
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<td>—</td>
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<td>47</td>
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<tr>
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<td>56</td>
<td>55</td>
<td>—</td>
<td>—</td>
<td>40</td>
<td>61</td>
<td>—</td>
<td>63</td>
<td>43</td>
<td>—</td>
<td>75</td>
<td>55</td>
</tr>
<tr>
<td>CB &amp; COL</td>
<td>83</td>
<td>87</td>
<td>—</td>
<td>73</td>
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<td>81</td>
<td>103</td>
<td>—</td>
<td>84</td>
<td>104</td>
<td>—</td>
</tr>
</tbody>
</table>

* One culture per determination.

* Parentheses indicate the total dpm × 10^-9 in the control cultures.

Experiment 1. Cells were plated at 5 × 10^6 live cells/100-mm plate (leg), 4 × 10^6 live cells/100-mm plate (breast), 1 × 10^6 cells/100-mm plate (L6), and 1 × 10^6 cells/100-mm plate (3T3). All cultures were labelled with 200 μCi [3H]fucose from 45 to 69 h of culture (leg, breast, L6) or from 18 to 42 h of culture (3T3) in complete medium after which the label was removed. The shed fraction was collected in chase medium for the next 6 h in the presence of various combinations of Con A (10 μg/ml), cytochalasin B (CB, 5 μg/ml), and colchicine (COL, 5 μg/ml). These same concentrations were used in experiments 2 and 3.

Experiment 2. Cells were plated at 1.5 × 10^6 live cells/60-mm plate (leg, breast), 3 × 10^6 cells/60-mm plate (L6), and 3 × 10^6 cells/60-mm plate (3T3). Cultures were labelled with 100 μCi [3H]fucose from 24 to 48 h of culture in complete medium. The shed fraction was collected in the presence of the various additions from 48 to 54 h.

Experiment 3. Cells were plated at 4 × 10^6 live cells/100-mm plate (breast), 3 × 10^6 cells/100-mm plate (L6), and 1.5 × 10^6 cells/100-mm plate (3T3). Cultures were labelled with 180 μCi [3H]fucose from 19 to 29 h of culture (breast) and from 24 to 44 h (L6, 3T3) in complete medium. The materials released into the medium from the next 4 h of culture were discarded and those shed in the presence of the additions during the following 6 h were collected.
Effects of Con A on shedding

that gel which are in all of the samples except those in which Con A was present during the release period. Most of these bands can be found between the top of the gel and the albumin marker. Inversely, there are bands present in Con A-treated samples which are more intense relative to other bands in the same sample than are the corresponding bands in the samples without Con A. The differential effect of Con A, regardless of the presence or absence of cytochalasin B or colchicine is also evident in Fig. 2. In the culture treated with Con A plus cytochalasin B (well 6) there are 3 bands of about 70,000, 44,000, and less than 44,000 Daltons which are not as intense in the other Con A-treated cultures presented in Fig. 2. This result occurred in only 1 experiment and will be discussed later. It does not, however, detract from the conclusion that Con A has a differential effect on shedding.

Con A inhibition of internalization and degradation

The overall reduction of the amount of [3H]fucose-containing glycoprotein shed in the presence of Con A could be caused by variations in 2 parameters: the amount of surface fucose-labelled glycoproteins capable of being shed and the rate of shedding. The degree to which each of these parameters is involved in the Con A effect was investigated next in breast muscle cell cultures. Because of the possibilities of cell surface recycling (Schneider, Tulkens, de Dure & Trouet, 1979) and cytoplasmic storage forms of plasma membrane (Doyle et al. 1978), it is not known for certain how much of the fucosyl-glycoprotein in the cells is at the surface or in the cytoplasm at any given time. Trypsin is usually thought to act only at the surface of viable cells. For this reason the Con A effect on the trypsin-sensitive and total cell bound fucosyl-glycoprotein was measured. The results are shown in Table 2. Con A increases dramatically the trypsin-sensitive surface fucosyl-glycoprotein. The reduction in shedding, however, does not completely account for the increased trypsin-sensitive surface glycoprotein.

