Blut, Band XXIV, Seite 388–397 (1972)

From the Department of Radiotherapeutics, University of Cambridge
Addenbrooke's Hospital, Trumpington Street, Cambridge

Surface Molecular Components of Ehrlich Ascites Tumour Cells

By Jitendra N. Mehrishi

Neoplasia seems to involve change in the properties of some cells and alterations in the cell surface components may be an important aspect of the differences between normal and cancer cells.

The occurrence of tumour specific antigens in experimental systems (*Klein* (1966); *Old,* and *Boyce* (1964); *Sjögren* (1965) [*27,44,52*]) suggests a change in the structure and presumably the chemical composition of the cell membrane. Neoplastic transformation of liver cells is accompanied by alterations in the antigenic composition of the cell surface (*Baldwin,* and *Moore* (1969) [*3*]). Histocompatibility antigens are located on the cell surface and there is evidence that only these antigens that are exposed at the cell surface are responsible for the host response leading to cellular damage. The isolation of a complex containing the tumour specific agglutination sites and studies on its location on the surface have been described [*9*]).

Many of the distinctive properties of neoplasm, such as local invasion, metastases and loss of contact inhibition are believed to depend on changes in the tumour cell membrane. These appear to be governed by the density of the antigen sites and the charge on the surface (reviews, see *Abercrombie* and *Ambrose* (1962); *Klein* (1970) [*1,26*]). Attachment of antibodies to the cell surface has been demonstrated by immunofluorescence (*Klein* and *Klein* (1964); *Lejneva* et al. (1963); *Pasternak* 1965) [*25,29,46*]). The adhesive material on the cell surface contains protein and antibodies-like protein on the surface of Ehrlich ascites tumour cells (EAT) has been detected (*Thunold* (1968) [*55*]). An analysis of the products of proteolytic digestion of EAT cells has shown that surface glycoprotein is associated with the cell membrane (*Cook* (1968); *Langley* and *Ambrose* (1967); *Molnar* et al. (1969) [*13,28,43*]).

It has been demonstrated in vitro that the destructive effects of sensitized lymphocytes can be prevented when cell surface antigens are coated with specific antibodies (*Perlmann* and *Holm* (1969) [*47*]). Cytotoxic action of lymphocytes on tumour cells can be prevented when cell surface antigens are coated with specific antibodies. If the antibodies do not kill the cells, they may block the antigen sites which are not accessible to the lymphocytes for forming an attachment (*Dumonde* et al. (1969); *Maini* et al. (1969); *Perlmann* and *Holm* (1969) [*16,30,47*]). The role of the cell surface in immunology is now generally accepted (*Klein* (1970) [*26*]): since antibodies (γ-globulins) are of large molecular weight and do not penetrate the cell membrane barrier, their effects are mediated via the interaction of the cell surface components. The exact mechanism of immunological damage is not known (*Perlmann* and *Holm* (1969) [*47*]). In particular a molecular basis for many of the reactions taking place on the surface of tumour cells has been lacking. For a direct insight into the nature of the receptors and surface components more information is necessary about the

Eingegangen am 16. 2. 1972.

surface proteins/peptides which maintain the cell membrane enzyme system(s), membrane integrity and govern cellular interactions.

The aim of my studies has been to obtain information on the surface molecular components of tumour cells (*Mehrishi* (1970a); *Mehrishi* and *Grassetti* (1969) [*37,38*]). The present paper presents data on the number of various types of groups on the surface of EAT cells on a per cell basis and their relative contribution to the electrokinetic charge/profile. The arrangements for the possible distribution of the groups in the cell periphery have been proposed. Although the EAT cell as the model chosen for this study may have limitations, it is hoped that the methods developed would be of some practical use in the characterization of some other cell types.

A combination of the biochemical and biophysical methods with electron microscopy on cells with blocked groups and other serological studies would hopefully provide answers about the topochemistry of the cell surface. Such information is essential for a better understanding of the highly specific biological phenomena involving the cell surface.

