Journal of Supramolecular Structure 11:175–187 (1979)
Tumor Cell Surfaces and Malignancy 121–133

Growth Control by Cell to Cell Contact

Richard Bunge, Luis Glaser, Michael Lieberman, Dan Raben, James Salzer, Brock Whittenberger, and Thomas Woolsey

Departments of Biological Chemistry and Anatomy and Neurobiology, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri 63110.

Control of cell growth by cell to cell contact is reviewed with particular emphasis on two systems – contact inhibition of growth observed with Swiss 3T3 cells and the mitogenic stimulation of Schwann cells by dorsal root ganglia neurites. In both cases the biological effect can be reproduced by the addition of surface membranes to the corresponding cells. In the case of contact inhibition of 3T3 cells, biological activity appears to correlate with membrane binding to the cells. An octylglucoside extract of 3T3 plasma membranes retains the biological activity (growth inhibition) of the original membranes.

Key words: growth control, 3T3 cells, Schwann cells, neurites, plasma membranes

While the purpose of this review is to discuss recent advances in the study of the effect of cell to cell contact on growth control, the subject can be put into the general context of specific cell to cell adhesion. The problem has been examined in a number of systems, of which the best studied are dissociated embryonic cells in which it has been demonstrated that specific cell to cell adhesion can occur [for review, see references 1–4]. These results imply that there are molecules present on the cell surface that can interact with the surface of other cells (either homologous or heterologous) and result in cell to cell adhesion. Such adhesion is believed to be important in terms of normal development, but in a number of instances it is also likely that specific cell to cell adhesion will have an effect on the physiological properties of the cells; that is, a cell in contact with certain other cells may behave in a different manner or respond in a different way to external stimuli than a cell that is growing in relative isolation. The problem with a number of the approaches that have been used to study cell to cell adhesion is that it is very difficult to distinguish biologically meaningful cell to cell adhesion – that is, adhesion between cells, which has a developmental or physiological consequence, in contrast to intercellular adhesion, which happens to represent a laboratory curiosity in the sense that two normally unrelated cell types fortuitously have ligands on the cell surface that can interact. Therefore, it is of great interest to study cell interactions in which there are physiological consequences of cell adhesion. This review concentrates on two such examples and mentions a few others that have been less well investigated but may represent fruitful areas for further investigation.

Received March 18, 1979; accepted June 18, 1979.

0091-7419/79/1102-0175$02.60

The two systems that will be discussed in detail are 1) contact inhibition of growth, a situation in which contact between 3T3 cells is followed by cessation of growth (in this situation a negative growth signal is generated) [3, 5–8], and 2) contact stimulation of growth, a situation in which contact between the surface of a neurite and a Schwann cell induces cell division in the Schwann cells (in this case a positive growth signal is generated) [9–11].

CONTACT INHIBITION OF GROWTH

The subject of contact inhibition of growth is an old one and a one that has been debated for a number of years. The specific question that has been asked is whether the cessation of growth observed at confluence with a number of "normal" fibroblastic cell lines at high cell density is due to a contact phenomenon or represents the failure of nutrients or other mitogenic factors in the medium to reach the cell surface. The latter could result either because the factors are depleted from the medium [12] or because a diffusion boundary layer exists near the cell surface [13, 14].

Classical experiments by Dulbecco [7] have shown that if a nongrowing confluent monolayer of cells is wounded, new cells will grow into the wound area, indicating that depletion of growth factors or other nutrients from the bulk medium was not responsible for contact inhibition of growth. A slightly different way of looking at such experiments is shown in Figure 1. The conclusion drawn from this experiment is essentially the same as that in the original Dulbecco experiments as well as similar experiments by Holley and Kiernan [15]; ie, that 3T3 cells at confluence do not grow, in spite of the fact that they are in a medium that can support the growth of sparse cells.

The question has been raised, however, as to whether growth is limited because of an inability of mitogenic factors to reach the cell surface at a rate rapid enough to have an effect on the cells, since the cells generally degrade or utilize mitogenic factors [13, 14]. A test of this proposal has been carried out by increasing the viscosity of the medium. This should result in a further reduction of cell growth if diffusion limitation of growth exists [16]. Since neither the rate nor the extent of cell growth was altered by changes in viscosity, it appears unlikely that growth under these conditions with Swiss 3T3 cells is diffusion limited.* A similar conclusion by a different approach was reached by Thrash and Cunningham [17], who showed that cell density as well as medium components can limit the density of Swiss and Balb 3T3 cells. We can, therefore, assume that among the signals for growth that a cell is capable of receiving there are both positive signals, such as those brought about by the binding of known mitogens to the cell surface, and negative signals, which are derived in the particular case that is under discussion by contact with other cells. Contact could therefore be considered in the same light as the interaction of hormones with a surface receptor. These interactions between cells ultimately produce an intracellular signal which at the present time has not been defined, and which is responsible for the cessation of growth.

Not all cultured "normal" fibroblastic cells exhibit the same extent of growth control as do 3T3 cells, and for some lines this property is only apparent in low concentrations of serum [3]. One of the simplest ways in which cell to cell contact could inhibit cell growth

*Some reinterpretation of these observations has been presented in the "Matters Arising" section of Nature (274:722, 1978) by H. G. Maroudas, B. Whittenberger and L. Glaser, as well as a different interpretation by R. W. Holley and J. H. Baldwin in Nature (278:283, 1979).

