Biochemical Pharmacology, Vol. 25, pp. 1333–1337. Pergamon Press, 1976. Printed in Great Britain.

COMMENTARY

FACTORS AFFECTING GROWTH OF NORMAL AND MALIGNANT CELLS *IN VITRO**

GEORGE LIPKIN, MARTIN ROSENBERG and MARGARETE E. KNECHT

Department of Dermatology (G. L. and M. E. K.) and Department of Pathology (M. R.), New York University School of Medicine, New York, N.Y. 10016, U.S.A.

Models *in vitro*, despite their artifactual nature, have long proven useful in the investigation of growth control mechanisms, permitting the systematic dissection and elucidation of a rapidly growing list of factors which participate in growth regulation. Recent experimental observations in this laboratory, demonstrating that it is possible to restore certain aspects of normal *in vitro* growth control to malignant cells by replacing a single cell surface-associated protein lacking in such neoplastic cells, have prompted us to review some of these known factors and to speculate upon the implications of new findings.

One of the most useful models for studying growth regulation is based upon the observation that normal cells in culture limit their growth by cellular interactions which depend upon population density. When such cells grow together to form confluent (and in some cases well-oriented) monolayers, they stop proliferating even though the culture medium is still capable of supporting growth at lower cell densities. Such arrest of growth occurs in the G_1 (Go) phase of the cell cycle, and is associated with a marked decrease in the rate of synthesis of DNA, RNA, and protein, increased turnover of certain cell surface proteins, and early diminution of transport of hexose and phosphate.

By contrast, malignant cell types, upon reaching confluence, fail to stop proliferating, but proceed to form multilayered (disoriented) heaps of cells, growing usually to much higher saturation densities than their normal counter-parts. Even in such crowded cultures considerable DNA synthesis may continue, and there is little decline in transport of either hexose or phosphate.

This limitation of growth in normal cells has been termed contact inhibition of growth [1], density-dependent inhibition of growth [2], or topoinhibition [3].

The relevance of contact inhibition of growth as a valid experimental model is based upon the observation that it is the property *in vitro* whose loss is most closely correlated with malignancy *in vivo* [4]. For example, among different strains of a malignant cell line, there is a direct correlation between the degree of loss of contact inhibition of growth (measured as increasing saturation densities of cultures) and ease of transplantability of tumors *in vivo* [5].

There is good evidence that cell contact plays a role in growth limitation *in vitro* [5–10]. Such a role is also favored by the cell contact-dependent regulation in normal, but not in transformed cells, of the transport of hexose [11] and phosphate [12], increase in membrane D-galactopyranosyl-like residues [13], redistribution of intrinsic membrane proteins [14], and synthesis of glycolipid [15].

Factors causing overgrowth of cells

At the same time it is essential to recognize that this density-dependent growth limitation can be removed by a variety of exogenous factors including increased serum concentration [16, 17], increased pH of the medium [18], alterations in the microenvironment [19], and even changes in metal ion concentrations [20]. Other factors which can increase the density at which cells cease proliferating include the presence of "co-promotors" or "conditioning factors" like insulin [21], heparin, histamine and serotonin [22] and corticosteroids [23]. Furthermore, a variety of "overgrowth" factors elaborated by transformed or embryonic cells can overcome the density dependence of non-transformed cells [24–30].

Transformed cells also have been shown to release into the culture medium increased amounts of proteases, particularly serine proteases capable of activating plasminogen to plasmin [31], and such proteolytic activities have previously been shown capable of initiating cell proliferation and release from density-dependent inhibition of growth [32, 33].

In addition, a number of "growth factors" presumably of normal origin have been reported to be able to initiate growth in quiescent cultures of their respective target cells. Fibroblast growth factor (FGF), a polypeptide purified from bovine brain and pituitary, stimulates division both of human diploid and murine heteroploid fibroblasts, overcoming the contact-inhibited state [34]. Also reported are an ovarian cell growth factor (OGF) [35], a fibroblast "multiplication-stimulating" factor [36], growth-stimulating factors in the sera of normal [37] or hepatectomized [38] rats, and a fraction from bovine brain which is mitogenic for myoblasts and amniotic cells [39]. Finally, a group of esteroproteolytic enzymes has been isolated from mouse submaxillary glands which includes a nerve growth-promoting factor (NGF) [40], an epidermal growth factor [41], an epithelial growth fac-

* Supported by grants from the USPHS (CA 16013 and CA 18034, NCI).