There are 3 possible interpretations of these results. First, if the trypsin-removable fucosyl-glycoproteins are assumed to represent the major portion of the fucosyl-glycoproteins in the cell surface and the trypsin-insensitive material consists of internalized recycling or precursor plasma membrane, then the increase of approximately 22 points in the percentage of total label in the culture that is trypsin-sensitive is much more than what can be accounted for by the decrease in shedding which is from 8 to 12 % of total label. This interpretation suggests that endocytosis is inhibited by Con A as well. Second, there is some evidence that trypsin can cause the release of cytoplasmic glycoprotein destined for secretion (Jett & Jamieson, 1973). This is probably not occurring here because the trypsin was added after 6 h of release in a fucose chase medium. It has already been shown that little labelled glycoprotein is secreted at this time (Doetschman, 1980). Third, it is possible that the cell-bound fraction represents the cell surface fucosyl-glycoprotein and that the Con A effect on trypsin sensitivity is due to a change in distribution, orientation, or configuration of surface materials as occurs in the micoredistribution and cooperative binding effects of Con A (Bornens, Karsenti & Avrameas, 1976; Karsenti, Bornens & Avrameas, 1977; Prujanski, Ravid & Sharon, 1978; Gordon & Young, 1979). A decrease in
Effects of Con A on shedding

internalization and degradation is not necessarily consistent with this interpretation of the Con A effect on shedding. However, since the total amount of fucosyl-glycoprotein per culture is greater by 13 ± 9% in the Con A-treated cultures (last column in Table 2), internalization and degradation are necessarily decreased.

Fig. 2. Electrophoretic profiles of the [3H]fucosyl-glycoproteins shed from leg muscle cell cultures in the presence of combinations of Con A, cytochalasin B, and colchicine. The samples were obtained from Experiment 1 of Table 1 and were prepared as described in the footnotes to Table 1. The amount of radioactivity in each sample can be calculated from Table 1. No additions (control), 2; Con A, 3; cytochalasin B, 4; colchicine, 5; Con A and cytochalasin B, 6; Con A and colchicine, 7; Con A, cytochalasin B, and colchicine, 8; and cytochalasin B and colchicine, 9. The molecular weights of the markers are 200,000 for myosin (m), 68,000 for albumin (a), and 44,000 for ovalbumin (o).

Fig. 1. Electrophoretic profiles of the [3H]fucosyl-glycoproteins shed in the presence of combinations of Con A, cytochalasin B, and colchicine. The samples were obtained from Experiment 2 of Table 1 and were prepared as described in the footnotes to Table 1. Leg (a), breast (b), 3T3 (c), L6 (d); Unlabelled molecular weight markers, 1 and 10; no additions (control), 2; Con A, 3; cytochalasin B, 4; colchicine, 5; Con A and cytochalasin B, 6; Con A and colchicine, 7; Con A, cytochalasin B, and colchicine, 8; cytochalasin B and colchicine, 9. The molecular weights of the markers are 200,000 for myosin (m), 68,000 for albumin (a), and 44,000 for ovalbumin (o).
Table 2. Effect of Con A on the cellular distribution of fucosyl-glycoproteins

<table>
<thead>
<tr>
<th>Time, h, of release</th>
<th>Treatment</th>
<th>Cell-bound</th>
<th>Shed</th>
<th>Trypsin-sensitive</th>
<th>Total, dpm/culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con A</td>
<td>Trypsin</td>
<td>Exp. 1</td>
<td>Exp. 2</td>
<td>Exp. 1</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>97</td>
<td>94</td>
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<td>0</td>
<td>-</td>
<td>+</td>
<td>73</td>
<td>65</td>
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<td>82</td>
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</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>42</td>
<td>31</td>
<td>14</td>
</tr>
</tbody>
</table>

Chick embryonic breast muscle cells were plated at 5.0 × 10⁴ live cells/100-mm plate and labelled with 20 μCi [¹⁴C]fucose from 24 to 42 h of culture. The label was removed and the cultures rinsed 4 times in macromolecule-free medium. At 42 h of culture 2 sets of cultures were treated with or without 0.05% trypsin in Ca²⁺, Mg²⁺-free PBS for 15 min. The cells were then scraped off the plates and centrifuged at 900 g for 10 min. Portions of the supernatants (trypsin-sensitive fucosyl-glycoprotein) and the cell pellets (cell-bound fucosyl-glycoprotein) were measured for radioactivity. The remaining cultures were incubated either in the presence or absence of 10 μg/ml Con A during a 6-h release period (42 to 48 h of culture). The shed fraction was then removed and the cells treated with or without trypsin as described above. The cell-bound, shed, and trypsin-sensitive fractions were then analysed for radioactivity. Data from 2 cultures were averaged for each determination.
DISCUSSION

The important findings of this study are that (1) 10 μg/ml of Con A inhibits shedding in general; (2) Con A differentially inhibits the shedding of fucosyl-glycoproteins that are in large lipid-containing particles; (3) even when combined with cytochalasin B or colchicine, Con A maintains its differential inhibitory effect on shedding; (4) the inhibition is due to a decreased rate of shedding rather than an increased internalization and degradation; and (5) internalization and degradation are also inhibited by Con A.