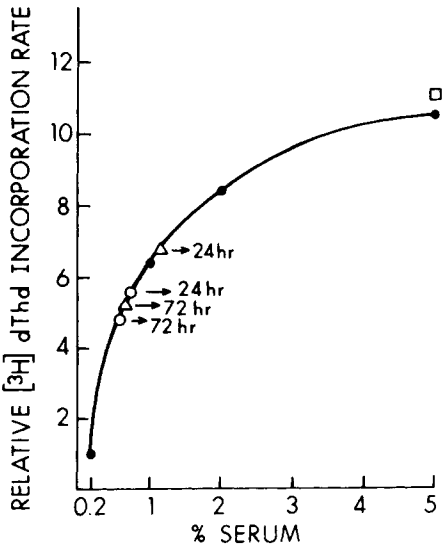

Fig. 1. Depletion of mitogenic activity from serum by confluent 3T3 cells. Cells were plated in both Linbro multi-well dishes (2 cm^2 per well, 1.2×10^3 cells per well) and Falcon 35 mm dishes (6×10^4 cells per dish) in DME/10% calf serum/L-[^{35}S]-methionine (1 μCi/ml). Medium was also plated on empty 35 mm dishes as a control. Seventy-two hours after plating, the medium in the Linbro dishes was changed to DME/0.2% calf serum/L-[^{35}S]-methionine (1 μCi/ml) (0.6 ml per well). Prior to the addition of the low serum medium the wells were rinsed twice with DME to remove residual serum. In addition, the medium in all the 35 mm dishes (including the dishes with no cells) was changed to DME/10% serum utilizing two different serum lots (this will generate 72-hour depleted medium). Forty-eight hours later the medium in one-half of the 35 mm dishes was changed (DME/10% calf serum, 2 serum lots) to generate 24-hour depleted medium. Twenty-four hours later the medium was removed from the 35 mm dishes (5 sets of samples: serum lots 1 and 2, both 24- and 72-hour depleted, and the medium over empty plastic for 72 h), brought to 1 μCi/ml in L-[^{35}S]-methionine (stock at 1,000 μCi/ml), and added to the starved cells in the Linbro dishes (1.0 ml/well). In addition, medium containing 0.2%, 1.0%, 2.0%, or 5.0% serum was also added to starved cells. The dishes were pulsed with [^{3}H]-dThd (6.7 Ci/mmole) from 21 to 25 hours after the medium change, and the data were collected and analyzed by the double-label technique previously described [37]. Symbols: •, standard curve determinations; △, serum lot 32060 (K.C. Biological); ○, serum lot 328074 (K.C. Biological); □, medium incubated over empty plastic. The cells from which the depleted medium was taken were assayed for DNA synthesis and had 10% the rate of a sparse culture for the 24-hour depleted medium (both serum lots) and 2% the rate of a sparse culture for the 72-hour depleted medium (both serum lots). Similar results have been obtained in independent experiments by autoradiography. The data show that incubation of medium with confluent cells results in only a partial depletion of mitogenic activity for sparse cells.

would be if cell contact changed either the number of receptors for mitogens and/or the ability of these receptors to function. The concentration of receptors for mitogenic factors in confluent density-inhibited cells has only been investigated in a limited number of cases. The concentration is generally similar to or higher than that observed in sparse cells [for example, see references 8, 18]. In BSC-1 cells, the level of receptor for epidermal growth factor decreases tenfold at confluence, perhaps as a consequence of cell to cell contact (our interpretation) [19].

If growth is in part controlled by the interaction of molecules present at the cell surface, then it is possible to consider that a plasma membrane fraction of such cells when added to sparse cells (ie, subconfluent cells, which do not have extensive cell to cell contact

with other cells), may result in the cessation of growth if the cells recognize membranes in the same manner as they recognize other cells.

The probability that such an experiment would actually succeed was greatly increased by the fact that, in a number of cases where specific cell adhesion had been examined, it was known that plasma membranes retain the ability to bind to cells with the same or similar specificity as the cells from which they were derived [see for example references 20, 21]. In addition, a limited number of experiments suggested that the developmental patterns of slime molds could be altered by the addition of homologous plasma membranes [22, 23]. In previous experiments in which adhesion of plasma membranes to cells was measured, it was concluded that only one of the two complementary cell surface adhesive molecules remained functional in isolated plasma membranes [20]. It is not known whether this is a general phenomenon, but the proposed experiments require that this component be the one that elicits a response in the target cell.

An extensive series of studies has therefore been carried out in order to determine whether a plasma membrane fraction derived from 3T3 cells can, in fact, induce the cessation of growth of a sparse culture of the same cells. The system is clearly one that needs to be approached with caution, because cessation of growth, which is the parameter to be measured, could be due to a variety of "toxic effects" that would not be related to contact inhibition of growth.

Plasma membranes from 3T3 cells when added to sparse cells lead to the cessation of growth in a time and concentration dependent manner. This effect is not due to general toxicity since the same membranes do not have an effect on SV40 transformed 3T3 cells. The effect is reversible, in that removal of the membranes by trypsinization followed by replating of the cells results in reinitiation of growth. Control experiments have shown that the membranes do not act by removing essential nutrients from the bulk medium which would be a trivial cause for the cessation of cell growth [24].

The cells that are arrested by the addition of membranes are arrested early in the G_1 phase of growth, a situation identical to that which obtains during contact at high cell density. The kinetics by which membranes interact with cells are such that in each cell cycle, even at saturating membrane concentrations, only a maximum of 40–50% of the cells are arrested. The remainder of the cells go through an additional cell cycle, and if membranes are still present about 40–50% of these cells will now be arrested in G_1. This leads to a model of growth control which suggests that during each cell cycle, presumably early in G_1, the sensitivity of the cells to contact to other cells (or in this case membranes), is reset at different levels. If this sensitivity is below some threshold value, the cells can be arrested by addition of membranes or, presumably, by contact with other cells. If this signal is set above some threshold value, then the cells can continue through the cell cycle. In the next cell cycle this sensitivity will again be randomized, and some cells will become sensitive to contact inhibition [25]. Previous experiments by Martz and Steinberg [26], in which the growth of 3T3 cells in contact with other cells was followed by cinematography, would be in agreement with this type of model, as would recent experiments on growth control by nutrient starvation [27]. In the experiments by Martz and Steinberg, cells with extensive contact with other cells were observed after cell division. About 50% of such cells divided again within 24 hours, whereas the other 50% failed to divide during the maximum time period of observation (72 hours).