B.P. 25/12—A

tor [42], a fraction which stimulates growth of mesenchymal cells [43], and one promoting overgrowth of rat hepatoma cells [44].

Factors inhibiting growth of cells

In juxtaposition to the many factors which act to promote cell growth and the loss of density-dependent restrictions are a variety of factors which inhibit growth *in vitro* and/or restore one or more morphologic features of the contact-inhibited state. These include: cAMP, phosphodiesterase inhibitors, PGE_1 and PGE_2, inhibitors of proteases and of hyaluronidase, "monovalent" plant lectin, dimethylsulfoxide, halogenated pyrimidine analogues, a factor found in cultures of chick fibroblasts, and a protein isolated from cultures of a contact-inhibited line of hamster melanocytes.

Thus, cyclic AMP and/or its dibutyryl analogue produce distinct morphological changes in cultured neoplastic cells of fibroblastic [45, 46], ovarian [47], hepatic [48], glial [49], neuroectodermal [50 51], and mast cell [52] origins. Such neoplastic cells are induced to express varying degrees of structural and functional differentiation or maturation, thus exhibiting a partial phenotypic reversion. These changes are accompanied by a decrease in DNA synthesis, slowing or cessation of growth, increase in size of soma and nucleus associated with increase of total protein content, and decrease in agglutinability by lectins. Such differentiated cells have a higher proportion of poly-A-rich cytoplasmic RNA [53].

There is evidence that the morphologic changes induced by cAMP are mediated by effects upon the microtubular-microfibrillar system. Intact microtubules and microfilaments are required for the morphologic expression of the differentiated state in cAMP-treated fibroblasts [45], Chinese hamster ovary cells [47], Schwannoma cells [54], and melanoma cells (unpublished data). It has been suggested that cAMP causes assembly, stabilization and orderly alignment of the microtubular system [55], resulting in morphological alteration of the cell surface and of cell shape. The latter may be mediated via actin-containing microfilaments known to be closely associated with the plasma membrane in several cell types. cAMP exerts a direct effect upon the activity of glycosyl transferases in plasma membranes of transformed, but not normal, mouse kidney cells, and therefore it has been suggested that such an effect of cAMP might be transmitted by the microfilaments [56]. It should be noted that the growth inhibition and morphologic changes induced by cAMP are not cell density dependent, occurring in subconfluent log phase cultures as well as at confluence [48, 55]. Not all neoplastic clones respond to cAMP (nor to PGE's or phosphodiesterase inhibitors), indicating that other factors, e.g. binding of cAMP to receptors, are important [57]. cAMP also increases the sensitivity of adenylate cyclase to various neurotransmitters [58], that of cAMP phosphodiesterase activity to divalent ions [59], and that of PGE_1-stimulated adenylate cyclase activity to guanosine triphosphate potentiation [58]. cAMP-mediated growth arrest involves the rapid, reversible inhibition of incorporation of precursors into DNA, with prolongation of the S phase of the cell cycle [60].

In view of the growth-suppressive effects of cAMP *in vitro*, it is of interest that a variety of tumors (but not all tumors) are growth-inhibited by cAMP *in vivo*, in a dose-dependent, reversible fashion [61, 62], and tumor lines which are induced to differentiate *in vitro* by cAMP may be less tumorigenic *in vivo* [63, 64]. Nevertheless, it should be emphasized that both basal and induced cAMP levels are variable in different systems [61] and an increase of intracellular cAMP is not the sole determinant of cAMP-induced tumor regression. Other cyclic nucleotides, in particular cyclic GMP, may play important roles as regulators of cell proliferation [65].

