The Surface Properties of Cancer Cells: A Review

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BIOLOGICAL ASPECTS

1. Local invasion. The malignant cells move outward from the focus of origin of the neoplasm, insinuating themselves amongst the surrounding normal cells.

2. Metastasis. As a result of invasion the malignant cells reach moving body fluids, by which they are passively transported and become widely disseminated.

3. Disorganization. The malignant cells as they multiply fail to form tissue of the arrangement characteristic of their normal counterparts.

4. Persistent growth. The malignant cell population keeps up a continuous growth by mitosis.

LOCAL INVASIVENESS

We must first consider what is the mechanism of movement of malignant cells when they invade their immediate surroundings. Probably the main mechanism is active "amoeboid" cell locomotion, which was first recorded for cancer cells nearly a century ago. Since the advent of tissue culture it has been observed in a very wide range of metazoan cells. Until the mechanics of this style of locomotion are better understood it seems preferable to put the term "amoeboid" to one side and to use the relatively noncommittal name "solid-substrate locomotion." This emphasizes one essential aspect of the mechanism: it involves adhesion, though transient at any one point, to a solid substrate. Only flagellated (or ciliated) cells can swim. The necessity for a substrate makes the nature of the cell surface, and of the surrounding solid structures with which the cell surface comes into contact, of profound importance for locomotor behavior.

A number of pathologists (e.g., Willis [146], Young [150]) consider that the pressure developed within a tumor by its growth provides the main motive power for invasion. The peripheral malignant cells, according to this view, are forced along paths of lesser resistance through the surrounding tissues, disrupting their structure and so intermingling with the normal cells. It may be recalled, however, that Wolff (147, 148) and his colleagues have shown that, when a malignant tumor is laid against an organ in culture, invasion of the latter occurs. In these circumstances it seems inconceivable that the growing tumor can exert any pressure. We shall, therefore, concentrate on active locomotion, though it is not, of course, justifiable to conclude that passive transport by means of pressure in no circumstances contributes to invasion.
THE NONINVASIVE STATE

The capacity to perform solid substrate locomotion is certainly observed among the majority of kinds of malignant cells when they are cultured in vitro (see for instance Coman [35]). However, it is also found among the majority of kinds of normal cells of a vertebrate; red cells and germ cells are the most obvious exceptions. The mere existence of an orderly pattern of cells in the body makes it, however, impossible to believe that, in normal circumstances, adult cells (white blood cells are an exception) undertake extensive locomotion of an invasive kind—i.e., involving penetration among other normal cells. Direct evidence as to whether, and if so in what circumstances and how far, normal adult cells move in vitro is of course hard to achieve, and there may be an unsuspected amount of small-scale movement. Nevertheless, it seems safe to suppose that the problem of invasiveness is as much a question of why normal cells stay substantially in place as of why malignant cells do not. We will, therefore, consider some ideas as to why a normal adult cell (other than a white blood cell) does not move extensively among other normal cells, even though it has the power of locomotion, under two headings: (a) mutual inhibition of movement by contact ("contact inhibition"); (b) other hypotheses of locomotor inactivation.

Contact inhibition.—It is a well known principle that "epithelium will not tolerate a free edge." Given a suitable substrate, a free edge will advance by movement of the epithelial sheet behind it. The idea is of long standing that the movement of such a sheet is stopped by contact of its edge with another epithelial edge, because this abolishes the polarization of the peripheral cells between the free space in front of them and their followers behind them (see Arey [21]). As early as 1913 Smith (124) examined the interaction of different epithelia from this point of view. Perhaps most important for the present review is the quantitative study by Lash (95) of the movement of epidermises over skin wounds in amphibian larvae. By marking individual cells he was able to show that, after excision of a small piece of skin, a rim of epidermises around the wound takes part in the movement and that there is a rather abrupt cessation of movement throughout this rim when the free edges meet in the middle of the wound. There remains an important obscurity about such epithelial movement, as to how cells behind the free edge move appropriately; but the cells at the edge seem to be the pacemakers, and their movement seems to be controlled by the state of their contacts with other cells.

That fibroblasts have a similar sort of contact reaction was demonstrated by Abercrombie and Heaysman (7), who investigated the interactions of chick heart fibroblasts moving in liquid medium culture on a glass surface. Interaction was obtained by placing two primary explants about 1 mm. apart. The movement and the distribution of the cells, before and after the outgrowth from the two explants made contact with each other, were measured. It was found that, as soon as a complete junction had been established between the two outgrowths, but not before, the cells showed a change of behavior: their speed dropped sharply, their direction of movement became random instead of predominantly outward from the explant, and the density of population in the inter-explant region became almost stable. The cells between the explants, in fact, largely ceased moving, undergoing apparently mere oscillations; and they remained distributed on the glass surface, if not in a perfect monolayer, at least with far fewer overlaps than was to be expected had they been disposed at random without mutual interference. No slowing of movement before junction of the two outgrowths was detectable. It was, therefore, concluded that the cells were inhibiting one another by mutual contact of cell surfaces, and the phenomenon was termed contact inhibition. A similar quantitative analysis was applied to the interaction of fibroblasts from chick embryo heart with those from neonatal mouse skeletal muscle, and they were found to inhibit each other in the same way by contact (8).

The cytological details of what happens during contact inhibition of fibroblasts were studied by Abercrombie and Ambrose (4). They found that usually, when a fibroblast made contact with a second one, the ruffled membrane that forms the leading edge of the first fibroblast joins closely to the second cell, at the same time ceasing its undulations. When the leading ruffled membrane is immobilized in this way, the cell bearing it ceases to move in the original direction. The region of contact between two cells, judging from microdissection studies (94) and from the distortion produced in the pair of cells when renewed locomotion in another direction draws them apart again until they separate with a sudden recoil (6), is as a rule a firm but not indissoluble adhesion between the two plasma membranes. Some of these observations have been substantially confirmed by Weiss (142).

The nature of the inhibitory effect of contact between fibroblasts is unknown. There are various hypotheses at present under consideration (3, 18, 40), according to which a change in a contacting cell may be produced; such a change can then
provide the basis for an alteration in locomotion. (a) The cell may in some way be paralyzed by the adhesive force in the region of contact (see 40). (b) The cell surface may alter in chemical constitution as a result of reaction with the surface molecules of the cell it contacts. (c) The cell may be unable to adhere at all to most (though obviously not all) of the surface of the cell it contacts, so that it cannot move across it. (d) Adhesion to the surrounding substrate may so exceed adhesion to other cells that it is impossible for a fibroblast to move itself from the substrate onto a neighboring cell. These four hypotheses do not seem equally likely on present information, but no strongly conclusive evidence is available. Since they all involve properties of the cell surface, they all bring contact inhibition within the scope of this review. There remains, however, a fifth hypothesis that does not necessarily involve surface properties: (e) diffusion of substances out of or into the cell will be changed where it is in contact with another cell, thus altering concentrations at the surface or further inside the cell. Now the diffusion gradients concerned extend by definition outside the cell boundary; yet no change in a ruffled membrane has been observed until it makes what under the light microscope appears as actual contact with another fibroblast. It seems probable, however, one explains this very short range of action of the chemotactic influence postulated by the hypothesis, that variability of cells and conditions would lead at least sometimes to an observed action at a distance. The hypothesis, though it seems to us at present an unlikely one, cannot, however, be dismissed out of hand.

Any mutually repulsive behavior between cells can immobilize them, since a cell may be so closely surrounded by others that no space free of repulsive force is presented to it. Contact inhibition is equivalent in effect to a repulsive force of very short range, and to immobilize a cell efficiently all the surrounding cells must be in contact with one another, so as to block all pathways. This readily happens on a plane surface, since the contacts between fibroblasts tend to be long-lasting, so that a population forms a meshwork. Essentially the same may hold when cells are within a three-dimensional gel, all available solid pathways being blocked by cell processes; but the behavior of cells in these circumstances has not been sufficiently studied.

For both epithelia and fibroblasts, therefore, reactions requiring contact are known which are capable of immobilizing them. There seems however, little doubt that mutual contact inhibition is not the sole mechanism tending to hold cells in place in vivo. We now consider the evidence for additional mechanisms, and how far the cell surface may be involved in them.

Other hypotheses of locomotor inactivation.—When explants are placed in culture they at once become surrounded by an expanse of cell-free substrate. Contact inhibition no longer exists at the external side of the peripheral cells of the explant (dead cells apparently do not for long inhibit living ones). Different tissues, however, differ greatly in their latent periods before they take advantage of the release from contact inhibition and in the rate at which cells subsequently emerge from the explant. Perhaps the most revealing differences are those between a normal adult tissue and the same tissue which by treatment before explantation has been thrown into a state of proliferation. Wounding a tissue in vitro, or calling on any of the other mechanisms that induce mitosis in it, makes its cells emigrate earlier and in greater numbers when it is subsequently explanted (see 2). Now the effect of this pretreatment on the propensity of the cells to move can hardly be via a change in their capacity to move over one another’s surfaces such as would be produced by a change in contact inhibition. Only a decrease in this capacity, corresponding to an increase in contact inhibition, would, by encouraging movement away from one another, tend to drive cells more readily out of an explant; and the extremely poor initial outgrowth from untreated adult tissue would then have to be interpreted as due to such a lack of contact inhibition that the cells were almost completely free to move within the explant and hence devoid of any reason for leaving it other than random diffusive movements. This is a condition that, as already discussed (p. 526), is most unlikely to hold in adult tissue. There seems to be, then, some additional influence (or constellation of influences), inversely correlated with mitotic activity, which tends to restrain cells. We shall refer henceforth to release from this influence as “mobilization.” We now consider possible hypotheses about the nature of the influence and in particular whether the cell surface may be concerned.