Membranes prepared from erythrocytes are not active in inhibiting the growth of 3T3 cells, and membranes prepared from SV40 transformed 3T3 cells show less activity than those prepared from 3T3 cells. Thus, one would conclude that SV40 transformed 3T3

cells, which do not show density-dependent inhibition of growth, have lost the ability to respond to contact with other cells but can still generate a signal by contact with normal cells. The relevant molecules are still present in the plasma membrane of SV3T3 cells, although apparently at a somewhat lower concentration than in the 3T3 cells.

Addition of membranes to cells will also reduce the rate of uptake of α-aminoisobutyric acid and uridine [28], an effect which resembles that observed at high cell density [for review see references 3, 29, 30]. On the other hand, addition of membranes has no effect on the rate of uptake of 2-deoxyglucose or inorganic phosphate by sparse 3T3 cells, and thus these two parameters seem to be dissociable from the cessation of growth seen at high density [28] – ie, contact inhibition of growth. These data are complementary to those obtained by Cunningham and co-workers, which also suggested that transport of glucose and inorganic phosphate was not directly related to growth control [29], and to the finding of Lever that contact did not alter the ability of membrane vesicles to transport glucose [31]. A summary of these data is shown in Figure 2A. (It should be noted that the use of membranes for such experiments has a number of advantages, since the cell density in the presence of membranes is low and any effects due to nutrient depletion by high cell density are therefore minimized.) SV40 transformed 3T3 cells show a weak response to the addition of membranes, in that the uptake rate of α-aminoisobutyric acid and uridine is decreased, but there is no effect on cell growth or on the rate of uptake of 2-deoxyglucose. Whether this response represents a decreased ability of these cells to respond to the same stimuli as 3T3 cells or a nonspecific effect is not known. At membrane concentrations that give maximal inhibition of uptake of 3T3 cells there is very little effect on the uptake rate of α-aminoisobutyric acid and uridine by SV3T3 cells, but at high concentrations of membranes

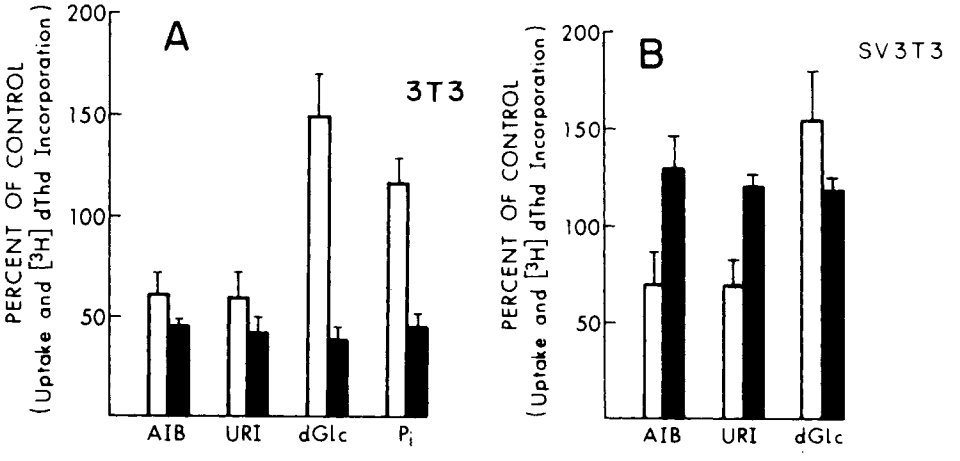

Fig. 2. Effect of 3T3 plasma membranes on solute transport. A. The bar graph summarizes a large number of experiments in which the rate of solute uptake was measured after 48 h of incubation of Swiss 3T3 cells with saturating levels of membranes (16 to 20 phosphodiesterase units per 35 mm dish) [28]. Open bars indicate rate of solute uptake; closed bars, the corresponding rate of DNA synthesis. B. Similar data obtained for SV3T3 cells (for details see text). The data shown are for 20 PDE units (alkaline phosphodiesterase [24]) per 35 mm dish. Maximal inhibition of solute uptake by 3T3 cells occurs at 4 PDE units/dish, a level at which SV3T3 cells show a very low response. Thus solute uptake (α-aminoisobutyric acid and uridine) is at least five times more sensitive to membrane addition in 3T3 cells than in SV3T3 cells. Abbreviations: AIB, α-aminoisobutyric acid; URI, uridine; dGlc, 2-deoxy-D-glucose; Pi, inorganic phosphate.

the inhibition can be as large as that observed with 3T3 cells (Fig. 2B). The data in Figure 2B were obtained at 20 phosphodiesterase units of membranes per 35 mm dish, whereas the maximum effect of membranes on transport with 3T3 cells is obtained at 4 phosphodiesterase units/35 mm dish, at which level there is very little effect on the rate of nutrient uptake by SV3T3 cells. The effect on nutrient uptake by SV3T3 cells is unlikely to be due to steric effects, since the rate of 2-deoxyglucose appears not to be affected and monosaccharides and amino acids are molecules of roughly equivalent size.