Phosphodiesterase inhibitors, e.g. theophylline or R020-1724, also may induce morphologic changes of differentiation, by facilitating the accumulation of cAMP [53]. PGE's have also been shown to decrease growth rate and lectin agglutinability of transformed cells while inducing differentiative changes, presumably by increasing adenylate cyclase activity [66, 67]. PGE_1 also increased survival of B16 melanoma-bearing mice [68].

The ability of "monovalent" Concanavalin A to restore to normal levels the density of transformed 3T3 cultures [8] may reflect experimental stabilization of membrane protein components, restoring to transformed cells those normal restrictions upon lateral translation of lectin-binding sites found in non-transformed cells.

The inhibition of cell growth *in vitro*, as well as of cutaneous carcinogenesis *in vivo* [69], by protease inhibitors is consistent with the known ability of very low concentrations of proteases to stimulate growth of normal cells [32], and with the demonstration that growth characteristics of transformed cells appear to require proteolytic, particularly plasmin activity [31].

In view of the apparent importance of the normal cell surface as a locus for mitotic regulatory control, as well as the obvious fact that differences in social behavior between benign and malignant cells *in vitro* must ultimately reflect differences in their surfaces and surface interactions, it is of interest that a number of workers have now described the selective deletion, following malignant transformation of chick embryo, mouse and hamster fibroblasts, of a high molecular weight (250 K) external glycoprotein [70 76]. The functional correlate(s) of these large external proteins is (are) not known, nor whether the latter are indeed the same or closely related species. However, we recently demonstrated that a line of contact-inhibited hamster melanocytes produces a high molecular weight (*ca.* 160,000) external protein (melanocyte contact inhibitory factor, MCIF) which is capable of restoring to malignant melanocytes the capacity for contact inhibition of growth [77]. It is significant that MCIF-induced contact inhibition of growth involves a G_1 arrest (unpublished data), as in the classical case of diploid fibroblasts. The effects of the isolated protein transcend both species and tissue barriers[78, 79], and it appears to be a potent reversible growth inhibitor for a very broad spectrum of cell types, both malignant and benign. A protein having identical electrophoretic mobility on polyacrylamide gels has been detected in cultures of other contact-inhibited fibroblastic cell lines of murine and human origin [80]. It will be of interest to determine whether,

in fact, MCIF is functionally identical with the above-mentioned external proteins which become deleted during viral transformation. Such identity would indicate the existence of a closely related class of surface-associated glycoproteins concerned with the regulation of normal cell–cell interaction permitting the generation and/or reception of signals leading to feedback inhibition of growth. The recently isolated major cell surface glycoprotein of chick embryo fibroblasts [81], obtained by a urea-extraction procedure, would also appear to function in a similar manner, since a factor released into culture medium from the chick cells by urea restores contact inhibition of growth to urea-"transformed" cells.

Possible interactions among proteases, protease inhibitors, and external glycoproteins

Since the regulation of cell division appears to depend upon cellular responses to environmental stimuli, mediated through the plasma membrane, it is natural to consider this organelle as the locus of receptor(s) for the "divide" signal; this does not exclude the possibility that alternate internal receptors may co-exist and respond to internally generated signals. Of various factors exerting effects on the cell surface, proteolytic enzymes have been the subject of much experimental effort [32, 33, 71, 82]. These enzymes are able to trigger cell division in resting cells, and also are present at elevated levels in transformed cells [83]. Non-dividing cells treated with protease acquire altered surface properties and cytoplasmic states similar to those observed in dividing cells [84]. Thus, it appears that proteolytic activation of cell division is a convenient model to study the environmental "divide" signal. The suitability of this model is strengthened by observations that certain tissue specific growth factors possess proteolytic (or esterolytic) functions [42, 44, 85, 86], and that a variety of protease inhibitors exhibit growth regulatory properties [69, 87–89]. A number of critical cellular events follow protease treatment, although not necessarily in this order: (1) loss of 250 K glycoprotein [82], (2) altered levels of cyclic nucleotides [90], (3) increased lectin agglutinability [91–93], (4) increased nutrient transport [94], (5) phosphorylation of histone and non-histone proteins [95], and (6) cell division [32, 33, 71, 82].