Methods and Processing of the data

Information about the surface regions of single cells in suspension can be obtained by cell electrophoresis. Electrophoretic examination of the chemically-modified cells, i.e., cells with surface group blocked after treatment with mild, specific agents, can yield information on the nature and the number of such groups in the cell periphery. In the 1960s electrokinetic studies on EAT cells had shown that the cells exhibited a net negative charge which was attributed to α-carboxyl groups of N-acetylneuraminic acid (pK_a 2.6) (*Cook* et al. (1962); *Ruhenstroth-Bauer* et al. 1961); *Wallach* and *Eylar* (1961) [*14,48,56*]). The pH mobility relationship suggested the presence of other ionizing groups as well-groups of pK about 10 and unidentified acidic acid groups (pK 3.5 to 4.0) (*Cook* et al., 1962 [*14*]). Ribonuclease susceptible phosphate groups also contribute to the electrokinetic charge (*Mayhew* and *Weiss* (1968) [*32*].

The characterization of the surface groups of high pK values (above 9, such as amino and sulphydryl) had proved extremely difficult because of the lack of suitable reagents (*Mehrishi* (1967), *Mehrishi* (1970c) [*33,37*]). The presence of -SH (*Mehrishi* and *Grassetti* (1969) [*38*]) and amino groups (*Mehrishi* (1970c) [*37*]) has now been established by reversible physicochemical studies on live intact cells. Such cells were treated at room temperature with mild specific and sensitive reagents at or near physiological pH and ionic strength (experimental time 10 to 20 min.). (2,3-dimethylmaleic anhydride and 6,6′-dithiodinicotinic acid were for the reversible blocking of the amino groups (*Mehrishi* (1970c) [*37*]) and sulphydryl groups (*Mehrishi* and *Grassetti* (1969) [*38*]) respectively.) It is necessary to emphasize that the interpretation of the electrokinetic data on cells treated with harsh reagents such as aldehyde or reagents at very high or low pH values is difficult and often suspect (Review *Mehrishi* (1970c) [*37*]).

Results

The electrokinetic method determines the net electron charge density. Once the contribution of the positively charged amino groups to the net charge was known (*Mehrishi* (1970c) [*37*]) the actual number of electron charges per unit surface area (μm^2) or per tumour cell surface area could be calculated (*Mehrishi* (1970c) [*37*]). For the purposes of this calculation the cell was assumed to have a spherical shape. Because of the wide variation in the size of the EAT cell, a median value of 900 μm^2 (range 600 to 1200 μm^2) was used for the calculations (*Mehrishi* (1969) [*34*]). The

numbers of amino, α-carboxyl groups of NANA, phosphate and -SH groups per cell surface area are given in Table 1. Since the calculated electron charge on the EAT cell surface exceeds the sum of the electron charge due to NANA and phosphate groups, the remainder of the electron charges are attributed to other unidentified anionogenic groups, including weak acidic groups of pK ca. 3.5 to 4.0 (Table 1). The relative densities of the various surface groups are represented by a histogram plotted in arbitrary units.

Charges or Groups	Number per cell $\times 10^7$	Area per Group or Charge Å^2	Av. distance between Neighbouring Groups Å	Ref.	Reference (Data processed from)
1. Total Electron Charges	8			(37)	Mehrishi (1970)
2. Electron Charges contributing to the Electro-kinetic Charge	6.88			(37)	Mehrishi (1970)
3. α-carboxyl (Neuraminidase-susceptible-N-acetyl-neuraminic acid)	2.35	3830	61.9	(14)	Cook, Heard & Seaman (1962) (cf. 37)
4. Phosphate Groups (RNase-susceptible)	1.19	7330	86.9	(32)	Mayhew & Weiss (1968)
3. Positively Charged Amino Groups	1.17	7700	87.6	(37)	Mehrishi (1978)
16. Unidentified anionogenic Groups	3.91	2300	48	(37)	Mehrishi (1970)
7. -SH Groups	3.69	2440	49.4	(38)	Mehrishi & Grassetti (1969)

Table 1: Cell Surface molecular Mosaic. Groups/Charges on the Surface of Ehrlich Ascites Tumour Cells. (Camb. Univ. Dept. of Radiotherapeutics strain.)