We have devised a technique for measuring the binding of membranes to cells on a dish by the use of ^{125}I-labeled plasma membranes, which retain the ability to block cell

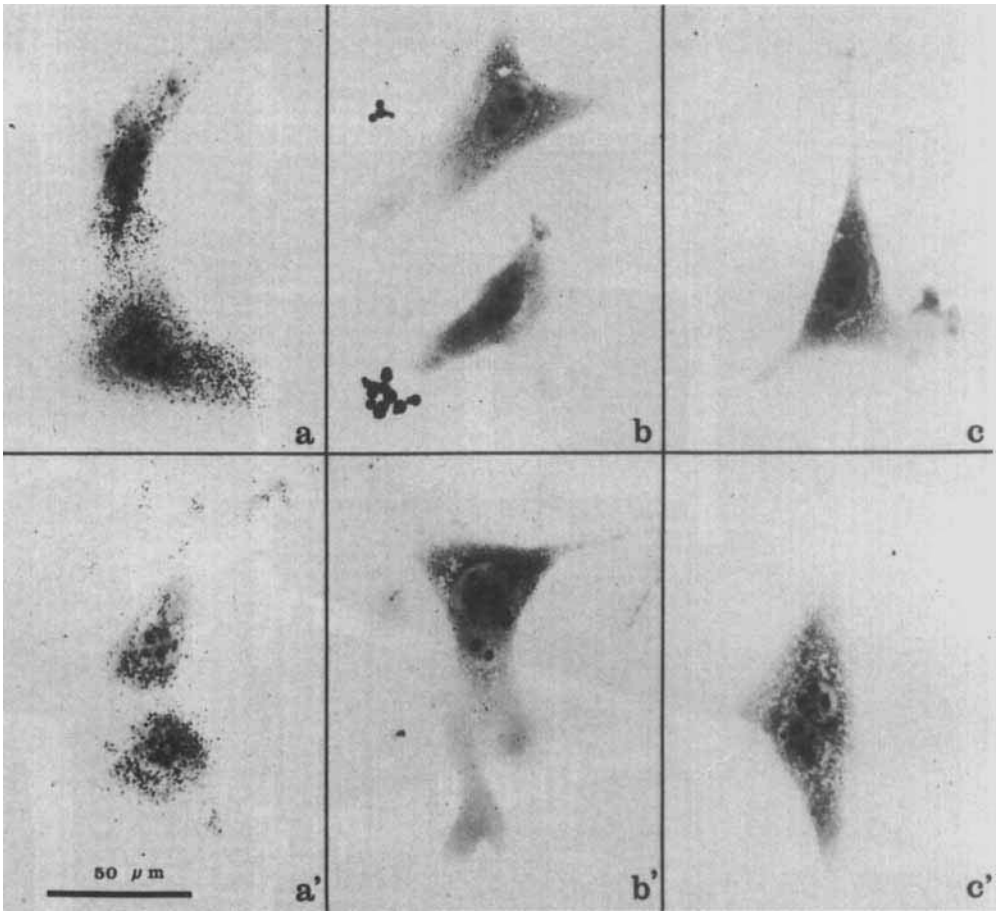

Fig. 3. Binding of ^{125}I-labeled plasma membranes to 3T3 cells. Plasma membranes were labeled with ^{125}I by the glucose oxidase lactoperoxidase method, and added to cells in Linbro wells at concentrations of 6 PDE units/dish (a, b, c) or 2 PDE units/dish (a′, b′, c′). After 48 h the medium was removed, the cells fixed with glutaraldehyde and dipped in emulsion. a,a′, 5% serum; b,b′, 25% serum; c,c′, 5% serum with heat-inactivated membranes. The levels of DNA synthesis as a percent of control were: a, 41%; a′, 51%; b, 77%; b′, 93%; c, 110%; c′, 124%. High serum reduces, or heat inactivation of membranes abolishes, both biological activity and binding of membranes to cells. Cells have been lightly stained with Giemsa. Note that panels b,b′ and c,c′ have very few grains compared to a and a′ and that an increase in membrane concentration (panel a compared to a′) increases the labeling of cells.

growth. By autoradiography it is possible to determine the amount of ^{125}I present on the cells as well as that present on plastic. The conclusions from a number of experiments, although still somewhat preliminary, suggest the following: 1) The plasma membranes from 3T3 cells will bind to the cells on the dish, but only a small fraction (less than 2% of added counts) is internalized. 2) Membranes that have been inactivated by heat treatment do not bind and do not block cell growth. 3) High concentrations of serum that compete with the biological activity of the membranes, in the sense that higher concentrations of membranes are needed to produce the same biological activity in the presence of high serum as in low serum, prevent the binding of membranes to the cells (Fig. 3), and 4) At the moment there appears to be a rough correlation between the amount of membrane bound and biological activity. Additional experiments, especially experiments with defined mitogens, will have to be carried out to determine whether, in fact, serum acts in part by preventing the interaction of membranes with cells and, by implication, the interaction of cells with each other. Should this possibility turn out to be correct, it would be a very exciting phenomenon and could be considered analogous to down regulation of hormonal receptors. In this case mitogens would decrease the affinity of receptors on the cell surface that can bind to other cells or membranes, thereby decreasing the effect of cell to cell contact.

It should be pointed out that experiments by Peterson et al [32] have shown that membranes will prevent the mitogenic stimulation of 3T3 cells by the defined mitogen epidermal growth factor using membranes prepared from a 3T3 mutant, which lacks epidermal growth factor receptors. Thus the blockage of the effect of epidermal growth factor cannot simply be due to trapping of this hormone by the membranes. Membranes appear to act in part by down regulating the receptors for epidermal growth factor at the cell surface, and it is attractive to speculate that the converse may also be true – that certain mitogens might decrease the concentration of the cell surface receptors that mediate the binding to other cells. This observation is not without precedence in that several growth factors, notably nerve growth factor and epidermal growth factor, have been shown to alter the adhesive properties of cells [33–35].