The most obvious suggestion is that mobilization and its opposite are due to variations in the adhesion of the cells to their surroundings. We have already seen that contact inhibition may be related to adhesiveness between cells: a strong mutual adhesion is a normal consequence of contact between fibroblasts, and it may indeed be the actual cause of the failure of the cells to move over one another (hypothesis [a] above). We are now considering a different result of adhesion: an anchoring down of the cells. Though both contact inhibition and immobilization in this sense may
Some degree of adhesion of a cell to its substrate is essential for locomotion. A cell moves by a little understood procedure of making and breaking adhesions (19), coupled with contractions and relaxations. Its adhesions may, however, be in absolute terms too high (or too low) to permit movement; or its adhesion at some part of its surface may be too high, relative to its adhesion to the substrate or its adhesion at some part of its surface may be too high, relative to its adhesion to the substrate available to its locomotor mechanism, for it to be able to pull away.

An explicit theory that cells are immobilized by the strength of their adhesions has been proposed by Coman (36, 37), developing a suggestion by Cowdry (39). He has proposed that it is the mutual adhesion of normal epithelial cells that prevents them from invading their surroundings; insofar as they break free they become able to move. If normal epithelial cells, however, are presented in tissue culture or in vivo with a suitable plane surface, they move across it so closely adherent to one another as to appear like a continuous sheet. They can move through a three-dimensional gel in vitro also in very close association, in the form of cords and strands. It is possible that the areas of mutual adhesion at any moment are reduced in the moving state as compared with the stationary state, but the cells do not need to break free from their fellows in order to move (see also Clark and Clark [92] for epithelial movement in ear chambers). Similarly, normal fibroblasts move despite a good deal of mutual adhesion. It is true that the more neighboring cells a fibroblast is adherent to, the slower its speed of locomotion (6); but there is compensation for this reduced speed in an improved consistency of the direction of movement (owing to contact inhibition at the points of adhesion—Abercrombie and Heaysman, unpublished) so that net displacement over a period of time will be considerably greater for a coherent sheet of cells than for isolated cells (Abercrombie and Gitlin, unpublished). It may be concluded, therefore, that it is by no means certain that adhesion to homologous cells necessarily reduces the displacement of epithelial cells, as Coman has suggested. For a cell to become anchored it must adhere firmly to something which does not itself move.

An example of the anchoring effect is probably to be found in the relation of Schwann cells to axons. Schwann cells emigrate freely from explants in vitro of peripheral nerves which have undergone a period of Wallerian degeneration, without reinnervation, before explantation (9, 88). They do not emigrate freely from explants of intact nerves. Wallerian degeneration is a promoter of proliferation, and as usual locomotion is also activated. When (again before explantation) new axons are allowed to penetrate a degenerating nerve, emigration in vitro of Schwann cells but not of fibroblasts becomes suppressed, though the proliferative state is unimpaired (10). A wave front of this suppression can be detected passing down the nerve behind the axon tips. In view of the well known close association of axons and Schwann cells, it seems that immobilization by adhesion has occurred. Myelination might seem the obvious form that the anchoring takes (61), which would make this case peculiar to nerve fibers; but there is some reason to think that a similar immobilization takes place in unmyelinated nerves (5).

Evidence is scanty that other types of cells, when aroused from their normal stationary state in vivo, are released from adhesion to their surroundings; this may, however, occur during healing in epidermis (95). The stimulation of cell emigration from adult explants by trypsin treatment (122) may also be adduced, though with some reservation, as suggesting the importance of adhesion to surroundings in limiting cell movement. However, even if clear evidence of the intervention of such adhesion is obtained, it is likely to be difficult to decide whether, when cells are mobilized, there is a primary change in their surfaces (or in the surfaces to which they are adhering) that is a decrease in the intensity of adhesion; or whether the primary change is the activation of the mechanism of movement, which then secondarily produces some degree of detachment, that is a decrease in the area of adhesion.

Summarizing these considerations about the holding still of normal cells in vivo, we may say that contact inhibition provides one possible mechanism, in that when all available solid substrate pathways bring a cell into contact with a homologous cell it will usually be prevented from making headway in any direction. This behavioral mechanism seems to occur among fibroblasts and among epithelia. The important problem of epitheliofibroblast contact relations is, however, as yet almost completely unexplored. The behavior of explants in culture indicates that contact inhibition, as it is known at present, cannot be the only mechanism holding normal cells still. There is evidently some other influence the operation of which is correlated with mitotic inactivity. How far the cell surface is primarily involved in this second mechanism or group of mechanisms is uncertain.

**The Invasive State**

Since malignant cells are not stationary in vivo, we must consider them in relation to the two inhibitory mechanisms we have discussed.
In the first place, if contact inhibition stops movement in vivo, malignant cells must somehow escape the influence of contact inhibition by the surrounding normal cells. Their immunity to it might theoretically be because as they move they bring about the bodily removal of the normal cells from their path, so that no prolonged contact inhibition can take place. They may do this either by so affecting the behavior of the normal cells that they move out of the way or by simply killing the normal cells. Both processes may occur to some extent. We have observed that a fibroblast population in vitro may appear to move aside from an encroaching outgrowth of sarcoma cells; the exact behavior mechanism has not, however, been analyzed. This may be the explanation of Leighton's observation (98) in sponge matrix culture of a great scarcity of fibroblasts among invading sarcoma cells. To be effective such a mechanism requires either that the fibroblasts to be shifted can find some place free of other cells to move to, or that they lose their contact inhibition by other cells. It is well known that malignant cells may kill nearby normal cells, either through output of substances or through competitive intake of nutrients. Like the possible repulsion of normal cells, this may to some extent eliminate cells from the path of moving cancer cells so that contact inhibition would have no chance to operate. However, there is no doubt that in practice malignant cells infiltrate among and in contact with a population of normal cells both in vivo and in vitro. It has been observed in vitro that destruction of normal cells occurs only after such infiltration, apart from the special case of penetration of an epithelium (147). It seems clear that, if our hypothesis is to be sustained, contact inhibition of invading malignant cells by invaded normal cells must be much reduced or absent.

The absence of contact inhibition of sarcoma cells by normal fibroblasts has been demonstrated, though only for a small number of mouse tumors. It has been shown that S-37 and S-180 cells are not inhibited by contact with normal fibroblasts (4, 7, 8), either mouse or chick. Subsequently, this was shown too for transplanted fibrosarcomas (MCIM and Klein's M3C, Abercrombie and Karthauser, unpublished) and for methylcholanthrene-induced primary tumors (Abercrombie and Karthauser, unpublished). From the point of view of invasion in vivo, such experiments suffer from the difficulty of testing contact inhibition by normal cells of the kind which actually are invaded by such tumors. The defect is to some extent mitigated by the fact that very different kinds of normal fibroblast inhibit one another. Furthermore, Temin and Rubin (129) have found that, when a monolayer culture of chick cells is infected with Rous sarcoma virus, the cells of an infected focus spread over the surrounding normal cells, indicating a failure of contact inhibition by homologous cells (see also Sachs and Medina [119]).

Although it is clear that mutual contact inhibition exists between normal epithelial cells, as already mentioned, it is not known how malignant and normal epithelial cells behave toward each other. More important, however, for the actual problems of invasion in vivo is, no doubt, the behavior of epithelial cells and fibroblasts to each other. Though a full analysis in terms of individual cell movements is lacking, it has been reported that in cultures some normal epithelia did not invade a fibroblast population, whereas in the same circumstances the carcinomas tested did invade (38, 120). There is, therefore, a clear suggestion that contact inhibition exists between nonmalignant epithelia and fibroblasts, at least in some conditions, but is absent when the epithelium is malignant. Further investigation is badly needed of this important relation.

Since contact inhibition is probably primarily a reaction of cell surfaces, these results suggest that a surface change plays a key part in the malignant transformation. The precise nature of the relevant surface change will remain obscure until the mechanism of contact inhibition is elucidated. The most likely hypotheses depend on adhesiveness of the cell surface, and, as will appear, a diminution of the general adhesive properties of malignant cells as compared with normal cells, suggested by Leford (102) and Cowdry (39), is now well authenticated. Curtis (40) has argued that the loss of contact inhibition by sarcoma cells depends on this general loss of adhesiveness; but it still remains a possibility that a more specific adhesiveness between cells of the same type is involved in contact inhibition (9).

According to our previous analysis, release from the constraint on mobility by contact inhibition is necessary but not sufficient to convert normal cells to invasive behavior. There is also the second mechanism of immobilization to be considered, which in normal tissues is correlated with mitotic inactivity. Cells from explants of a malignant tumor isolated in vitro give the impression of emigrating more freely than do cells from explants of the corresponding normal tissue, though there seem as yet to be no quantitative data standardized for the number of cells within the explant. (It may be noted here that the stromal cells of a tumor are to some extent mobilized—Drew [48]). Since in these conditions, where contact inhibition does not operate, the emigration of nonmalignant cells is correlated with their degree of mitotic stimul-
tion, the migratory activity of the malignant cells, which are mitotically active, is entirely to be expected. The nature of this mechanism of mobilization of normal cells has already been discussed, and we concluded that diminution of adhesion of the cells to their surroundings may play a part, though there is little evidence that it does. The evidence that lack of adhesion plays a part in the mobility of malignant cells is stronger in that we at least know that their adhesiveness is reduced. Already in 1912 Hanes and Lambert (70) described how in vitro individual carcinoma cells easily break free from one another. Coman (34) showed that mutual adhesion is much less between carcinoma cells than between normal epithelial cells by direct measurement. There is evidence pointing the same way, but less direct, for sarcoma cells (4, 62). Furthermore, there are miscellaneous data suggesting a generalized lack of adhesiveness of malignant cells to substrates of various kinds (Ludford [102] early remarked on this) as well as to homologous cells (we have already referred to the possibility that such lack of adhesiveness, to normal cells, accounts for the failure of contact inhibition). The relatively unflattened shape of cancer cells when cultured on a planar surface (see, for instance, Ludford [102]; Gey, Shapiro, and Borysko [63]) is probably a reflection of it; and it is well known, in the case of ascites forms, that many tumor strains can be selected for a remarkably low degree of adhesion to each other and to other objects.