Discussion

Research into the structure, composition and function of cell membranes is of considerable interest in medicine and biology. At present increasing attention is also being paid to the study of the cell surface in connection with transformation of cells by **(I)** oncogenic viruses (*Berwald* and *Sachs* (1965); *Borek* and *Sachs* (1966); *Borek* and *Sachs* (1967); *Borek* and *Sachs* (1968); *Burger* (1969); *Burger* (1970); *Burger* and *Noonan* (1970); *Dulbecco* and *Eckhart* (1971); *Inbar* and *Sachs* (1969); *Sachs* (1966); *Shevliaghyn* and *Karazas* (1970); *Stoker* (1968); **(II)** carcinogens (*Berwald* and *Sachs* (1965); *Sachs* (1966) and **(III)** X-irradiation (*Borek* and *Sachs* (1966); *Borek* and *Sachs* (1967); *Borek* and *Sachs* (1968); *Sachs* (1966) [*5,6,7,8,10,11,12,15,24,49,50,53*]).

In view of the information on the chemical nature of the surface molecular components (Table 1), let us consider their importance in some cell membrane properties and function.

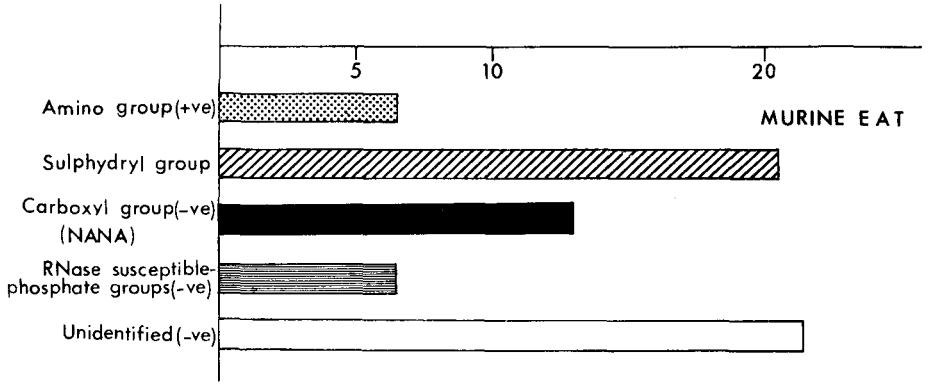

Fig. 1: Relative densities of the five different types of groups present in the cell periphery. The heights of the columns have been drawn in arbitrary units for a qualitative comparison, enough to demonstrate the relative contributions of the various groups to the electrokinetic charge.

Filled in	: α-carboxyl groups of neuraminidase-susceptible N-acetylneuraminic acid (—ve) [*14*].
Cross-hatching	: -SH group (negligible contribution of physiol. pH) [*38*].
Vertical lines	: Phosphate groups (—ve) (Ribonuclease-susceptible) [*32*].
Dots	: Amino groups (+ve) [*37*].
Blank	: Unidentified weak acidic groups (—ve) [*37*].

The number of cell surface groups were calculated from the data given in the reference numbers given in brackets (also see Table 1).

Significant contribution of the radiobiological effects of ionizing radiation due to cell membrane damage has been proposed (*Alper* (1972); *Bacq* and *Alexander* (1961); *Flemming* et al. (1968); *Mehrishi* (1970a) [*2,19,35*]). The integrity and the function of the cell membrane is believed to be dependent on the content and the orientation of the membrane -SH groups. Indirect evidence has been obtained in support of the postulate that some of the primary targets of ionizing radiation in the cell are certain thiol groups (*Barron* (1950); cf. *Eldjarn* and *Pihl* (1968); *Gronow* (1965a) [*4,17,21*]). Therefore the information about the cell surface -SH groups is important in radiobiological studies, particularly because of its relevance to radiosensitization and radioprotection. Cell surface -SH groups have been suggested to be the target of biochemical damage of the cell membrane following irradiation of the cell as reflected in changes in the cell membrane permeability to potassium ions (*Flemming* et al. (1968); *Mehrishi* (1970a); cf. *Sutherland* et al. (1967) [*19,35,54*]).