As summarized in Table I, all the data obtained to date suggest that the interaction of isolated plasma membranes with cells parallels the biological effects observed at high cell density. It therefore seemed reasonable to proceed to attempt to solubilize the membrane component or components responsible for this action, and this has been accomplished by the use of the detergent octylglucoside [36]. As shown in Figure 4 and Table II [37], the extract basically appears to act in the same way as the membranes, and efforts to purify the components present in this extract are in progress. We conclude from these data that contact inhibition of growth mediated by molecules on the cell surface is one of the important signals that regulates cell growth under culture conditions. Only if the appropriate mole-

TABLE I. Characteristics of Growth Inhibition of 3T3 Cells by Plasma Membranes

1) Concentration is time dependent and reversible.
2) Maximally 50% of cells are arrested in each cell cycle.
3) Cells are arrested in G_1 at a restriction point probably identical to that observed in low serum (G_0).
4) Membranes induce decrease in rate of uptake of α-aminoisobutyric acid and uridine but not of Pi and glucose.
5) The membranes compete with mitogenic factors and serum.
6) Membranes bind to cells and are not extensively internalized.
7) Membrane activity and membrane binding to cells is prevented by mild heat treatment.

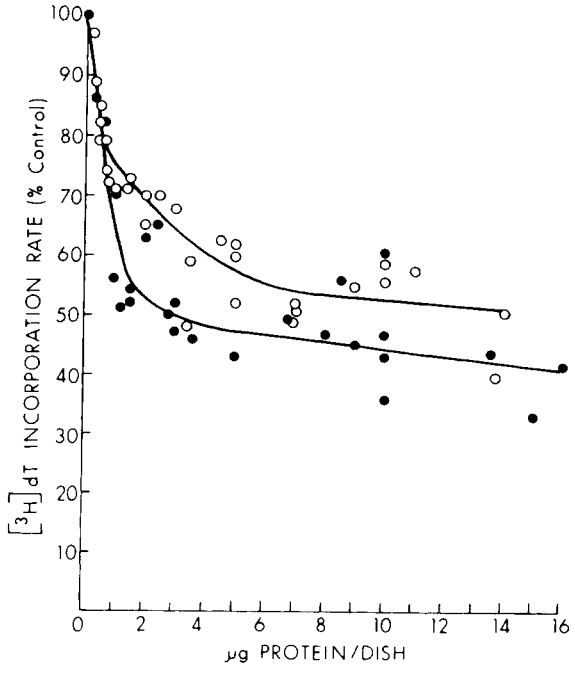

Fig. 4. Effect of an octylglucoside extract of membranes on DNA synthesis. Sparse 3T3 cells were incubated with the indicated concentrations of membranes (•) or an octylglucoside extract of membranes (○) for 24 hours, after which the rate of thymidine incorporation into DNA was measured during a 2-hour pulse with [^{3}H]-dThd as described. Eight separate experiments are included in this figure [37].

TABLE II. Biological Activity of Octylglucoside Extract of 3T3 Plasma Membranes

1) Inhibits DNA synthesis in 3T3 cells in a concentration-dependent manner to a maximum of 50%.
2) Inhibition is reversible.
3) Inhibition of 50% is due to a steady state of cells becoming inhibited and escaping from inhibition.
4) Inhibition can be blocked by high concentrations of serum and other mitogens.
5) Inhibitory activity is heat labile.

cules are purified and their interaction with the cell surface is defined will we be able to get past the current descriptive phase of these investigations. It is important to point out again that the inhibition of growth by contact is *only one* of the signals that control cell growth and that under different conditions cell growth can be controlled by the availability of nutrients or mitogens, as has been discussed in detail by Thrash and Cunningham [17]. This distinction is important to keep in mind when assessing a number of observations in the literature.

Recently a cell surface component [38, 39] has been described which is obtained by urea extraction of cells and which also blocks growth of 3T3 cells. The reversibility of this effect is not known, and the precise portion of the cell cycle in which these cells are arrested has not yet been determined. This surface component is inactivated by incubating cells with UDP-GlcNAc. When we incubate 3T3 cells with UDP-GlcNAc and then isolate from these cells a plasma membrane enriched fraction or an octylglucoside extract of these

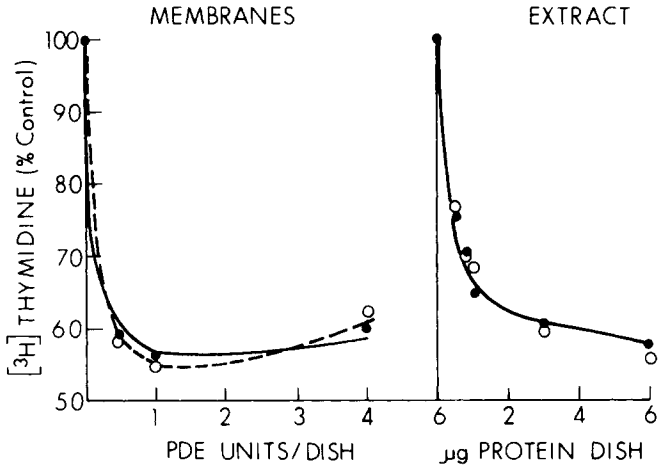

Fig. 5. Effect of UDP-GlcNAc on inhibitory components present in 3T3 membranes. Swiss 3T3 cells were grown to confluence in 1,585 cm^2 roller bottles. To 2 roller bottles at confluence was added enough sterile UDP-GlcNAc in culture medium to bring the final concentration to 0.5 mM. Controls received an equivalent quantity of medium with no nucleotide addition. After 5 hours a plasma membrane fraction was prepared from these cells and an aliquot of the membrane fraction was extracted with octylglucoside. The enrichment of these membrane fractions for phosphodiesterase was identical in control membranes and those prepared from UDP-GlcNAc-treated cells. Panel A shows the ability of these membranes to inhibit DNA synthesis in 3T3 cells after 24 hours of incubation with cells, and panel B shows similar data for the octylglucoside extract. •, Control membranes or extract; ○, membranes or extract from cells incubated with UDP-GlcNAc. Note that the membranes and extracts prepared from cells incubated with UDP-GlcNAc give results identical to those observed with control samples.

membranes, their activity is identical to that of control membranes (Fig. 5). Thus the molecules present in the plasma membrane fraction that reversibly inhibit cell growth appear to be different from those obtained by urea extraction.