All this suggests that if diminution of adhesion to their surroundings plays a primary role in the mobilizing of cells, the mobilized state of cancer cells is easily explained. Coman’s theory, already mentioned in connection with the behavior of nonmalignant cells, attempts to explain the whole of the invasiveness of malignant cells on these lines, taking, however, lack of mutual adhesion as the key. He supposes that a malignant epithelial cell, easily liberated from the general mass, can for that reason freely move away. We have, however, already pointed out that freedom from mutual adhesion is not an essential condition for movement and that, even if it were, it would not be sufficient for movement if contact inhibition were also present. The theory, therefore, seems inadequate to account completely for invasiveness. Nevertheless, the lack of mutual adhesion which Coman has so ingeniously demonstrated is probably a very important discovery about cancer cells. It probably reflects a more general loss of adhesiveness, which could be the basis for the absence of contact inhibition exerted by the normal cells invaded, and could also, as suggested above, play some part in mobilization by diminishing anchorage to station-ary structures. Furthermore, its great importance for metastasis can hardly be doubted.

We have discussed invasion as if the only point was that the invading cells should be able to undertake locomotion among the invaded cells. However, the direction of locomotion must also be considered. A localized population of cells which are undergoing random changes of direction, or as random as the fact that cells cannot simultaneously occupy the same position in space allows, will no doubt slowly spread by a process analogous to molecular diffusion reinforced by the inevitable nonrandom element in their movement. Such spread will probably be very slow, and it seems most improbable that this is how malignant invasion occurs. It is not how nonmalignant cells in tissue culture extend into a cell-free medium; they have various mechanisms of orientation. A particular problem is raised here, since it seems that a defect in contact inhibition may be one of the changes which makes malignant invasion possible, and yet the operation of contact inhibition is one of the main mechanisms producing the directed dispersal of a group of fibroblasts so as to occupy cell-free space. The problems of orientation of cell movement must therefore be briefly considered, since the cell surface is evidently involved.

Any mechanism by which the members of a cell population repel each other, even over short distances, greatly increases the efficiency with which the population spreads. Mutual repulsion may be by negative chemotaxis, which requires that each cell is surrounded by a diffusion gradient to which other cells react. It is possible that wandering cells react to each other in this way, and Twitty and Niu (130) have found evidence that amphibian embryonic melanoblasts do also. There is no evidence yet that fibroblasts or sarcoma cells have such a behavior mechanism. Contact inhibition, however, produces a form of negative taxis, permitting movement apart and discouraging closer approach than the first contact, and so tending to promote movement into space where contact inhibition does not operate. This interaction is probably responsible for the radial emigration of fibroblasts from an explant in culture on a glass surface. It could be an important element in radial movement of sarcoma cells from a malignant focus into surrounding tissue, if the sarcoma cells to some extent inhibit one another’s movement by contact but are not inhibited by surrounding normal cells. No quantitative investigation has, however, yet been made on the mutual interaction of sarcoma cells.

The structure of the region under invasion is likely to be an important influence on invading
cells; it is expressed in the contact guidance of Weiss (138, 139). An orientated structure of the substrate correspondingly orients the movement of the cells upon it. In tissue culture in or on a gelated medium, a focus of dense population such as an explant tends to produce a radially orientated structure in the medium around it. Under these conditions the radial spread of a culture, impelled by both contact guidance and contact inhibition, is highly efficient. Two explants near together tend to produce a pathway between themselves of orientated medium so that they become linked by a bridge of cells—the 2-center effect of Weiss (141). When one or both of the explants is of sarcoma cells, the 2-center effect still occurs (Abercrombie and Heaysman, unpublished). The sarcoma cells both orientate the medium, and are themselves very susceptible in their movement to the orientation. Carcinoma cells probably also show contact guidance (58, 97, 98). The change in surface properties associated with malignancy has in no way weakened this reaction. Since sarcoma cells lack contact inhibition they are able to treat fibroblasts as a substrate; and if the fibroblasts are themselves orientated, this orientation is imposed on the sarcoma cells. In mixed cultures made on a glass substrate, the fibroblasts orientate each other in a radial direction by mutual contact inhibition, and the sarcoma cells orientate themselves to the fibroblasts by contact guidance. It is even possible that orientated sarcoma cells themselves further orientate sarcoma cells moving on them. The tendency for invasion in vivo to occur along preferred pathways is no doubt an expression of these various mechanisms of guidance, and perhaps of others as yet undetected.

The failure of fibroblasts to produce contact inhibition of sarcoma cells does not, in the present state of our knowledge of the mechanism of contact inhibition, imply that sarcoma cells must fail to produce contact inhibition of fibroblasts. In fact, however, fibroblasts in vitro invade a sarcoma cell population (8), so that the failure of contact inhibition is qualitatively reciprocal. The locomotion of fibroblasts among sarcoma cells we have tested is, however, quantitatively much depressed as compared with their locomotion into cell-free space; the locomotion of sarcoma cells into a colony of fibroblasts is unaffected or even promoted as compared with their locomotion into cell-free space. This effect of sarcoma cells on fibroblasts may be complex, but it is no doubt connected with the diversion of fibroblasts from an advancing sarcoma outgrowth already mentioned. The effect seems to vary considerably with different kinds of sarcoma (see also Carrel [29], Ludford and Barlow [108], Jones [85]). In part, if not wholly, it operates by means of diffusive substances and so is not related to a special property of the malignant cell surface, except perhaps insofar as this by its leakiness promotes change in the medium around a sarcoma cell colony.

There are, of course, normal cells that invade in vivo, in the sense of infiltrating by locomotion among other cells. Leukocytes and macrophages do so. Correspondingly, tested in tissue culture, these cells show no contact inhibition by normal fibroblasts (Oldfield, unpublished). Their mutual adhesiveness is low (see Levi [99], Coman [34]). Macrophages are perhaps potentially of great interest from our point of view, because they seem to be able to pass between an invasive state (the ordinary migratory form) and an immobile state (the tissue histiocyte form). The trophoblast presumably makes a similar transition, and indeed numerous orderly morphogenetic cell movements of embryogenesis and of later histogenesis need to be investigated from this point of view. Some of these migrations, such as the dispersal of the neural crest, appear to be episodes of temporary invasiveness (limited to certain substrates [41]), and a reversible and controlled counterpart of malignant invasiveness may be part of the repertoire of behavior of many normal cells. Curtis (40) has considered how progressive changes in invasiveness, initiated artificially by disaggregation, may explain the sorting out of cells in reaggregates.

We must now draw together these considerations about the role of the cell surface in the invasive state of malignant cells. We suggested that for a population of normal cells to become invasive two changes were necessary: it must cease to be contact-inhibited by the surrounding cells which are to be invaded, and it must become "mobilized," since actual movement does not necessarily follow the lifting of contact inhibition. Those sarcomas tested have proved to be immune to contact inhibition by normal fibroblasts. Carcinomas, however, have not been precisely compared with normal epithelia in this respect. It is, nevertheless, a permissible hypothesis in the present state of the evidence that contact inhibition is a limitation on normal fibroblast and epithelial movement which disappears in malignancy. This change is probably in the surface of the cell and may be concerned with its adhesiveness. As to the second change, since "mobilization" is normally associated with mitotic activity it is not surprising that malignant cells have acquired this characteristic. It, too, may conceivably be connected with a change in adhesiveness of the cell surface; but if so, since normal cells may be mobilized without losing contact in-
hibitition, this change in the cell surface is different at least in degree from that which we suppose eliminates contact inhibition. There are, however, no strong grounds at present for thinking that mobilization is primarily due to a change in the cell surface.

**METASTASIS**

The passive spread of cancer cells via flowing body fluids, and the consequent establishment of new foci of growth and invasion, probably involve the properties of the malignant cell surface. It can hardly be doubted that adhesiveness plays a major part in determining the initial detachment of the cancer cells, and it may also play a part in their arrest in other parts of the body.

It is no doubt usually through the invasion discussed in the previous section that the malignant cells make contact with a flowing body fluid. In order to be swept away they must already be separated or be readily separable into single cells or into small groups. This implies that their mutual adhesiveness must be low. It may also require that their adhesiveness to the endothelium bounding the body fluid should at least be below a certain threshold. (A similar suggestion has been made about the liberation of red cells from the marrow into the circulation [91]). We have already discussed the strong evidence for diminished adhesiveness of sarcoma cells, mutual and to other structures, in the previous section and need not repeat it here.