The role of -SH groups in radiosensitization by 2-methyl-1,4-naphthaquinol bis(disodium phosphate) (MNDP-Synkavit, Roche Products Ltd.,) should also be considered (rev. *Mitchell* (1971); *Mitchell* (1960); *Mitchell* (1967) [*39,40,41*]). *Mitchell* and his collaborators have studied the radiosensitizing properties of MNDP (*Marrian*

et al. (1961); *Mitchell* (1971); *Mitchell* (1960); *Mitchell* (1967); *Mitchell* and *Marrian* (1965); *Gronow* (1965b); *Gronow* (1965a); *Flemming* et al. (1968); *Mehrishi* (1970b). *Mitchell* (1960); (1967) [*19,21,22,31,36,39,40,41,42,*]) has suggested that in vivo the active principal of MNDP is menadione which is formed as a result of dephosphorylation and oxidation. In view of the ease of the reaction of naphthaquinones with -SH compounds, he has postulated that menadione, formed in or on entering cells, could react with vital -SH groups. During discussions, Professor J. S. *Mitchell* has pointed out that the biological effects do not depend solely on the reaction with -SH groups, though this reaction seems to play some part.

The radiation chemistry of the nitrogen-containing compounds in aqueous media is frequently determined by the neighbouring substituent groups. Under certain conditions, however, the reactivity of the nitrogen locus is manifested as the characteristic radiation-chemical property (for example, see *Garrison* (1964) [*20*]). Elimination of the surface amino groups by ionizing radiation should also be considered. Oxidation of surface -SH groups and amino groups could cause spatial rearrangement and changes in the cell membrane property and function. Combination of chemicals with the cell surface -SH and amino groups could alter the cell membrane characteristics (*Pardee* (1964) [*45*]) to modify radiation damage leading to radioprotection or radiosensitization. It is hoped that studies on irradiated cells, with the surface amino and -SH groups blocked, will throw some light on the mechanisms of the action of ionizing radiations on the tumour cell membrane and the surface enzyme system(s) which govern the cation transport across the cell membrane and membrane integrity (*Mehrishi* (1970a) [*35*]).

Change in the properties of some cells is thought to be the primary cause of cancer and alterations in the cell surface may be an important aspect of the differences between normal and cancer cells.

Striking changes which accompany the transformation of cultivated cells by oncogenic viruses and which can be detected by agglutination tests with plant agglutinins are of considerable interest (e.g. see *Burger* (1969); *Burger* (1970); *Burger* and *Noonan* (1970); *Dulbecco* and *Eckhardt* (1971); *Inbar* and *Sachs* (1969); *Shevliaghyn* and *Karazas* (1970) [*10,11,12,15,24,50*]). The exposure of agglutinin sites or some concomitant change in the cell surface molecular components may well play an integral part in the establishment and/or maintenance of neoplastic transformation. Transformation by the small DNA viruses SV40 and polyoma, the genomes of which specify probably less than ten proteins, results in surprisingly complex changes in the surface antigens of the transformed cells. It is believed that SV40 and polyoma viruses somehow induce the specific uncovering of antigenic components which are present in the membranes of untransformed cells but are masked and are inaccessible to the immune system.

Membrane protein biosynthesis is under direct genetic control and the gene DNA carries the information necessary to code for a given primary amino acid sequence. Any changes in the amino acid sequence of the cell surface proteins/peptides and any intrinsic difference between the surface of different cells could give valuable information about the genetic material controlling the synthesis of the cell surface proteins. Changes which result in modified antigenicities would clearly determine the nature of interaction between tumour cells and their hosts.

It would be of considerable interest to establish if the cell surface molecular components of populations of normal and transformed cells could be used to distinguish one type of cell from another. It is hoped that the methods of reversible blocking of the surface groups will be useful in such studies.