GROWTH CONTROL OF SCHWANN CELLS

In 1976 Wood and Bunge [9, 10] developed methods for the preparation of dorsal root ganglia from 16-day-old rat embryos free of fibroblasts and containing only neurons and Schwann cells. When these ganglia were maintained in culture these investigators could show that the neurons extended neurites and that Schwann cells would proliferate to ensheath these neurites. When the ganglion somata were removed and the neurites were allowed to degenerate the Schwann cells remained quiescent. Addition of a ganglion (which itself was devoid of any Schwann cells because of a prior treatment with antimitotic agents) to such a culture of quiescent Schwann cells restored their ability to grow and, after a period of several weeks, myelin was formed in relation to the axons growing from the ganglia. It was suggested that this growth control of Schwann cells might be related to contact with the neurites.

We have, therefore, carried out experiments whose design and principle is similar to that used for 3T3 cells [11]. Quiescent Schwann cells, which on 24-hour labeling with [^{3}H]-thymidine show less than one nucleus in 200 labeled with thymidine, were incubated for two days with a membrane fraction prepared from dorsal root ganglia neurites (Fig. 6).

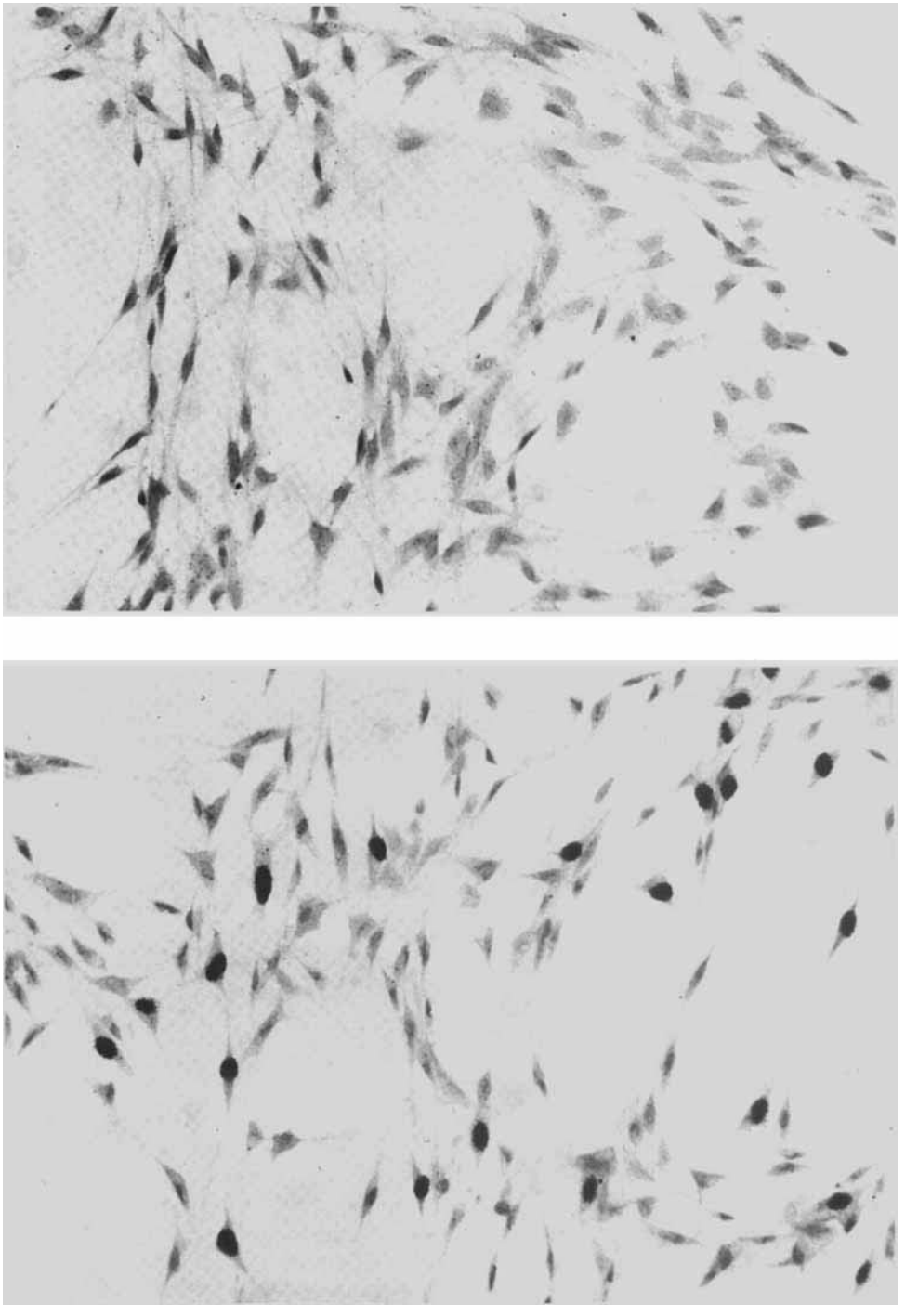

Fig. 6. Mitogenic effect of neurite membranes on Schwann cells. Quiescent Schwann cells [10, 11] were incubated for two successive 24-hour periods with neurite membranes and labeled with [^{3}H]-thymidine for the second 24-hour period followed by autoradiography. Top panel: control cells not incubated with neurites. Bottom panel: cells incubated with neurites. Note presence of labeled nuclei in bottom panel and their absence in top panel. (Labeled nuclei appear black due to high grain density toluidine blue stain; magnification, × 300.)