To form a successful metastasis a tumor embolus must settle down in a place favorable for growth. Where it settles will depend partly on the size of the embolus; and, insofar as the embolus is multicellular, its size is likely to reflect mutual adhesiveness. Coman (35) has, for instance, shown that large clumps in the vascular system may tend to stick in arterioles where conditions for growth are unsuitable. Since the settling of a tumor embolus may involve adhesion to endothelium (149), where it settles may also be partly determined by the degree of its adhesiveness in this respect. Willis (146) as early as 1952 suggested that it might depend on surface charge of the tumor cell. It has, however, already been suggested that too great an affinity for the endothelium may hinder embolism. This would imply an optimal range of adhesiveness reminiscent of that required of ascites cells (Klein [93]) which need to be relatively nonadhesive to occupy their fluid habitat, and somewhat adhesive to evoke ascitic fluid from the peritoneum. Quantitative differences in adhesiveness to vascular endothelium may perhaps explain such observations as that some tumor cells pass readily through the lung circulation, whereas others, equal in size or larger, do not (152). Large ascites tumor cells, likely to be very nonadhesive, may circulate for a long time after intravascular injection (90). It may be that there are also qualitative differences in adhesiveness to substrate which, along with its diminished adhesiveness to endothelium may hinder embolism. However, already been suggested that too great an affinity for the endothelium may hinder embolism. Furthermore, the whole concept of the establishment of a metastasis involves several unknowns, and the whole concept of qualitatively specific adhesion between cells is at present in a somewhat uncertain state (40), so such speculation is premature. It should be added that for these problems the arrest of tumor emboli the plasticity of cell shape must also be considered (151).

**DISORGANIZATION**

It is well known that malignant tumors often lack the typical pattern of corresponding normal tissues. This is an expression partly of the dedifferentiation of intracellular specializations, partly of a simplification of cell shape, partly of a disorganization of cell arrangement. This section of the review does no more than call attention to the probability that the simplification of shape and the disorganization of arrangement may be, at least in part, an expression of surface abnormalities of the cell. The importance of the cell surface for embryonic development of the form and association of cells has been reviewed by Holtfreter (78) (see also Birbeck and Mercer [23]).

That the form characteristic of many kinds of cells in situ is not maintained by internal structural rigidity, but rather by cell surface relations, is shown by the manner in which these cells round up into a sphere when suspended freely in liquid. The potent influence of adhesion to surfaces on shape is also well known to the tissue culturist who compares the effects of different patterns of solid substrate, such as meshworks of fibrin with strands of different caliber (148). On the whole the malignant cell, observed in relatively standardized conditions such as on a plane surface, shows a tendency towards a reduced surface/volume ratio (see for instance Ludford [102], Gey, Shapiro, and Borysko [83]). This suggests a general diminution in its adhesiveness to substrate which, along with its dimi-
nution of mutual adhesion (34), could contribute to the simplification of shape actually found.

The cellular arrangement of a complex tissue, composed of different types of cell, will naturally be disorganized if only one of the cell types grows in population and invades the others. The disorganization extends, however, to the mutual arrangement of the malignant cells, all of the same type. Normal cells of a given type, at any rate be disorganized if only one of the cell types grows in population and invades the others. The disorganization of epithelial cells is plausibly interpreted as an outcome of mutual contact reactions. It seems at present that they must involve a pattern on each cell of surfaces favorable and unfavorable for mutual adhesion. With the surface changes, manifested by the general diminution of adhesion among malignant cells, there may go a partial or complete loss of the power of mutual arrangement. The partial retention of this power is presumably what Leighton (96) is concerned with in his "aggregate replication" of carcinomas, the increase in numbers of nests or aggregates of malignant cells during the growth of the tumor.

GROWTH AND MITOSIS

The last main characteristic of malignant tumors to be mentioned is the persistent replicative activity of a proportion of the malignant cell population, accompanied by the rhythm of repeated mitosis which usually goes with such growth. Can this characteristic be linked to a change in the cell surface? If it is assumed that growth is normally limited by intake of nutrients, it is clear that it may (125), particularly if pinocytosis is important for such intake (82, 90). Increased pinocytosis in malignant cells has been reported (82, 100). It has been suggested that the pinocytosis of normal cells was stopped where they form the adhesions associated with contact inhibition; and these mutual adhesions are much less in evidence with malignant cells (4). There is, therefore, some possibility that the growth activity of malignant cells may be linked with persistent surface activity associated with diminished adhesiveness.

CONCLUSIONS

We now attempt to draw together what has been said about the biological evidence that changes in the cell surface may be an important element in the differences between normal and malignant cells.

The mitotic activity of a population of malignant cells, and its capacity to migrate on a noncellular substrate (which we have called its mobilization) do not distinguish it from a population of normal cells in a state of proliferation. The malignant change, as far as these properties are concerned, is simply to put the population into a state of permanent proliferation. However, a second set of properties appears in most malignant cells which is unusual among proliferating normal cells: the capacity to migrate with other cells serving as substrate—i.e., to invade (which we refer to a lack of contact inhibition); the liability to passive dissemination; and the disorganization of structure.

The two properties in the first group, mitosis and mobilization, are certainly closely linked together in normal cells (though the correlation is not perfect [2]). That they are linked in malignant cells is, therefore, not surprising. It has been tentatively suggested in this review that their onset in a population of cells, when it is stimulated to proliferate or become malignant, may be related to some change in cell surfaces; but the connection is speculative, and if it exists it is not clear which is cause and which is effect. On the other hand, there is good reason for supposing that the appearance in a population of the three characteristics of the second group—lack of contact inhibition, dissemination, disorganization—are largely an expression of a surface change. The possibility has frequently emerged in this discussion that lowered adhesiveness is the fundamental aspect of this surface change. Passive dissemination seems to require lowered adhesiveness for the critical process of disintegration from the main mass; diminution of adhesion seems capable of accounting for much of the disorganization of cancers; and though the nature of contact inhibition is still uncertain, diminished adhesiveness is at least the basis of some plausible hypotheses about it.

On the evidence presented, then, a generalized diminution of adhesiveness seems to be an important part of the malignant transformation; but this hypothesis must not be pushed too far. It is evident that invasion, dissemination, and disorganization may vary among different tumors in an independent way (59). Even in the absence of quantitative methods for the assay of these characteristics, it seems that a theory based simply on a nonspecific loss of adhesiveness cannot account for their distribution in different tumors. A more sophisticated theory is probably required.

If it is difficult to unify these three characteristics by making them exclusively the result of a single kind of surface change, it is clearly more difficult still to add in the persistent mitosis and mobilization of malignant cells as well. However, even though this may mean that qualitatively dis-
tinct changes are involved in the successive steps of carcinogenesis, it becomes tempting to think of the cell surface as at least one of the primary sites of carcinogenic action. Two of the more mysterious forms of carcinogenesis, that produced by implantation of sheets of material and that produced by long culture in vitro, might fit with such a conception, since they may involve presenting cells with prolonged abnormal contacts (which may, however, act selectively rather than adaptively). At least one chemical method may act initially on the cell surface (60). Malignancy is, however, usually a lasting change which becomes independent of whatever induces it; it must, therefore, involve the mechanisms of replication. It is conceivable that the cell surface may be a component in one or more replication cycles, so that it takes part in replicating itself and hence is a possible site for a primary carcinogenic action. But what the changes in replication are that bring about lasting transformations like differentiation and malignancy is really still obscure, and it is wiser for the present merely to say that the cell surface, at whatever remove from the primary transformation it is altered, seems to play an important part in the manifestation of malignancy.

**PHYSICAL ASPECTS**

**INTRODUCTION**

In the biological summary the importance of the cancer cell surface in relation to the invasive behavior and adhesive properties of the cells was emphasized. We shall now attempt to examine the physical and chemical aspects of these changes under three headings: (a) the adhesiveness of normal and tumor cells, (b) mechanisms of cellular locomotion, and (c) the ultrastructure of cell contacts. This part of the review will be concluded with a summary (d) of the more general biochemical aspects of the structure of the tumor cell membrane with particular reference to possible future lines of research.

The primary function of the plasma or cell membrane is to provide a barrier of low permeability which will isolate and protect the internal structure of the cell. It might therefore be expected that lipides, which are largely impermeable to hydrophilic molecules, would play an important part in such structures. This was originally suggested by Overton in 1895 (114), who found that fatty substances readily penetrate cells, whereas substances that are insoluble in fats penetrate very slowly, if at all. But measurements of the surface tension of cells by Harvey (71) indicated that the tension was much lower (0.2–0.08 dynes/cm) than would be expected for a pure lipide layer. Danielli and Harvey (43) and Danielli (42) were able to show that lipides readily adsorb protein at the lipide-water interface; protein can reduce the surface tension to the magnitude observed in living cells.