Changes in the cell surface have also been suggested to be the basis for several characteristics of cancer cells, like the invasive properties, loss of contact inhibition and metastases (*Abercrombie* and *Ambrose* (1962); *Pardee* (1964); *Weiss* (1967) [*1,45, 57*]). The invasiveness and decreased adhesiveness of tumour cells (and other properties), at least in some systems, were attributed to the high electron charge density (anodic electrophoretic mobility, zeta potential) of tumour cells (Rev. *Abercrombie* and *Ambrose* (1962) [*1*]).

However, electrokinetic studies on embryonic cells (*Heard* et al. (1961) [*23*]), regenerating liver cells (*Eisenberg* et al. (1962) [*18*]), a small range of mouse normal and tumour cells (*Simon-Reuss* et al. (1964) [*51*]), TA3 ascites tumour cells in different strains of mice (*Weiss* and *Hauschka* (1970) [*58*]) have not shown any correlation between the surface charge density of cells and 'malignancy'.

Furthermore, the negative charge of the tumour cell membrane increased to high values with both the polyanions poly I. poly C. (tumour inhibitory) and chondroitin sulphate (tumour growth promoting (*Mehrishi* (1970b) [*36*]. It was therefore necessary to emphasize that great caution in the interpretation of the electrokinetic data on cells must be exercised; the concept of charge *alone* is not sufficient to explain all the phenomena observed [*Mehrishi* (1970b) [*36*].

Cell surface topochemistry

Assuming that the five types of groups are uniformly distributed over the entire cell surface area, a figure for the effective area of a group was obtained (Table 1 column 3). The square root of this figure was taken as the minimum average distance between two neighbouring groups of the same type (Table 1, column 4). These groups may then be present at the corners of squares or equilateral triangles (hexagonal packing) with the sides of the appropriate calculated dimensions (Table 1, column 4). In Fig. 2 only the half of the cell surface area is shown: the top half of the picture shows the groups arranged at the corners of equilateral triangles and in the bottom half of the picture the groups have been placed at the corners of squares. One unit in the figure represents a cluster of 10^6 groups.

So far no evidence has been obtained to suggest a preference for either of the two models (Fig. 2). It is also possible that the cluster of groups on the surface membrane appear in patches with a certain pattern in the distribution of the groups *within* the cluster. In considering the models proposed, the very serious limitations of the assumptions and the experimental approach must not be forgotten.

Note added in proof

Recently, similar speculative distribution of anionic sites on the surface of Ehrlich ascites cells has been proposed [*69,71*]. This is based on electrokinetic and electron microscopic study of the negatively charged sites at the surface of cells with and