The removal of the 250 K glycoprotein may be an important event leading to cell proliferation. The absence of this protein from the surface of transformed cells suggests that either it is not made by the cells, or that it is continuously removed—perhaps by the cells' own proteases [96]. Similarly, the melanocyte contact inhibitory factor is a high molecular weight membrane glycoprotein found in contact-inhibited, but not in non-contact-inhibited cultures, which restores growth regulation to the latter [77, 78]. Such surface glycoproteins may themselves be regulatory molecules, and may act by combining with enzymes (proteases?) involved in initiating growth or transformation of cells. It is significant that tumor promotors increase proteases by combining with specific receptors on the cell surface [97]. In this connection, the surface glycoproteins may instead block such specific receptors and prevent appearance of the proteases.

In such a scheme, changes in cyclic nucleotide levels might be secondary to changes in levels of surface regulatory macromolecules. In the above formulations, the presence (or absence) of the latter macromolecules at the cell periphery would directly influence subsequent events. In fact, when trypsin is added to cells in culture, it triggers cell division and removes the 250 K glycoprotein. However, thrombin can stimulate cells to divide (more effectively than trypsin) without removing the 250 K glycoprotein [82]. Moreover, chymotrypsin can remove this protein without initiating cell division. On the basis that trypsin and thrombin preferentially attack at lysine or arginine sites in the peptide chain, one might assume that the triggering region has an exposed lysine or arginine residue, i.e. trypsin and thrombin trigger cell division, while chymotrypsin does not, despite its effect on the 250 K protein. Thus, there are apparently at least two sites of tryptic attack on the cell surface: (1) the 250 K glycoprotein, and (2) a "thrombin" site, seemingly more proximal to direct triggering of cell division; removal of the 250 K glycoprotein may not be an absolute requirement for cell division.

An alternative possibility is that these membrane glycoproteins may act as receptors for external regulatory molecules. There is some evidence for this; partially purified MCIF appears to be associated with one or more protease inhibitory species which, in turn, may derive from serum.* As pointed out previously, protease inhibitors (including ovomucoid) [87] can indeed inhibit the growth of transformed cells. Serum protease inhibitors can also inhibit the neutral protease produced by transformed cells [98, 99]. In fact, the relation between serum factors and cell growth needs to be more clearly defined. It is possible that the mitogenic impulse may be determined by an equilibrium between protease and anti-protease at the cell surface. Excess protease (whether from exogenous or endogenous sources) would cause a shift toward mitosis; conversely, excess inhibitor would favor arrest of growth. The latter effect would be considerably enhanced by a surface glycoprotein capable of binding protease inhibitors. These surface glycoproteins which are deleted from transformed cells could play a regulatory role in mitogenesis by serving in such a binding role. Absence of these glycoproteins in tumour cells [71] might permit continuous triggering of cell division by endogenous protease. The neutral protease reported in tumor cells, does in fact, attack at an arginine site [100]. In untransformed or non-dividing cells, the presence of MCIF or 250 K may bind sufficient inhibitor to prevent proteolytic activation of mitosis by ambient levels of protease.

It is thus apparent that patterns of cell growth *in vitro* reflect the complex interplay of numerous factors modulating the expression of cell surface characteristics. A more complete knowledge of the interactions among these factors is certain to suggest new approaches to therapeutic intervention and control of neoplastic growth processes. Such strategies may well be based upon the premise that it is not necess-

* M. Rosenberg, M. E. Knecht and G. Lipkin, unpublished data.

ary to eradicate cells to control neoplastic disease; functional "repair" may be an effective alternative.

Acknowledgement- We thank Dr. Walter Troll for thoughtful discussion and suggestions.