The model for the structure of the cell membrane as proposed by Danielli is, therefore, as shown in Chart 1. It consists of a double layer of lipide in which the polar regions lie toward the aqueous phase while the hydrocarbon regions lie perpendicular to the surface and are packed in a manner similar to that which is known to occur in crystals of long-chain fatty acids. The protein chains are much longer than the fatty acid chains and may be expected to lie parallel to the surface of the membrane. Although modified in certain respects by more recent work, the Danielli model has proved satisfactory in accounting for most of the general properties of cell membranes. There is much experimental evidence in favor of a double lipide layer. The most direct evidence concerning the chemical nature of the lipides comprising the cell membrane has been obtained by the analysis of erythrocytes. The total lipide content of these cells is just sufficient to provide a bimolecular layer over the surface, which strongly suggests that the
lipide analyzed is membrane material. This ghost material has the composition: 70 per cent phospholipide, 25 per cent cholesterol, 5 per cent cholesterol ester, 0.4 per cent cerebroside (55); in human erythrocytes the phospholipide contains: 30 per cent lecithin, 15 per cent phosphatidyl ethanolamine, 18 per cent phosphatidylinerine, 19 per cent sphingomyelin, 10 per cent other lipide (45). The chemical constitution of the more commonly occurring lipides is shown in Chart 2. It will be seen that the phospholipides can readily be fitted into a model of the Danielli type, with the long fatty acid chains providing the hydrocarbon portion while the phosphate and amino groups provide the polar regions. In general, phospholipides display a number of the physicochemical properties of cell membranes, as has been mentioned by Holtfreter (78). For example, lecithin when placed in water spreads out to produce the familiar threads of myelinic form. Optical studies have indicated that the molecules in these myelinic forms are arranged in a series of double layers with a radial orientation. The myelinic threads are hollow tubes with the polar ends of the lipide molecules at the lipide-water interfaces. Myelinic forms occur even more readily with mixtures of lecithin and cholesterol, which suggests that both phospholipide and steroid may produce a particularly stable double-layered structure. X-ray diffraction studies of phospholipides and of the sheath of myelinated nerve, which resembles a myelinic form in many respects, indicate that the spacing between layers has a characteristic value for individual lipides but reaches a stable and independent value for mixtures of lipides. The spacing obtained for nerve myelin is 171 Å for two double layers. A method of packing phospholipide and steroid into a double-layered structure has been suggested by Finean (57). The choline portions are turned upward toward the polar ends of the cholesterol; the phosphate groups lie at the surface.

When extracted from whole tissues, phospholipides are generally tightly bound to protein, as might be expected from Danielli's observations. They can only be separated from protein by comparatively polar solvents, such as methyl alcohol. The neutral lipides, on the other hand, may be removed by comparatively nonpolar solvents such as ether. The general pattern of the lipoprotein in myelin consists of double lipide layers which are 52 Å thick, whereas the protein layers are 28 Å thick. Comparatively little is known about the chemical and physical properties of the protein fraction of cell membranes. Stromatin as isolated from red cell ghosts has an amino acid content rather similar to keratin but with an extremely low content of cystine (0.7 per cent) (145). The arginine content is relatively high. It is, therefore, likely to be combined with the phospholipide by van der Waals' attraction between side chains and by electrostatic linkages between carboxyl, phosphate, and choline; primary chemical bonds are unlikely to be involved.

ADHESIVENESS OF NORMAL AND TUMOR CELLS

The adhesiveness of surfaces depends upon the relative magnitudes of the attractive and repulsive forces. The forces are exerted between the macromolecules, as in the case of the lipides, or between the macromolecules and the substrate on which they are adsorbed. The magnitude of the forces depends upon the physical and chemical properties of the macromolecules and the substrate. The forces are similar in magnitude to those found in the case of the lipides, but they are not as well understood. The forces are not as well understood for the case of the lipides, but they are similar in magnitude to those found in the case of the lipides.
forces between them. In general terms, the repulsive forces can be understood simply as arising from the electrostatic repulsion between charged surfaces. These repulsive forces operate over considerable distances and in the case of Coulomb forces fall off inversely as the square of the distance between the charges. The attractive forces in general decrease more rapidly with increasing distance. The attractive forces are also more complex; for example, they may involve van der Waals' and related forces and electrostatic attractions between acidic and basic groups; these attractive forces can operate over considerable distances. In addition, if the surfaces come into close contact, hydrogen bonds can form between the >NH and >C = O groups of proteins, —OH groups of steroids, amide linkages in phospholipide, etc. (These bonds involve energies of 2–5 Kcal/mole, whereas covalent bonds require 20–50 Kcal/mole to separate the combining atoms.) Linkages between negatively charged groups such as carboxyl by bivalent ions such as calcium may also take place. All the cell-substrate adhesions brought about in this way are readily reversible and can be broken moderately easily at the temperature of the body.

Intercellular adhesions exhibit more complex behavior. Cells such as erythrocytes, which are comparatively nonadhesive both to substrate and to each other, carry a high negative surface charge (17). This is consistent with the principles already mentioned which indicate that the main repulsive forces between surfaces are due to electrostatic repulsion between like charges. Insofar as the attractive forces between cells are concerned, calcium ions play a predominant role, at least in the early stages of embryonic development. Amphibian embryos at the blastula stage may be disaggregated by low concentrations of EDTA, which removes calcium by chelation. If the calcium in the medium is replaced, the cells will reaggregate and eventually sort themselves out to produce normal structures. In addition to forming linkages between carboxyl groups (—COO Ca++ OOC—) calcium ions will reduce the net negative charge on the cell surface. Both these factors may be of importance in the establishment of intercellular adhesions. In mixed cultures of mammalian cells it is also found that the cells eventually associate with cells of a similar type (109, 140). Selective affinities are also observed in organ culture (147). The tissue specificity does in fact appear to be more organ-specific than species-specific. Rat liver cells will associate with mouse liver cells, for example, rather than with other tissue cells of the rat. The presence of specific reactions of this type possibly suggests the presence of a reaction rather similar to antigen-antibody interactions. Specific surface antigens are well known, as in the agglutination tests for blood group substances on erythrocytes. Reactions of this type, however, appear to be more characteristic of the individual than of the organ. For example, Kebus, Gerner, and Coombs (80) have detected the H-blood group substance on Hela carcinoma cells after 8 years of continuous tissue culture. This blood antigen is associated with the 'O' group of the original donor of the carcinoma cells. It would certainly not be expected that rat and mouse liver cells would associate preferentially according to accepted views of antigen-antibody reactions. It is not likely, therefore, that blood group substances provide the main component for organ specificity. Intercellular adhesions differ from antigen-antibody reactions in another important respect. They occur between the surfaces of identical cells and would not, therefore, be expected to possess the 

complimentary character proposed for antigens and antibodies. In this connection the sensitivity of embryonic tissues to calcium may be important. Identical surfaces of specific structure could clearly be bound by bivalent cations as already mentioned. An alternative possibility would be the sequestration of a cementing material which is deposited between the cells. As will be mentioned later, electron microscopy has shown clearly that in certain tissues a dense secretion of layers takes place between individual cells. Recent work by Easty and Mutolo (53) suggests that in adult tissues a protein cement may be considerably more important than calcium in maintaining intercellular adhesions. In agreement with Essner, Sato, and Belkin (56) they have found that proteolytic enzymes (trypsin, pepsin, and chymotrypsin) are effective in breaking up islands of hepatoma cells and liver cells. Diisopropyl fluorophosphate-inactivated trypsin, on the other hand, is without effect, indicating that proteolytic hydrolysis is involved in the separation. Erythrocytes which have been agglutinated with antiserum (γ-immune globulin) are not dispersed by trypsin, unlike the intact tissues. This result suggests that the protein cement is not of the same type as antibody protein. It is therefore possible that in embryonic tissues and tissue cultures an early stage of intercellular adhesion occurs which is sensitive to calcium in the medium. This adhesion probably takes place directly between the cell membranes. In adult tissues this reaction appears to be at least partially replaced by a definite secretion of a cementing protein. L. Weiss (136, 137) has demonstrated the importance of protein in determining the adhesiveness of both normal and tumor tissues. He has found that trypsi-
treated cells require a higher concentration of serum in the medium to make them adhere to glass than is required by untreated cells. The constituent of whole serum which appears to be mainly responsible for the adhesive properties is the $\beta_2$ lipoprotein-rich serum fraction.

A more physical approach to the problem of cellular adhesiveness has been given by Curtis (40), who has developed a theory to account for the properties of the cell membrane based on the known rheological properties or flow characteristics of protein monolayers and on the Verwey and Overbeek (133) theory of adhesions between surfaces, which throws some light on the mechanism leading to cellular adhesions. Protein films are known to exhibit viscosity changes with shear, the viscosity decreasing with increasing shear at low concentrations giving the phenomenon of thixotropy (tendency to become fluid at high rates of shear). A rise in viscosity occurs with shear at high concentrations giving rise to rheopexy (tendency to become rigid at high rates of shear); it is to be expected that the cell membrane will also exhibit effects of this kind. According to the Verwey and Overbeek (133) theory of adhesion, surfaces of similar constitution should attract one another by van der Waals' forces and repel one another if they differ considerably; the electrical charges of the two surfaces will drive them apart if of similar sign and draw them together if of opposite sign. Van den Teupel (131) has examined experimentally an oil-water-oil system and has found that the two surfaces are stabilized at a distance of 100–200 Å. This result is in good agreement with the Verwey and Overbeek theory, which predicts such a distance for balance between the attractive and repulsive forces. According to Curtis it is therefore possible to account for the separation of the parallel membranes at ~150 Å, as seen in the electron micrographs (Chart 5). More recent methods of fixation have shown that a deposit does in fact lie between the membranes. But Curtis has pointed out that this may occur at a later stage after the membranes have been initially stabilized.

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**Chart 3.**—(a) Cross section through a moving Amoeba. L = leading pseudopodium; T = tail; S = solid substrate. (b) Undulating contacts between a filamentous alga and the solid substrate. C = contacts between membrane and substrate. (c) Mechanism of cellular locomotion involving undulating contacts between membrane and substrate. C, C' = moving contact; T = transverse undulations of membrane; M = compressional wave traveling in cytoplasm.