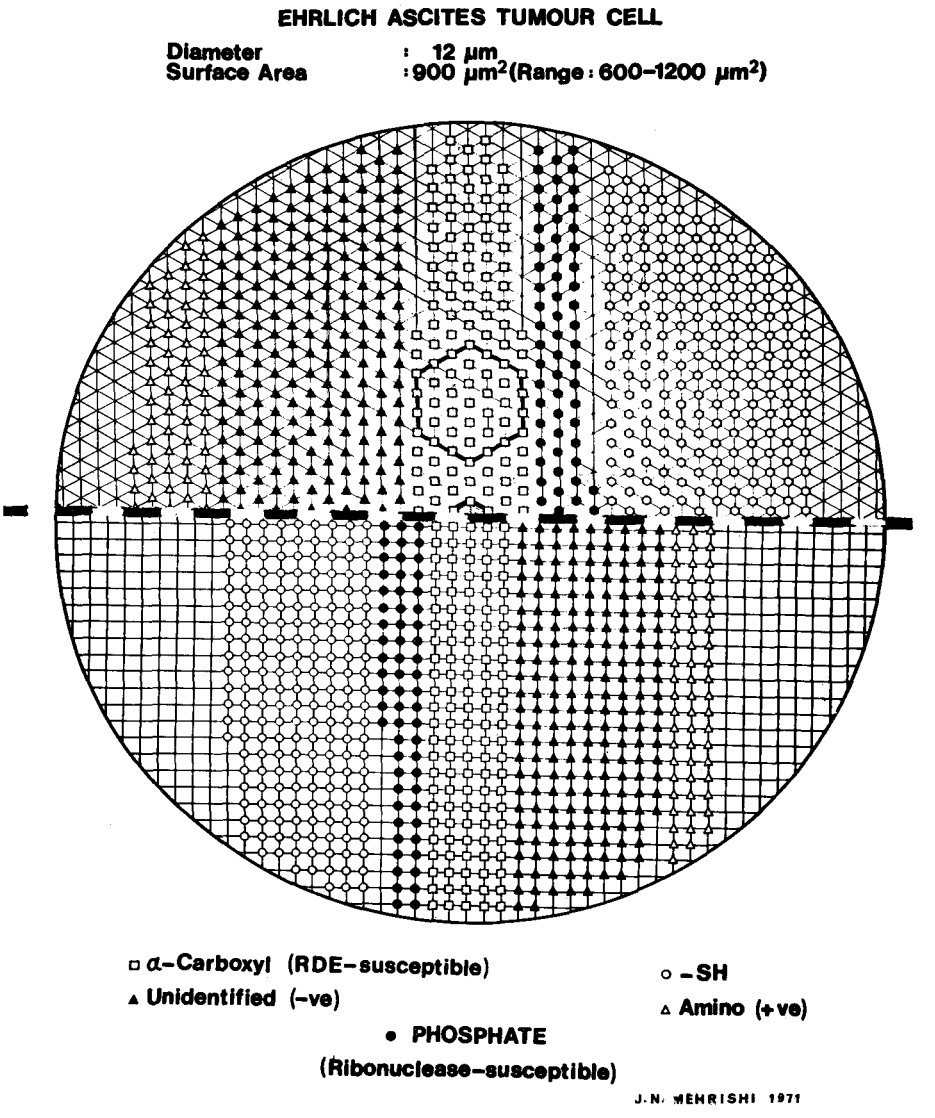

Fig. 2: The cell is assumed to have a spherical shape with a diameter of 12 μm and a median value of the surface area of 900 μm² (range 600 to 1200 μm²). For a schematic representation of the models, it is adequate to show only half of the cell surface. The top half of the picture shows the half of the cell surface area with the groups arranged at the corners of equilateral triangles (hexagonal packing) and the lower half of the picture shows the half of the cell surface area with the groups arranged at the corners of squares.

Squares	: α-carboxyl groups of neuraminidase-susceptible N-acetylneuraminic acid [*14*].
Open Circles	: -SH groups [*38*].
Closed Circles	: Phosphate groups (—ve) (Ribonuclease-susceptible) [*32*].
Open Triangles	: Amino groups (+ve) [*37*].
Filled in Triangles	: Unidentified (—ve) [*37*].

without prior incubation with neuraminidase and (or) ribonuclease and fixation with glutaraldehyde. Subsequently, the cells were treated with net positively charged colloidal iron oxide particles and processed for electron microscopy.

The authors postulate that the enzyme-sensitive cell surface anionic sites are arranged in higher-than-average charge density clusters and that the anionic sites not susceptible to these two enzymes, were spread more homogeneously over the cellular electrokinetic surface, see also *Mehrishi* [*62*] and [*60,61*] for other cell types.

In the case of erythrocytes, the neuraminidase-susceptible sialic acids at the surface are arranged in clusters having a higher-than-average charge density and *Weiss* et al. (1972b) [*70*] postulate that the clusters are distributed uniformly over the cell surface 60. Based on studies on erythrocytes treated with anti-A, anti-B and anti-D sera *Sachtleben* and *Ruhenstroth-Bauer* [*66,67*] and *Sachtleben* [*64*] had also proposed that on the cell surface there were areas of higher-than-average charge density.

However, it must be pointed out that electrokinetic data on enzyme-treated cells must be treated with great caution [*68,62,65*]. Some studies on the effect of anti-neuraminidase sera (Ab-RDE) on erythrocyte mobility recently concluded, would appear to indicate that the reduction in the mobility of cells treated with neuraminidase may not *only* be due to the release of N-acetyl-neuraminic acid (NANA) from the cell periphery, but also because of the enzyme "anchored" on the cell surface masking some surface sites (*The Umbrella Effect*) [*65*].