REFERENCES

1. G. J. Todaro, G. K. Lazar and H. Green, *J. cell. comp. Physiol.* **66,** 325 (1965).
2. M. G. P. Stocker and H. Rubin, *Nature, Lond.* **215,** 171 (1967).
3. R. Dulbecco, *Nature, Lond.* **227,** 802 (1970).
4. S. A. Aaronson and G. J. Todaro, *Science, N.Y.* **162,** 1024 (1968).
5. R. E. Pollack, H. Green and G. J. Todaro, *Proc. natn. Acad. Sci. U.S.A.* **60,** 126 (1968).
6. T. Gurney, *Proc. natn. Acad. Sci. U.S.A.* **62,** 906 (1969).
7. R. Dulbecco and M. G. P. Stoker, *Proc. natn. Acad. Sci. U.S.A.* **66,** 204 (1970).
8. M. M. Burger and K. D. Noonan, *Nature, Lond.* **228,** 512 (1970).
9. J. B. Baker and T. Humphreys, *Proc. natn. Acad. Sci. U.S.A.* **68,** 2161 (1971).
10. T. Humphreys, in *Cell Interactions* (Ed. L. G. Silvestri), p. 264. North Holland Publishing, Amsterdam (1972).
11. S. K. Bose and B. J. Zlotnick, *Proc. natn. Acad. Sci. U.S.A.* **70,** 2374 (1973).
12. L. Harel, M. Jullien and C. Blat, *Expl Cell Res.* **90,** 201 (1975).
13. G. L. Nicolson and M. Lacorbriere, *Proc. natn. Acad. Sci. U.S.A.* **70,** 1672 (1973).
14. R. E. Scott and L. T. Furcht, *Proc. natn. Acad. Sci. U.S.A.* **70,** 3631 (1973).
15. S. Hakomori, *Proc. natn. Acad. Sci. U.S.A.* **67,** 1741 (1970).
16. R. W. Holley and J. A. Kiernan, *Proc. natn. Acad. Sci. U.S.A.* **60,** 300 (1968).
17. G. D. Clarke, M. G. P. Stoker, A. Ludlow and M. Thorton, *Nature, Lond.* **227,** 798 (1970).
18. C. Ceccarini and H. Fagle, *Proc. natn. Acad. Sci. U.S.A.* **68,** 229 (1971).
19. M. G. P. Stoker, *Nature, Lond.* **246,** 201 (1973).
20. H. Rubin, *Proc. natn. Acad. Sci. U.S.A.* **72,** 1676 (1975).
21. A. Vaheri, E. Ruoslahti, T. Hori and S. Nordling, *J. cell. Physiol.* **81,** 355 (1973).
22. K. Norrby, *Virchows Arch. Abt. B. Zellpath.* **15,** 75 (1973).
23. D. Gospodarowicz and J. S. Moran, *Proc. natn. Acad. Sci. U.S.A.* **71,** 4584 (1974).
24. H. Rubin, *Science, N.Y.* **167,** 1271 (1970).
25. B. K. Nair and K. B. De Ome, *Cancer Res.* **33,** 2754 (1973).
26. R. R. Burk, *Proc. natn. Acad. Sci. U.S.A.* **70,** 369 (1973).
27. H. Rubin, *Expl Cell Res.* **41,** 138 (1966).
28. P. E. Austin, E. A. McCulloch and J. E. Till, *J. cell. Physiol.* **77,** 121 (1971).
29. E. R. Stanley, T. R. Bradley and M. A. Sumner, *J. cell. Physiol.* **78,** 301 (1971).
30. M. G. P. Stoker, G. D. Clarke and M. Thorton, *J. cell. Physiol.* **78,** 345 (1971).
31. L. Ossowski, J. P. Quigley, G. M. Kellerman and E. Reich, *J. exp. Med* **138,** 1056 (1973).
32. M. M. Burger, *Nature, Lond.* **227,** 170 (1970).
33. B. M. Sefton and H. Rubin, *Nature, Lond.* **227,** 843 (1970).
34. D. Gospodarowicz, *Nature, Lond.* **249,** 123 (1974).
35. D. Gospodarowicz, K. L. Jones and G. Sato, *Proc. natn. Acad. Sci. U.S.A.* **71,** 2295 (1974).
36. N. C. Dulak and H. M. Temin, *J. cell. Physiol.* **81,** 153 (1973).
37. M. L. Dolan, M. L. Coetzee, M. Spangler and P. Ove, *J. natn. Cancer Inst.* **54,** 163 (1975).