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**Chart 4.**—Morphology of cell membranes and cell contacts as shown in the electron microscope.

a) Intercellular contact with separation of ~150 A between densely staining membranes.

b) Less continuous contacts seen between cells in region of active cell proliferation in the hair root.

b') Higher power picture of contact showing each membrane resolved into two layers ~70 A apart.

c) Region of intercellular contacts showing dense deposits (P) in cytoplasm. (Desmosomes.)

d) Multilayered deposit between cells seen in the upper part of the hair root and some other differentiated tissues.
at a distance of ~150 Å by the balance between the attractive and repulsive forces. By combining the rheological and the adhesion model, Curtis has been able to account for a number of the properties of cell membranes. An expansion of the surface of the cell may be expected to increase its adhesiveness because the van der Waals–London forces will be unaffected, whereas the charge density may be expected to decrease. This effect can explain the increased adhesiveness of spreading membranes and can account for the changes in contacts which occur continuously during locomotion by means of undulations between the moving cell and the solid surface, to be described later. When two moving cells meet in opposition the effective shear will be greatly increased, the surface viscosity will fall, and the membranes will expand; this will lead to increased adhesiveness, and an adhesion will form between the two cells. Contact inhibition can be understood in these terms.

How are we to explain the reduced adhesiveness of the tumor cell surface in such terms? Since the repulsive force between the cells is mainly due to repulsion between like charges, a method of examining the charge on the surface of tissue cells may throw some light on the adhesive process. Cell electrophoresis can be used for this investigation. The tissues are gently dispersed and suspended in a medium in which they are known to remain viable for many hours. The velocity of individual cells in an electric field is then determined in a chamber mounted under the microscope. Experiments of this kind have been carried out on normal and tumor cells from kidney cortex and on normal liver cells and hepatoma cells. The hamster kidney tumors were originally induced with stilbestrol. Both the normal and tumor cells have been grown in culture (15) and their contacts examined. The normal kidney epithelium grows as discrete islands of cells with closely adhering contacts between the membranes. The tumor cells grow much more independently with fewer stable adhesions between their membranes. The culture studies show that the membranes of these tumor cells are less adhesive than the normal cells from which they are derived. According to the electrophoretic studies the tumor cells also have almost twice the mobility of the normal cells from which they are derived (Chart 5). This increased mobility can be explained by an increase in the net negative charge per unit area carried by the tumor cell membrane. An increased negative charge was also found when hepatoma cells were compared with normal liver cells. This method of measurement is expected to demonstrate surface effects—e.g., Moyer (110) has shown that collodion particles when coated with protein display the same mobility as the protein molecules in solution. With tumors containing cells of varying size, the mobility is found to be independent of size, which also supports this view. In addition, the known electrical resistance of the cell membrane (44) ensures even more effectively that the cell surface is practically isolated from the interior in electrical studies of this type. The increase in negative surface charge can be correlated directly with a reduced mutual adhesiveness of the cells.
displayed in biological experiments. For example, various sublines of a single tumor have been examined by this method (MCIM sarcoma). A number of sublines of this tumor have been obtained by Klein and Klein (93) using selective transplantation. The MSS strain is a solid subcutaneous tumor which does not produce ascites cells but grows again as a solid tumor when implanted in the peritoneum. It does not metastasize readily. Metastases in the lung are found as discrete emboli which do not infiltrate into the lung tissue. MTLF is an intermediate form of solid subcutaneous tumor which gives rise partly to ascites cells and partly to solid tumor in the peritoneum. MAs is a solid subcutaneous tumor which produces an ascites tumor in the peritoneum at the first implant. It metastasizes readily, with a high degree of infiltration into the lung tissue. MAA is the actual ascites tumor, transplanted continuously in the peritoneum. In Chart 6 it can be seen that there is a progressive increase in the net negative charge carried by the cells as one passes from the MSS to the MAA strains. Lack of adhesiveness between the tumor cells can therefore be most readily explained by a high electrical charge resulting in electrostatic repulsion between the surfaces. How could such an increase in surface charge be brought about? The simplest explanation would be that the membranes have a reduced capacity to bind positive ions, notably calcium. A reduced calcium content of tumor tissue as compared with normal tissue has been found by Beebe (24), Brunsching, Dunham and Nichols (28), Clowes and Frisbie (33), de Long, Coman, and Zeidman (47), Dunham, Nichols, and Brunsching (49); de Long, Coman, and Zeidman have suggested that the decreased calcium content may be related to the decreased adhesiveness of the tumor cells. They have pointed out that in normal growth conditions, as in embryonic development, changes in the sodium/potassium ratio occur, but no decrease in calcium content is found (47). Alternatively, there may be some alteration in the quantity and distribution of the actual charged chemical groupings themselves on the surface of the tumor cell. One way of investigating this distribution is to examine the way in which long-chain polymers carrying positive charges are absorbed on the surface. For example, Katchalsky and his collaborators (112) have found that polylysine readily combines with the erythrocyte surface, neutralizing the negative surface charge of the cell and leading to rapid cellular agglutination. Ambrose, Easty, and Jones (16) studied the agglutination of suspensions of Walker carcinoma cells by various positively charged polyelectrolytes of this type. Comparisons were made with erythrocytes and spleen cells. It was found that several of the polymers showed selective effects. One polymer, polyethylene imine, agglutinated the tumor cells selectively, having less affinity both for spleen cells and erythrocytes. Erythrocytes carry a high negative surface charge like tumor cells, but these results suggest that, at least in this transplanted tumor, the distribution of charges on the cell surface differs from that of the erythrocytes.

It may be concluded that the adhesions between cell surfaces are probably due to the protein or mucoprotein component of the membrane. An increase in the net negative charge on the cell surface reduces adhesiveness, and this change probably explains in part the reduced adhesiveness of tumor cells.

MECHANISMS OF CELLULAR LOCOMOTION

The cell surface plays a fundamental role in all types of cellular locomotion. We shall now examine some physical theories of such locomotion with particular reference to the invasiveness of tumor cells. Unicellular organisms and almost all tissue cells of higher animals are capable of migrating on solid surfaces under suitable conditions. Most of the earlier observations of these movements were carried out on protozoa, particularly the large amoeba, Amoeba proteus. Goldacre (67) proposed the mechanism for locomotion illustrated in Chart 3. a. Contraction of the proteins in the tail of the organism causes the cytoplasm to flow forward toward the leading pseudopodium. Allen has
proposed that the driving mechanism for protoplasmic streaming is located in the leading pseudopodium. But whichever view of the location of the driving forces is accepted, evidently the membrane of the leading pseudopodium expands and flows forward in the direction in which the cell is moving. Meanwhile, the membrane in the tail is retracted and flows into the cytoplasm. In other words old membrane is withdrawn from the tail, is carried forward by protoplasmic streaming, and new membrane is formed continuously at the leading edge. This mechanism of locomotion is usually called amoeboid movement. It has been observed in a number of organisms, including mammalian white blood cells. It implies the presence of a labile membrane into and from which new molecules or micelles can be introduced and readily withdrawn. The model illustrated in Chart 3, a, is satisfactory from this point of view. It is by no means true, however, that cells with labile membranes are unique in being able to migrate across solid surfaces. An extreme case of organisms with coherent membranes which can still migrate are the blue-green filamentous algae. They consist of cells linearly arranged with a moderately rigid membrane, as commonly occurs in plant cells. The continuous flow of cytoplasm associated with amoeboid movement is excluded in these organisms, yet a number of them migrate rapidly on surfaces. By the use of a new type of microscope (19, 18) it has now become possible to observe the locomotor mechanism directly. This microscope depends upon the reflection of light at the surface of a glass prism and enables those regions of the cell which are in contact with the surface to be examined individually; the interior of the cell is not illuminated. In this microscope, it is seen that the locomotion of Oscillatoria and other algae is accompanied by waves of contact between the mem- brane and substrate which travel continuously along the length of the filament. These waves can readily provide a mechanism of locomotion as illustrated in Chart 3, b. If these waves are accompanied by a compressional wave, also traveling along the length of the organism as indicated in Chart 3, c, the membrane of the organism will make intermittent contact with the surface, each contact occurring at a different point on the membrane, if the compressional wave is out of phase with the transverse wave. In this way the organism is able to move along the surface, rather like a Gastropod.

An examination of mammalian tissue cells such as fibroblasts and epithelial sheets indicates that a similar mechanism is involved in their migration. Undulating contacts between the membrane and the substrate surface are again involved (Chart 3, c). Undulations of the membranes are also seen when migrating tissue cells are examined by time-lapse filming with the interference microscope (4). These undulations can be seen in phase contrast by focusing carefully on the upper surface—i.e., the cell-liquid interface. Observations of similar cells under the surface contact microscope, however, immediately show that these undulations are also occurring at the contacts between the cell and the surface of the solid substrate (18). The undulating contacts are particularly conspicuous in the advancing regions of epithelial sheets. When groups of fibroblasts streaming from an explant are examined, they are all seen to exhibit rapid undulations of the membranes and contacts. Migrations of tissue cells by this process can explain a number of otherwise unrelated phenomena. Fibroblasts and epithelium rarely exhibit streaming of the cytoplasm in the direction of migration, as is the case with Amoebae. Intermittent streaming of the cytoplasm takes place away from the leading edge of the cell, as can be seen when droplets are enclosed by pinocytosis and carried toward the nucleus. When groups of closely adhering fibroblasts and epithelial cells in culture are examined, they are often seen to stream uniformly across the substrate in the direction of a free border. In interference contrast and under the surface contact microscope the free borders of sheets are seen to exhibit extremely active undulations. These undulations travel backward from the leading edge of the cell. The leading edge therefore acquires dominance in guiding the general movements of the group of adhering cells. In the biological section mention was made of possible blocking mechanisms which could play a part in the phenomenon of contact inhibition. Physical or chemical factors affecting the intermittent contacts between cell and substrate of the type shown in Chart 3, c, could possibly operate in this way.