These studies have shown that a major portion of the anchored enzyme can eventually be removed by treatment with Ab-RDE, resulting in the exposure of the masked sites and an *increase* in the mobility of neuraminidase-treated cells [*65*].

Usually, more NANA is released after treatment of cells than can be accounted for from electrophoretic measurements [*68,59*]. *The Umbrella Effect,* in part, may account for the lack of correlation in such studies [*65*].

The remarkable effectiveness of glutaraldehyde as a protein cross-linking reagent which leads to the irreversible protein modification, possible disordering, partial loss of lysine and the expected interaction with amino groups (Table 1) should also be borne in mind [*63*].

I should like to thank Professor J. S. *Mitchell* for continued support and discussions, Mr. D. *Juett* for writing the computer (TITAN) programmes (Cambridge Multiple Access System) which eased the tedium of handling an enormous number of calculations, and Dr. N. *Taptiklis* for helpful criticisms. I also thank Mrs. Susan *Howroyd* for help with the calculations and preparation of the models, and the Cancer Research Campaign for financial support.

Summary

On the surface of Ehrlich ascites tumour cells various types of ionizable groups are present -sulphydryl groups, positively charged amino groups and at least three types of anionogenic groups.

The numbers of the surface groups has been calculated on a per cell basis and their relative contribution to the electrokinetic make up determined.

Two possible arrangements for the distribution of the groups in the cell periphery have been proposed.

The importance of the surface groups in radiobiology and the other associated phenomena involving the cell surface membrane has been considered. The difficulties of the interpretation of the electrokinetic data concerning the role of the surface charge as a basis for malignancy have been pointed out. Since the surface charge of tumour cells can be increased to high negative values by tumour growth promoting and tumour growth inhibiting polyanions, extreme need of caution in the interpretation of the data has been emphasized.

Zusammenfassung

Auf der Oberfläche der Ehrlich-Aszites-Tumorzellen findet man verschiedene Typen ionisierbarer Gruppen (Sulphydryl-Gruppen) positiv geladene Aminogruppen und mindestens drei Typen negativ geladener Gruppen.

Die Zahl der Oberflächengruppen wurde an Hand der Zellbasis und ihrer relativen Zuteilung zur elektrokinetischen Struktur festgestellt. Es wurden zwei Möglichkeiten vorgeschlagen, die Gruppen in der Zellperipherie zu verteilen.

Die Bedeutung der Oberflächengruppen in der Radiobiologie und anderer Phänomene, die an der Oberflächenmembran beteiligt sind, wurde erörtert. Außerdem wurde die Schwierigkeit der Interpretation der elektrokinetischen Daten, die die Bedeutung der Oberflächenbeteiligung am Malignen betreffen, betont. Da die Oberflächenbeteiligung bei Anwendung von Tumorwachstums- oder hemmungspolyanionen zu hohen negativen Werten anwachsen kann, ist äußerste Vorsicht geboten.