Con A effect is not due to cell death

It is unlikely that the shedding inhibition caused by Con A is a cell death-related phenomenon. First, the inhibition is of the same magnitude in the L6 cells as in the primary cultures (Table 1), yet the amount of cell death in these 2 culture systems is quite different, 2 and 15%, respectively (Doetschman & Jewett, 1980). Similarly, the inhibitory effect is quite different in the L6 and 3T3 cultures (Table 1) whereas the amount of cell death is the same (Doetschman & Jewett, 1980). Second, the 31 to 55% decrease in shedding caused by Con A (Table 1) cannot be quantitatively accounted for by a maximum of 18% contamination due to cell death (Doetschman & Jewett, 1980). Third, if the differential effect of Con A on shedding were due to cell death then one would expect less of this effect in the L6 and 3T3 than in the primary cultures. This is definitely not the case (Fig. 1). Fourth, no cell-death differences due to Con A were found in another study (Dunlap & Donaldson, 1978).

Con A effect is on shedding

The Con A inhibition of the release of fucosyl-glycoprotein into the culture medium is shown to be an effect on shedding rather than on secretion. This is consistent with previous results which showed that under the same labelling and release conditions used here, nearly all of the labelled, released fucosyl-glycoproteins are shed and not secreted. The reason for this is that after a prolonged labelling period the cell surface becomes so heavily labelled that the amount of label released by secretion is nearly undetectable (Doetschman, 19806). The methods used do not indicate whether secretion is also inhibited by Con A.

Shedding at cell surface sites

The inhibition of shedding caused by Con A at a concentration that inhibits the mobility of some cell surface materials (Schlessinger et al. 1976) suggests that there is a shedding site(s) to which surface glycoprotein must migrate (Bretscher, 1976; Stern & Bretscher, 1979; Ukena, Borysenko, Karnovsky & Berlin, 1974; Edelman, 1976; Bourguignon & Singer, 1977) before shedding can occur. Cell surface caps (Karnovsky et al. 1972; Leonard, 1973; Stanbridge & Weiss, 1978; Nicolson, 1979) and coated pits (Brown et al. 1979) are possible sites for cell surface shedding. Since cell surface sites are also involved in receptor-mediated endocytosis via capping (Unanue & Karnovsky, 1973; Albertini & Anderson, 1977) or coated pits (Goldstein,
Anderson & Brown, 1979), Con A would be expected to have an inhibitory effect on endocytosis as well. The evidence presented here is consistent with this possibility.

If there are shedding sites on the cell surface it is possible that the shed materials occur in the form of membrane vesicles as in endocytosis. Suggestive evidence for naturally occurring shed vesicles has been found in several cell culture systems (Peterson & Rubin, 1969; Vitetta & Uhr, 1972; Nowotny et al. 1974; Doetschman, 1980a).

Proteolysis is not a major shedding mechanism

Cell surface proteolytic activity has been suggested as one of the mechanisms of cell surface shedding (Kapeller et al. 1973; Hynes, 1974, 1976; Doljanski & Kapeller, 1976; Baumann & Doyle, 1978; Mosher & Vaheri, 1978; Parry, 1978). If a significant amount of shedding is caused by proteolytic activity, then Con A, by increasing the exposure of cell surface glycoproteins to proteolytic attack (Table 2), should increase shedding. On the contrary, Con A decreases shedding. This does not rule out the possibility that some shedding occurs by proteolysis, but it does suggest that serine proteases contribute at most only a small degree to shedding. Another study has also shown that the serine protease inhibitor phenylmethyl sulphonyl fluoride has no inhibitory effect on shedding (Doetschman & Jewett, 1980).