Migration of cells by amoeboid movement or by membrane undulations requires continuous supply of energy by the cell. Goldacre (67) has proposed that contractile proteins are stimulated in the tail of the Amoeba and that these proteins are carried forward to the leading pseudopodium, where they unfold. An ATP enzyme system similar to actomyosin has in fact been isolated from Amoebae. Little is known at present about the source of energy of membrane undulations, but there are a few clues. Weber (134) has shown that glycerol-extracted fibroblasts contract in the presence of ATP. Ambrose and Curtis (18) have found indications by surface-contact microscopy that the amplitude of membrane undulations in living fibroblasts is increased in the presence of ATP in the
The mechanism of locomotion in tumor cells has been studied by interference microscopy (Abercrombie and Ambrose [4]) and by surface contact microscopy (Ambrose, unpublished). As with normal tissue cells, undulations of the membrane in contact with substrate are generated with both sarcoma and carcinoma cells. The main locomotor mechanism in tumor cells is evidently by the same intermittent contacts (Chart 4, c) between cell and substrate, as in the case of normal tissue cells. Qualitatively, however, the type of movement in tumor cells differs from that of normal cells. The movements of tumor cell membranes tend to be less coordinated than those of their normal counterparts. Normal fibroblasts tend to develop a number of less expansive pseudopodia and in the anaplastic, more rounded types the membrane may be divided into large numbers of small and independently moving villi. This is probably just another manifestation of the general degeneration of control mechanisms observed with so many aspects of tumor cell behavior. In the biological section it was pointed out that complete independence of cells so that they were able to move like protozoa was not necessarily the sine qua non of migration. Normal epithelial cells in tissue culture or in vivo will move on a suitable plane surface while so closely adherent to each other as to appear like a continuous sheet; they can move through a three-dimensional gel in vitro in very close association in the form of cords or strands. Locomotion by membrane undulations giving rise to intermittent contacts with the substrate can account for such movements. It is only necessary that a free border be present to generate the leading membrane movements, when the whole group of cells will migrate in the direction of the free border, each cell being coordinated in its movements by the leading cell. Similarly, in the early stages of tumor invasiveness, it is not necessary to postulate complete loss of contact inhibition between all tumor cells. Some local loss of contact inhibition—e.g., between tumor cells and the surrounding normal tissues—may be all that is required. The tumor cell mass may then be able to migrate by membrane undulations on the normal tissue as substrate, while maintaining close contacts with neighboring tumor cells. There are suggestions, from histological sections, that the early stages of invasiveness can occur in this way. Only later, when tumor cells of much reduced adhesiveness have appeared, do independent cells break away from the tumor mass and give rise to metastases.

ULTRASTRUCTURE OF CELL CONTACTS

Electron microscopy has been used for the study of the morphology of cell membranes and cell contacts, both with normal and tumor tissues. The preparation of specimens for electron microscopy involves both fixation and drying. With a delicate colloidal system such as the lipoprotein complex of cell membranes, it might be expected that satisfactory fixation would be difficult to achieve. Mention has already been made, however, of the studies of nerve myelin by Enström and Finean (54, 57) using x-ray diffraction and electron microscopy. They have also examined the effect of successive stages of fixation and drying on the x-ray diffraction pattern of myelin and have compared the results with direct observation in the electron microscope. Although some qualitative changes in spacing occur, the basic structure appears to be preserved after fixation. It has been tempting to suggest that the plasma membrane of other cells might correspond to a single layer of the type observed in the multiple structure of the myelinic sheath. Evidence in support of this view has been obtained by Mercer from studies of Amoeba proteus, using fixation with osmium tetroxide. There are two outer layers of densely staining material with a central more transparent region. These three layers could correspond to the two outer layers of protein with a lipid layer between as envisaged by Danielli (48) (Chart 1). With other types of cell the membrane is usually seen as a single layer, but it may be resolved into a three-layered structure similar to Amoeba proteus, particularly if potassium permanganate fixation is used (Robertson [116]; Mercer, private communication). Contacts between neighboring cells also show interesting effects in the electron microscope. This has been clearly illustrated by Birbeck and Mercer's systematic study of the developing hair follicle (23). This is a particularly interesting system in which to examine cellular differentiation. In the hair root, where cells are actively dividing, the membranes show convoluted forms, and continuous close contacts between cells are not observed (Chart 4, b). In those regions where contacts do occur the membranes of adjoining cells lie side by side, at a distance apart of 150-200 Å. With osmium tetroxide fixation this space appears to be transparent, but with other methods, for example after staining with lead salts (Mercer, private communication), the space between the membranes is at least as dense as the general cytoplasmic back-
plaques were considered to correspond to the pro- 
of studying the cell surface by electron microscopy 
a. The membranes lie parallel to one another and 
insoluble in lipide solvents. Occasionally in these regions of thick- 
ened cytoplasm adjoining a contact, tonofibrils 
may be seen. These are fibers which lie at right 
angles to the cell surface. They are also conspic- 
uous in the region of the contact between muscle 
cells. They are thought to provide attachments 
between the cell contact and the interior of the 
cytoplasm, so that considerable tension can be 
maintained between adjoining cells. Further up the 
haft cuticle (Chart 4, d), in the region where kera- 
tinization is taking place, a definite secretion of a 
layered structure between the cells can be de- 
tected. Similar results have been obtained more 
recently by Rogers (117).

The presence of multi-layered structures in nerve 
myelin has also been demonstrated in a most in- 
teresting way by Robertson (116). The Schwann 
cell is considered to have wound itself round the 
nerve axon to produce a spiral structure. The outer 
surfaces of the Schwann cell membrane in the un- 
swollen condition are separated from one another 
by a distance of ~150 A, as in the case of tissue 
cells as previously described. It is interesting to 
note that on the cytoplasmic side the inner sur- 
faces of the membranes come into intimate con- 
tact. The individual membranes have also been 
resolved by Robertson into two layers ~20 A 
 thick, with a lighter zone ~35 A thick lying be- 
tween—i.e., the individual membranes have the 
same general appearance as the plasma membranes 
illustrated in Chart 4, b. Apart from the keratin- 
ized regions of the hair follicle and the myelin 
sheath, multilayers are not generally observed at 
cell contacts. Contacts between differentiated tis- 
sue cells generally take the form shown in Chart 4, 
a. The membranes lie parallel to one another and 
are separated by ~150 A. A more sensitive method 
of studying the cell surface by electron microscopy 
is to use some shadowing procedure to show up the 
surface contours instead of sectioning the tissue. 
For example, Hillier and Hoffman (72) prepared 
erythrocyte ghosts and shadowed the surface with 
evaporated metal in a high vacuum. When this 
procedure was carried out after careful washing, an 
interesting plaque formation was observed. These 
plaques were considered to correspond to the pro- 
tein portion of the membrane, because they were 
insoluble in lipide solvents.

Intercellular adhesions between normal cells of 
the type just described may have more general 
effects in the actual organism. For example, prob- 
lems of embryonic development are concerned 
with mechanisms of communication between cells. 
In the past, emphasis has been placed on organizer 
substances, chemical messengers which are se- 
creted by one cell and act upon a receptor in a 
neighboring cell. Emphasis has turned in recent 
years, however, toward a study of direct reactions 
between cells (77, 78, 140). Control by simple 
physical contact is clearly illustrated in the phe- 

omenon of contact inhibition (6, 7) described in 
the biological section. The adhesions between cell 
membranes which give rise to the contact inhibi- 
tion effects described in the biological section, af- 
cet the movements of cells. But they may also 
modify the cells in other respects. As already men- 
tioned, electron microscopy indicates that, in 
the region of an adhesion between membranes, a 
thickening may occur, just within the cyto- 
plasm. Evidently an increased secretion of some 
kind has been stimulated by the contact.

In a few cases actual passage of vacuoles from 
one cell to another has been observed. Mela-
nocytes secrete melanin into cells by this process 
(106). Cell-to-cell interactions, arising from con- 
tacts of an electrical nature, may also arise. This 
has been extensively investigated with growing 
plants by Lund and his collaborators (104). They 
have shown that differences in electric potential of 
up to 60 millivolts can be detected between the 
apex and proximal region of the cotylone of growing 
seeds. These potentials, which occur in many types 
of cell, are considered to arise from differences in 
the ionic permeability of the membrane in various 
regions of the cell. In mammalian nerve cells, the 
transfer of the action potential is known to be ac- 
companied by a reversible change in the permea-
bility of the cell membrane to potassium and sodium 
ions (73, 82). Goldacre (66) has demonstrated 
with electronic models that a small number of elec- 
trical interactions between units can give rise to 
quite complex patterns of behavior. The study of 
bioelectric potentials and currents in relation to 
cell-to-cell interactions generally may provide an 
interesting field for future research, particularly 
their possible failure and lack of coordination in 
cancer tissue.

When tumor tissues are examined by electron 
microscopy the morphology of the membrane in sec-
tion is similar to that of normal cells. The plasma 
membrane is observed as a continuous layer ~70 
A thick. However, an altered appearance of the 
contacts with neighboring cells can generally be 
observed in anaplastic tumors. In these tumors, 
the contacts are similar to those observed in the
actively dividing region of the hair root, as indicated in Chart 4, b. The membranes exhibit small areas of contact with neighboring cells, the remaining regions showing a highly convoluted form. Where contacts do occur they appear to be morphologically similar to those of normal cells. The membranes are separated by a distance of ~150 Å, and the cytoplasm in the region adjoining the contact tends to produce a desmosome structure (Mercer, private communication). These observations support the earlier studies of tissue cultures filmed under the interference microscope and the micromanipulation experiments which indicate that anaplastic tumor cells move considerably more independently and are less adhesive than the homologous normal cells.