38. C. G. Morley and H. S. Kingdon, *Biochim. biophys. Acta* **308,** 260 (1973).
39. D. Gospodarowicz, J. Weseman and J. Moran, *Nature, Lond.* **256,** 216 (1975).
40. R. Levi-Montalcini, *Science, N.Y.* **143,** 105 (1964).
41. S. Cohen, *J. biol. Chem.* **237,** 1555 (1962).
42. R. O. Jones and M. R. Ashwood-Smith, *Expl Cell Res.* **59,** 161 (1970).
43. D. G. Attardi, R. Levi-Montalcini, B. S. Wenger and P. U. Angeletti, *Science, N.Y.* **150,** 1307 (1965).
44. A. Grossman, K. P. Lele, J. Sheldon, I. Schenkein and M. Levy, *Expl Cell Res.* **54,** 260 (1969).
45. G. S. Johnson, R. M. Friedman and I. Pastan, *Proc. natn. Acad. Sci. U.S.A.* **68,** 425 (1971).
46. J. R. Sheppard, *Proc. natn. Acad. Sci. U.S.A.* **68,** 1316 (1971).
47. A. W. Hsie and T. T. Puck, *Proc. natn. Acad. Sci. U.S.A.* **68,** 358 (1971).
48. R. Van Wijk, W. D. Wicks and K. Clay, *Cancer Res.* **32,** 1905 (1972).
49. J. P. Perkins and E. H. Macintyre, *Fedn Proc.* **30,** 1097 (1971).
50. K. N. Prasad and A. W. Hsie, *Nature, Lond.* **233,** 141 (1971).
51. J. W. Krieder, M. Rosenthal and N. Lengle, *J. natn. Cancer Inst.* **50,** 555 (1973).
52. J. Davis and R. K. Ralph, *Cancer Res.* **35,** 1495 (1975).
53. S. C. Bondy, K. N. Prasad and J. L. Purdy, *Science, N.Y.* **186,** 359 (1974).
54. J. R. Sheppard, T. H. Hudson and J. R. Larson, *Science, N.Y.* **187,** 179 (1975).
55. K. R. Porter, T. T. Puck, A. W. Hsie and D. Kelley, *Cell* **2,** 145 (1974).
56. T. Sudo and K. Onodera, *Expl Cell Res.* **91,** 191 (1975).
57. K. N. Prasad, B. Mandal, J. C. Waymire, G. J. Lees, A. Vernadakis and N. Weiner, *Nature, New Biol.* **241,** 117 (1973).
58. K. N. Prasad, K. N. Gilmer, S. K. Sahu and G. Becker, *Cancer Res.* **35,** 77 (1975).
59. S. Kumar, G. Becker and K. N. Prasad, *Cancer Res.* **35,** 82 (1975).
60. R. Van Wijk, W. D. Wicks, M. M. Bevers and J. van Rijn, *Cancer Res.* **33,** 1331 (1973).
61. Y. S. Cho-Chung, *Cancer Res.* **34,** 3492 (1974).
62. M. J. Seller and P. F. Benson, *Eur. J. Cancer* **9,** 525 (1973).
63. K. N. Prasad, *Cytobios* **6,** 163 (1972).
64. E. E. Smith and H. H. Handler, *Res. Commun. chem. Path. Pharmac.* **5,** 863 (1973).
65. N. D. Goldberg, M. K. Haddox, R. Estensen, J. G. White, C. Lopez and J. W. Hadden, *Cyclic AMP, Cell Growth, and the Immune Response* (Eds. W. Braun, L. M. Lichtenstein and C. W. Parker), p. 247. Springer, New York (1974).
66. K. N. Prasad, *Nature, New Biol.* **236,** 49 (1972).
67. S. M. Wolfe and N. R. Shulman, *Biochem. biophys. Res. Commun.* **35,** 265 (1969).
68. A. Khan, J. Kogler and J. M. Hill, *Proc. Am. Assoc. Cancer Res.* **15,** 30 (1974).
69. W. Troll, A. Klassen and A. Janoff, *Science, N.Y.* **169,** 1211 (1970).
70. R. H. Bussell and W. S. Robinson, *J. Virol.* **12,** 320 (1973).
71. R. O. Hynes, *Proc. natn. Acad. Sci. U.S.A.* **70,** 3170 (1973).