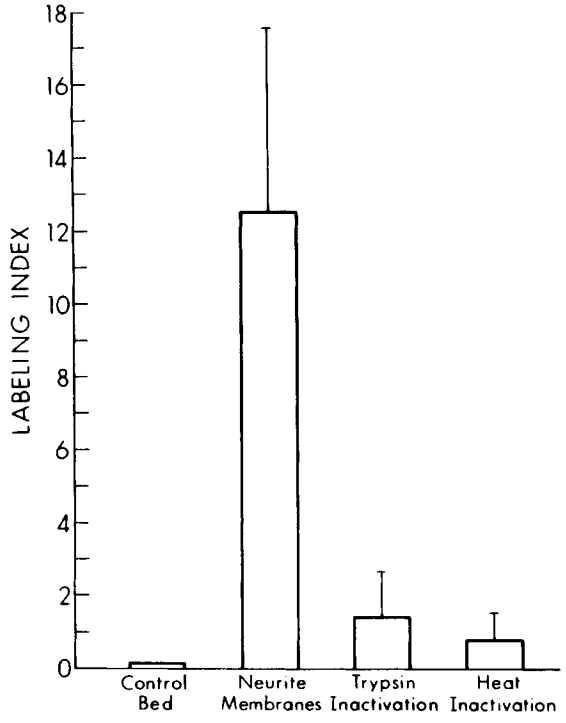

Fig. 7. Effect of various treatments on mitogenic activity of neurite membranes. The bar graph summarizes a series of experiments in which quiescent Schwann cells were incubated either with neurite membranes or with neurite membranes prepared from dorsal root ganglia pretreated with trypsin (0.05% at 34° for 30 or 60 min) before removal of the ganglia and preparation of the neurite membranes. Trypsin was inhibited with trypsin inhibitor at the end of the incubation. Heat-inactivated membranes were prepared by heating neurite membranes at 60° for 10 min. Note that pretreatment of intact ganglion before isolation of neurite membranes abolishes biological activity. Incubation with membranes was for 48 h – in the last 24 hours, in the presence of 1 μC/ml of [^{3}H]-thymidine. Labeling index is the percent of labeled nuclei. Note the very large stimulation of [^{3}H]-thymidine incorporation by Schwann cells incubated with neurite membranes.

Under these conditions, 20% and sometimes up to 30% of the cells can be labeled with thymidine during a 24-hour incubation. Thus the membrane fraction appears to be mitogenic for Schwann cells. A series of control experiments has shown that the relevant ligand appears to be on the surface of the neurite since it is inactivated by a short treatment with external trypsin (Fig. 7) and appears to be absent from the cytoplasmic fraction of the neurites.

This system is one that lacks some of the drawbacks of the 3T3 system discussed previously – namely, the effect of membranes on cells is a positive one; that is, heterologous cell to cell contact stimulates the Schwann cells to enter into S phase. The detection of this positive signal by itself directly excludes any toxic effect that the membranes might have on cells. The system is one that is of considerable biological interest and is highly specific. The membranes prepared from neurites cannot be replaced by a number of mitogenic compounds or with plasma membranes fractions obtained from a variety of other neuronal and non-neuronal cells in tissue culture. The isolation of the neurite membrane components responsible for this effect will require the development of a source of this mitogenic material other than the primary cultures now in use, since they yield insufficient material for successful biochemical characterization of the components.

Schwann cells have also been prepared from sciatic nerve by Raff and coworkers [40–42] and a Schwann cell-containing fraction has been prepared from chick sympathetic ganglia [43, 44]. These Schwann cell preparations show several different properties from the ones that have been used in the experiments summarized above; most notably, they grow, although slowly, in the absence of any neurites or neurite membranes. Their relation to the cells that have been prepared by the method of Wood is not entirely clear. Both cell types will respond to cholera toxin as a mitogen, although cells prepared by the method of Wood do so only weakly. The Schwann cell-enriched fraction of Hanson and Partlow [44] is stimulated to grow by a neuronal homogenate, and the Schwann cells prepared by Raff et al respond to pituitary extracts [40]. It remains for the future to ascertain the relation between these various types of Schwann cells. It should be clear, however, that the response to a surface mitogenic signal derived from neurites and that to an apparently soluble component present in pituitary extracts are not necessarily mutually exclusive phenomena.

Schwann cells can be defined by morphology, antigenicity, presence of S-100, and, most stringently, the ability to myelinate appropriate axons. Not all the available preparations of Schwann cells in culture have been subjected to all the above tests, and cells prepared by different procedures may differ, either because they are derived from different cell populations or because their properties have altered as a result of culture conditions.

GENERAL CONCLUSION

At this time the principal conclusion from this work is that cell to cell contact can have physiological consequences for cells and these can be mimicked by suitably prepared surface membrane fractions. This is clearly only the first step in the elucidation of the chemical components present in membranes responsible for these effects. Systems that respond in this way have clear advantages over systems in which cell to cell adhesion is simply examined as an adhesive phenomenon, not the least of which is that the biological effect represents an amplification of the binding signal that facilitates the assay for specific binding. There are a number of systems more or less well defined in the literature in which it is possible that cell to cell contact also results in biological response such as induction of specific proteins. Two specific examples that come to mind are the induction of S-100 protein at high cell densities, as first described by Pfeiffer et al [45, 46], and another, more recent description of induction of enzymes and transmitter synthesis in pheochromocytoma PC12 [47]. Both of these systems should be amenable to the types of investigation that have been described for 3T3 cells and for Schwann cells. Different systems that appear less amenable to chemical investigation are the formation of junctions between cells, observed in a number of systems, where the formation of the junction clearly must follow cell contact, but where the formation of the junction requires the presence of two living cells [for some recent examples, see references 48–51].

We have not attempted to present in this brief review a comprehensive list of references. A more detailed set of references to work in this field can be found in published papers as well as in two recent comprehensive reviews [41, 52].