As in the case of normal cells, the most sensitive method of studying the cell surface by electron microscopy is to shadow the surface layers. Several investigators have compared normal and tumor cells by this method. The most favored procedure has been to prepare carbon replicas. The surface of the cell is coated with carbon in high vacuum. The surface contours of the carbon are accentuated by shadowing with gold or chromium. The cellular material is then dissolved away by enzymatic digestion to leave the carbon replica for examination in the electron microscope. This method was first used for comparisons between rabbit Vx2 epidermoid cells and epithelial cells separated mechanically from the rabbit’s ear by Coman and Anderson (37). The normal epithelial cells exhibited a number of large projections or prickles, but the general texture of the membrane surface was comparatively smooth over the range from 30 to 300 Å. The carcinoma cells, on the other hand, did not exhibit prickles but showed many irregularities in the range from 30 to 800 Å. More recently, Berwick (26) has carried out similar observations using trypsin digestion to separate the cells. He has also found that the surface of the Vx2 carcinoma cells is rougher in the range from 50 to 300 Å. In the case of a benign tumor (Shope papilloma), the texture more closely resembled that of the normal epithelial cells. However, Easty and Mercer (58) have shown that, when cells which have not been subject to mechanical or enzymatic treatment are examined after fixation in tissue culture (59), the texture in the range from 50 to 300 Å is similar both with normal and tumor cells. The contacts between neighboring cells, however, show distinct differences. In the case of normal kidney epithelium, the shadowing indicates that the membranes between adjoining cells of the sheets are in close contact with uniform and parallel membranes running around the circumference of the cells, as in Chart 4, a. The adjacent membranes are separated by about 100–150 Å. Kidney tumor cells, on the other hand, exhibit a number of long processes or pseudopodia (0.1 μ diameter) around the cell borders, with very few parallel contacts with neighboring cells. The decrease in the mutual adhesiveness of the membranes in cancer cells is clearly demonstrated by the shadowing procedure. Nowell and Berwick (113) have also compared normal and leukemic lymphocytes by the shadowing method. Here again, no difference between the texture of the normal and tumor cells could be detected in the range from 50 to 300 Å. On biochemical grounds it is hardly to be expected that differences between the lipoprotein structure of normal and tumor cell membranes would show up in the 100 Å range. They would be expected to occur in the range from 10 to 100 Å. The changes observed by Coman and Anderson and by Berwick, with cells separated from tissues, may perhaps correspond more to changes in the elastic or other physical properties of the plasma membrane of cancer cells (see also Catalano, Nowell, Berwick, and Klein [30]), rather than to permanent morphological changes.

It may be concluded that on the whole the studies of ultra-structure provide additional evidence for altered surface properties associated with less coherent intercellular contacts, at least in anaplastic tumors.

BIOCHEMICAL PROPERTIES OF TUMOR CELL MEMBRANES

In the three previous sections of this part of the review we have tried to learn something of the physical factors underlying the changes in biologic behavior of the cell surface during carcinogenesis. We shall now consider the biochemistry of the complete plasma or cell membrane in more general terms.

That the internal structure of the membrane as opposed to the cell surface may be changed during carcinogenesis is suggested by observations of cell permeability. Particularly striking have been the observations indicating that a considerable leakage of enzymes takes place from the cytoplasm in growing tumors. For example, Malmgrén, Sylvest, and Révéz (105) found that ascites tumor cells release a considerable number of proteolytic enzymes into the ascitic fluid. By careful tests it was established that this release took place from the living cells and was not due to the presence of dead or dying cells in the suspension. In 1957, Libenson and Jena (101) prepared samples of interstitial fluid from transplanted human tumors. From electrophoretic studies on fluids incubated for from 24 to 96 hours at pH 4, they concluded that the deg-


The release of enzymes into the interstitial fluid found that ribose-5-phosphate, glucose, and fructose were metabolized by enzymes liberated into tumor fluid. Sylvén (126) has carried out an extremely detailed and thorough analysis of this problem by a microanalytical method. A fine capillary was used to withdraw interstitial fluid from unicentric mouse transplants, from the surrounding normal tissue, and from the peritoneum. In all cases, care was taken to ensure that regions containing only living and actively metabolizing cells were tested.

The cell-free tumor fluid had a protein content about 100 per cent higher than that of the peritoneal fluid, but the 'over-all' dipeptidase activity was increased 40–100 times; the arginase and glutathione reductase activities increased 5–20 times; the catheptic activity at pH 4.5 increased 8 times over the corresponding normal plasma. The amino peptidase and LDH activity were also found to be increased dramatically (Sylvén, personal communication). The interesting point here is that no mitochondrial enzymes are released — i.e., only those enzymes which are in a soluble state in the cytoplasm are released. These increases cannot be due to a general retention of protein in the tumor compartment, and it appears that the local increase in enzyme concentration is due mainly to leakage from the tumor cells. This is supported by other in vitro work by Sylvén and Bois (127). Evidence for the leakage of other types of enzyme from tumor cells has been obtained by Bosch (27). He found that ribose-5-phosphate, glucose, and fructose were metabolized by enzymes liberated into the extracellular medium by ascites tumor cells. The release of enzymes into the interstitial fluid may play an important role in tumor invasiveness, particularly the catheptic and peptidase activity which may break down the normal stroma.

According to the studies of de Grier and van Deenen (46) there is a direct correlation between permeability and phospholipide composition when erythrocytes from various species are compared. A correlation was found between permeability to glycol and percentage of lecithin in a range of erythrocytes. This result suggests that the altered permeability of tumor cells may be related to changes in phospholipide composition. Veerkamp, Mulder, and van Deenen (182) have in fact carried out a detailed analysis of the lipides of normal rat liver and a primary hepatoma. No striking differences could be observed in the various phospholipides. In addition, however, the constituent fatty acids of the phospholipides were compared by use of gas chromatography. A considerable change in the ratio of stearic to oleic acid was found. This ratio has a value of approximately 1:4:1 for normal liver and approximately 1:2 for the hepatoma. This ratio was found when the phospholipides were extracted from various regions of the cell, suggesting that the phosphatides may have a constant proportion of fatty acids irrespective of their position in the cell. The proportions of fatty acids differed only when cells from different tissues or different species were compared. It is, therefore, possible that van Deenen and his collaborators have detected an alteration in fatty acid composition of the phosphatides of tumor cell membranes.

Earlier evidence for a change in total tumor phospholipide was obtained by Rapport, Skipski, and Alonzo (115), who isolated a glyosphingolipide which they could not detect in normal tissues. Kasaki, Ikoda, Katani, Nakawa, and Saka (87) isolated a characteristic phospholipide from several human tumors which they could not detect in normal tissues. There is also some more indirect evidence from enzyme studies. Easty, Easty, and Ambrose (51) examined the effect of sixteen enzymes on the adhesiveness of viable ascites tumor cells to clear glass. They found that only four of these had a marked effect on the adhesiveness; these were wheat germ lipase, acid phosphatase, elastase, and trypsin. When the effect of enzymes on growing cultures of the stilbestrol-induced kidney tumor normal kidney epithelium already described were compared (15), it was found that the lipase showed the most strikingly selective action on the tumor cultures. The lipase inhibited the growth of the tumor cells at a lower concentration than that required for the normal cells. Cormack, Easty, and Ambrose (38) showed by the use of fluorescent labels that there was a characteristic difference between the reaction of the lipase with the normal and tumor-cell membranes.

Concerning the protein or mucoprotein component of the tumor cell membrane little is known at present. Taylor (128) has shown that the presence of serum or other proteins in the medium decreases the adhesiveness of tumor cells to clean glass. It is therefore probable that the adhesive material on the surface is secreted by the cells and is not adsorbed from the medium. Bryn Jones (84) has made use of a counterstaining procedure for proteins and has demonstrated a characteristic color reaction with tumors, which he considers may be due to an alteration in the state of tanning of the tyrosine residues in the surface protein.

Serological methods have provided some evidence for altered surface antigens in tumor tissue. Weiler (135) demonstrated several years ago that there was an antigenic loss in stilbestrol-induced kidney tumors of the hamster when compared with...
normal kidney tissue, using antibodies with fluorescent labels. Hughes, Louis, Duneen, and Spector (80) have shown that such effects can be produced by nonspecific reactions, but correspond to some biochemical change in the composition of the tumor cells. More recently, Nairns, Richmond, McEntegard, and Fothergill (111) have detected staining of the cell membranes in normal tissues using antibodies with fluorescent labels. This staining did not occur with homologous induced tumor tissue. Easty and Ambrose (80) obtained evidence with an anti-serum prepared against transplanted ascites tumors and adsorbed against normal tissue, for the presence of a specific reaction which appeared to take place initially with the plasma membrane of the tumor cells. Kay (88) has demonstrated the loss of a surface antigen in some human tumors, while Green (68) has proposed on the basis of his immunological studies that carcinogenesis is due to the loss of a tissue-specific lipoprotein antigen, present in the endoplasmic reticulum and possibly in the plasma membrane.

The biochemical characterization of the tumor cell membrane and surface must await the development of more refined physicochemical and biochemical techniques. When isolated and uncontaminated plasma membranes have been separated from a number of tumor cells and their normal counterparts and subjected to biochemical analysis, it may then become possible to relate the altered biological properties of the tumor cell surface to actual changes in metabolic pathways within the cell.

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