72. C. G. Gahmberg and S. I. Hakomori, *Proc. natn. Acad. Sci. U.S.A.* **70,** 3329 (1973).
73. G. G. Wickus and P. W. Robbins, *Nature, New Biol.* **245,** 65 (1973).
74. N. M. Hogg, *Proc. natn. Acad. Sci. U.S.A.* **71,** 489 (1974).
75. S. Steiner, M. Steiner and J. L. Melnick, *Proc. Am. Assoc. Cancer Res.* **15,** 74 (1974).
76. M. Rieber, J. Bacalao and G. Alonso, *Cancer Res.* **35,** 2104 (1975).
77. G. Lipkin and M. E. Knecht, *Proc. natn. Acad. Sci. U.S.A.* **71,** 849 (1974).
78. G. Lipkin and M. E. Knecht, *Proc. Am. Assoc. Cancer Res.* **16,** 140 (1975).
79. G. Lipkin and M. E. Knecht, *Schweiz. med. Wschr.*, **105,** 1360 (1975).
80. M. E. Knecht and G. Lipkin, *J. invest. Derm.* **64,** 211 (1975).
81. K. M. Yamada and J. A. Weston, *Proc. natn. Acad. Sci. U.S.A.* **71,** 3492 (1974).
82. N. N. H. Teng and L. B. Chen, *Proc. natn. Acad. Sci. U.S.A.* **72,** 413 (1975).
83. L. Ossowski, J. C. Unkeless, A. Tobia, J. P. Quigley, D. B. Rifkin and E. Reich, *J. exp. Med.* **137,** 112 (1973).
84. R. O. Hynes, *Cell* **1,** 147 (1974).
85. L. A. Green, J. T. Tomita and S. Varon, *Expl Cell Res.* **64,** 387 (1971).
86. J. Taylor, S. Cohen and W. Mitchell, *Fedn Proc.* **29,** 670 (1970).
87. J. G. Collard and L. A. Smets, *Expl Cell Res.* **86,** 75 (1974).
88. H. N. Chou, P. H. Black and R. O. Roblin, *Proc. natn. Acad. Sci. U.S.A.* **71,** 1748 (1974).
89. I. E. Goetz, C. Weinstein and E. Roberts, *Cancer Res.* **32,** 2469 (1972).
90. J. Otten, G. S. Johnson and I. Pastan, *Biochem. biophys. Res. Commun.* **44,** 1192 (1971).
91. M. Inbar and L. Sachs, *Proc. natn. Acad. Sci. U.S.A.* **63,** 1418 (1969).
92. B. A. Sela, H. Lis, N. Sharen and L. Sachs, *Biochim. biophys. Acta* **249,** 564 (1971).
93. G. L. Nicolson and J. Blaustein, *Biochim biophys. Acta* **266,** 543 (1972).
94. B. M. Sefton and H. Rubin, *Proc. natn. Acad. Sci. U.S.A.* **68,** 3154 (1971).
95. K. Letnansky and F. Seelich, *Experientia Speciala* **31,** 434 (1975).
96. R. O. Hynes, *Cell* **3,** 113 (1974).
97. W. Troll, T. Rossman, J. Katz, M. Levitz and T. Sugimura, in *Proteases and Biological Control* (Eds. E. Reich, D. B. Rifkin and E. Shaw), p. 977. Cold Spring Harbor Press, N.Y. (1975).
98. D. Loskutoff and E. Reich, *J. Cell Biol.* **63,** 200a (1974).
99. D. B. Rifkin, J. N. Loeb, G. Moore and E. Reich, *J. exp. Med.* **139,** 1317 (1974).
100. J. C. Unkeless, K. Danø, G. Kellerman and E. Reich, *J. biol. Chem.* **249,** 4295 (1974).