ACKNOWLEDGMENTS

This work was supported by grants from NIH, GM 18405, EY 01255 and NSF PCM 77-15972. M. L. is supported by a fellowship from the Damon Runyon-Walter Winchell Fund (DR6-168-F); D. R. by grant GM 07067; J. S. and B. W. by grant GM 02016; M. L. by Damon Runyon Fellowship DRG-168-F. Authors have been listed in alphabetical order.

REFERENCES

1. Shur BD, Roth S: Biochim Biophys Acta 415:473–512, 1975.
2. Marchase RB, Vosbeck K, Roth S: Biochim Biophys Acta 457:385–416, 1976.
3. Pardee AB, Dubrow R, Hamlin J, Kletzien R: Ann Rev Biochem 47:715–750, 1970.
4. Frazier WA, Glaser L: Ann Rev Biochem 48:491–523, 1979.
5. Todaro GJ, Green H, Goldberg BD: Proc Natl Acad Sci USA 51:66–73, 1963.
6. Stoker MGP, Rubin H: Nature 215:171–172, 1967.
7. Dulbecco R: Nature 227:802–806, 1970.
8. Westermark B: Proc Natl Acad Sci USA 74:1619–1621, 1977.
9. Wood PM, Bunge RP: Nature 254:662–664, 1975.
10. Wood PM: Brain Res 115:361–375, 1976.
11. Salzer J, Glaser L, Bunge RM: (in preparation).
12. Holley R, Kiernan J: Proc Natl Acad Sci USA 60:300–304, 1968.
13. Stoker MGP: Nature 246:200–203, 1973.
14. Stoker M, Piggott D: Cell 3:207–215, 1974.
15. Holley RW, Kiernan JA: In Wolstenholme GEW, Knight J (eds): Ciba Found Symp: "Growth Control in Cell Cultures." London: Churchill-Livingstone, 1971, pp 3–15.
16. Whittenberger B, Glaser L: Nature 272:821–823, 1978.
17. Thrash CR, Cunningham DD: J Cell Physiol 86:301–310, 1975.
18. Thomopoulus P, Roth J, Lovelace E, Pastan I: Cell 8:417–423, 1976.
19. Holley RW, Armour R, Baldwin JH, Brown KD, Yeh YC: Proc Natl Acad Sci USA 74:5046–5050, 1977.
20. Santala R, Gottlieb DI, Littman D, Glaser L: J Biol Chem 252:7625–7634, 1977.
21. Obrink B, Kuhlenschmidt MS, Roseman S: Proc Natl Acad Sci USA 74:1077–1081, 1977.
22. McMahon D, Hoffman S, Fry W, West C: In McMahon D, et al (eds): "Developmental Biology Pattern Formation, Gene Regulation." Palo Alto, California: W. H. Benjamin, 1975, pp 60–75.
23. Tuchman J, Smart JE, Lodisch HF: Dev Biol 51:77–85, 1976.
24. Whittenberger B, Glaser L: Proc Natl Acad Sci USA 74:2251–2255, 1977.
25. Whittenberger B, Raben D, Glaser L: J Supramol Struct 10:307–327, 1979.
26. Martz E, Steinberg MS: J Cell Physiol 79:189–210, 1972.
27. Riddle VGH, Rossow PW, Pardee DB: J Cell Biol 79:11a, 1978.
28. Lieberman MA, Raben D, Whittenberger B, Glaser L: J Biol Chem 254:6357–6361, 1979.
29. Barsh GS, Cunningham DD: J Supramol Struct 7:61–77, 1977.
30. Parnes JR, Isselbacher KJ: Prog Exp Tumor Res 22:79–122, 1978.
31. Lever JE: J Cell Physiol 89:779–787, 1976.
32. Peterson SW, Vale R, Das M, Fox CF: J Supramol Struct Suppl 2:126, 1978.
33. Merrell R, Pulliam MW, Randono L, Boyd LF, Bradshaw RA, Glaser L: Proc Natl Acad Sci USA 72:4270–4274, 1975.
34. Schubert D, Whitlock C: Proc Natl Acad Sci USA 74:4055–4058, 1977.
35. Aharonov A, Vladovsky I, Preiss RM, Fox CF, Herschman HR: J Cell Physiol 95:195–202, 1978.
36. Baron C, Thompson TE: Biochim Biophys Acta 382:276–285, 1975.
37. Whittenberger B, Raben D, Lieberman MA, Glaser L: Proc Natl Acad Sci USA 75:5457–5461, 1978.
38. Natraj CV, Datta P: Proc Natl Acad Sci USA 75:3859–3862, 1978.
39. Natraj CV, Datta P: Proc Natl Acad Sci USA 75:6115–6119, 1978.
40. Brockes JP, Fields KF, Raff MC: Nature 266:364–366, 1977.
41. Raff MC, Smith AH, Brockes JF: Nature 273:672–673, 1978.
42. Raff MC, Abney E, Brockes JP, Smith AH: Cell 15:813–822, 1978.
43. McCarthy KD, Partlow LM: Brain Res 114:415–426, 1976.
44. Hanson GR, Partlow LM: Brain Res 159:195–210, 1978.
45. Pfeiffer GE, Herschman HR, Lightbody J, Sato G: J Cell Physiol 75:329–340, 1970.
46. Labourdette S, Mahony JB, Brown IR, Marks A: Eur J Biochem 81:591–597, 1977.
47. Lucas CA, Edgar D, Thoenen H: Exp Cell Res (in press).
48. Pitts JA, Burk RR: Nature 269:762–764, 1977.
49. Griepp EB, Peacock JH, Bernfield MR, Revel JP: Exp Cell Res 113:273–282, 1978.
50. Lawrence TS, Beers WH, Gilula NB: Nature 272:501–506, 1978.
51. Lowenstein WR, Kanno Y, Socolar SJ: Nature 279:133–136, 1978.
52. Glaser L: Rev Physiol Biochem Pharmacol 83:89–122, 1978.