Roles of microfilaments and microtubules on cell shedding

By using the cytoskeletal disruptors cytochalasin B and colchicine, evidence was obtained which suggests that microtubules and perhaps microfilaments are involved in the shedding process. Alone and in conjunction with Con A colchicine inhibits shedding. There is some evidence that cytochalasin B may stimulate shedding but the effect is very small. With one exception (Fig. 2) these effects are not differential in the sense that some shed materials are affected to different degrees. Likewise, these drugs do not change the differential nature of the inhibition of shedding caused by Con A. In other words, although microtubules and microfilaments may affect the rate at which surface materials are shed, they confer little selectivity on the major portion of shed materials. This does not seem to be the case for the shedding of IgM and IgD which are inhibited by cytochalasin B and colchicine, respectively (Emerson & Cone, 1979). This suggests that cell surface receptors with specific functions may require specific regulation mediated by cytoskeletal elements.

It is not uncommon for cytoskeletal perturbants to alter Con A effects in some cases and not in others. Carraway et al. (1979) point out that it may be important to distinguish the global effects of Con A, where the lectin localized to one small area of the cell surface immobilizes cell surface proteins over the entire surface (Edelman, 1976), from the short-range (Bornens et al. 1976; Karsenti et al. 1977; Gordon & Young, 1979) effects of Con A. Applying this distinction to the results presented here, cytoskeletal elements may not interfere with the global protein-immobilizing effect of Con A but at the short-range level they may alter the shedding of individual surface molecules. This may be the reason for the exception to the cytochalasin B results found here.
Effects of Con A on shedding

The results are inadequate for analysing the roles that microtubules and microfilaments may play in the shedding process. However, the partial reversal of the Con A inhibition of capping (Edelman, 1976) and cell surface mobility (Schlessinger et al. 1977) caused by colchicine was not observed with respect to shedding. This makes it unlikely that shedding involves any kind of capping process in these cells.

Effects of mitogenic vs. non-mitogenic Con A concentrations on shedding

Evidence has been found that Con A stimulates release (Jones, 1973; Schmidt-Ullrich, Hoelzl-Wallach & Ferber, 1974). In both cases mitogenic concentrations of Con A were used. It has been shown that at these concentrations the lectin does not have inhibitory effects on capping (Edelman, 1976) or receptor mobility (Schlessinger et al. 1976). Moser, Schneider & Falke (1978) found that Con A at 2 to 5 μg/ml increased shedding from the cell surface of BHK21 cells but it is unknown whether this concentration inhibits surface mobility. Wortmann, Prinz, Ullrich & von Figura (1979) have investigated the effects of high concentrations (non-mitogenic) of Con A on the metabolism of glycosaminoglycans in primary fibroblast cell culture. The results are consistent with the view that the lectin decreases receptor-mediated endocytosis. Con A was shown to increase the amount of cell-associated glycosaminoglycan and specifically the amount of trypsin-sensitive surface glycosaminoglycan. It also increased the amount of glycosaminoglycan in the medium, but this was shown to be the result of decreased endocytosis of medium Con A-glycosaminoglycan complexes rather than of increased release of glycosaminoglycans.

At this point some comments of a speculative nature can be made concerning the mechanisms of cell surface shedding and their relationship to surface turnover. There are several lines of evidence which suggest that the shedding sites may be similar to or the same as endocytic sites, or that they may come under common regulation. First, inhibition of lateral surface mobility inhibits both shedding and endocytosis. Second, Brown et al. (1979) find that Epidermal Growth Factor (EGF) is normally internalized (in coated vesicles) by fibroblasts, but when antibody to EGF is present the factor is released rather than internalized. This suggests that some kind of a filtering system may exist at coated pits which selects some surface materials for endocytosis and some for shedding. A cell surface site with some of these properties has been hypothesized previously (Bretscher, 1976; Stern & Bretscher, 1979). Third, in a system unperturbed by surface or cytoskeletal effectors, the shedding and turnover of fucosyl-glycoprotein both have monophasic kinetics and the shed molecules appear to be a subset of the cell-bound fucosyl-glycoproteins (Doetschman, 1980b). Fourth, endocytosis and possibly shedding can occur in the form of vesicles, and the kind of shedding most inhibited when cell surface protein is immobilized is the kind that results in the release of large, lipid and fucosyl-glycoprotein-containing particles.

The importance of shedding with respect to evasion of the immune system in tumours (Nicolson, 1976), parasites (Bloom, 1979), and embryos (Alexander, 1974), and the potential importance of shedding with respect to all biological systems in which intercellular communication occurs, for example in development (Schubert et al. 1973; Truding, Shelanski & Morell, 1975), suggest that cell surface shedding is
worthy of further attention. It is hoped that the study presented here provides some insights into the phenomenon of cell surface shedding and raises some questions upon which future studies can be built.

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