References: 1 Abercrombie, M. and E. J. Ambrose: Cancer Res. *22,* 525 (1962). — 2a Alper, T.: Proc. Int. Atomic Energy Agency, Vienna, 171 (1971). — 2 Bacq, Z. M. and P. Alexander: Fundamentals of Radiobiology 2nd ed. Pergamon, London 1961. — 3 Baldwin, R. W. and M. Moore: Europ. J. Cancer *5,* 475 (1969). — 4 Barron, E. S. G.: Symp. in Radiobiology. J. Wiley & Sons, New York 1950. — 5 Berwald, Y. and L. Sachs: J. Nat. Cancer Inst. *35,* 641 (1965). — 6 Borek, C. and L. Sachs: Nature (Lond.) *210,* 276 (1966). — 7 Borek, C. and L. Sachs: Proc. Nat. Acad. Sci. U.S. *57,* 1522 (1967). — 8 Borek, C. and L. Sachs: Proc. Nat. Acad. Sci. U.S. *59,* 83 (1968). — 9 Burger, M. M.: Nature (Lond.) *219,* 499 (1968). — 10 Burger, M. M.: Proc. Nat. Acad. Sci. U.S. *62,* 994 (1969). — 11 Burger, M. M.: Nature (Lond.) *227,* 170 (1970). — 12 Burger, M. M. and K. D. Noonan: Nature (Lond.) *228,* 512 (1970). — 13 Cook, G. M. W.: Biol. Rev. *43,* 363 (1968). — 14 Cook, G. M. W., D. H. Heard and G. V. F. Seaman: Exp. Cells Res. *28,* 27 (1962). — 15 Dulbecco, R. and W. Eckhardt: Proc. Nat. Acad. Sci. (Wash.) *67,* 1775 (1971). — 16 Dumonde, D. C., R. A. Wolstencroft, G. S. Panayi, M. Matthew, J. Morley and W. T. Howson: Nature (Lond.) *224,* 38 (1969). — 17 Eldjarn, L. and A. Pihl: Mechanisms in Radiobiology 2. Acad. Press, New York 1968. — Eisenberg, S., S. Ben-Or and F. Doljanski: Exp. Cell Res. *26,* 451 (1962). — 19 Flemming, K., J. N. Mehrishi and J. A. F. Napier: Int. J. Radiat. Biol. *14,* 175 (1968). — 20 Garrison, W. M.: Rad. Res. Suppl. *4,* 158 (1964). — 21 Gronow, M.: Int. J. Radiat. Biol. *9,* 123 (1965). — 22 Gronow, M.: Int. J. Radiat. Biol. *10,* 103 (1965). — 23 Heard, D. H., G. V. F. Seaman and I. Simon-Reuss: Nature (Lond.) *190,* 1009 (1961). — 24 Inbar, M. and L. Sachs: Proc. Nat. Acad. Sci. U.S. *63,* 1418 (1969). 25 Klein, E. and G. Klein: J. nat. Cancer Inst. *32,* 547 (1964). — 26 Klein, E.: Europ. J. Cancer *6,* 15 (1970). — 27 Klein, G.: Ann. Rev. Microbiol. *20,* 223 (1966). — 28 Langley, O. K. and E. J. Ambrose: Biochem. J. *102,* 367 (1967). — 29 Lejneva, O. M., L. A. Zilber and E. S. Levleva: Nature (Lond.) *206,* 1163 (1963). — 30 Maini, R. N., A. D. M. Bryceson, R. A. Wolstencroft and D. C. Dumonde: Nature (Lond.) *224,* 43 (1969). — 31 Marrian,

D. H., B. Marshall and J. S. Mitchell: Chemotherapia *3,* 225 (1961). — 32 Mayhew, E. and L. Weiss: Exp. Cell Res. *50,* 441 (1968). — 33 Mehrishi, J. N.: Brit. Emp. Cancer Campn. A. R. *45,* 225 (1967). — 34 Mehrishi, J. N.: Europ. J. Cancer *5,* 427 (1969). — 35 Mehrishi, J. N.: 2nd Int. Symp. Radiosensitizing and Radioprotective Drugs 1969. In: Radiation Protection and Sensitization. Ed. H. L. Moroson and M. Quintiliani, p. 265. Taylor & Francis, London 1970. — 36 Mehrishi, J. N.: Nature (Lond.) *228,* 364, 375 (1970). — 37 Mehrishi, J. N.: Europ. J. Cancer *6,* 127 (1970). — 38 Mehrishi, J. N. and D. R. Grassetti: Nature (Lond.) *224,* 365 (1969). — 39 Mitchell, J. S.: Cancer, if curable, why not cured? W. Heffer & Son Ltd., Cambridge, 1–134 (1971). — 40 Mitchell, J. S.: Studies in Radiotherapeutics. Blackwell Sci. Public., Oxford 1960. — 41 Mitchell, J. S.: Deutscher Röntgenkongreß München, Teil 3, Urban & Schwarzenberg, München 1967. — 42 Mitchell, J. S. and D. H. Marrian: Biochemistry of Quinones, p. 503, Ed. R. A. Morton, Academic Press, London 1965. — 43 Molnar, J., G. Markovic and H. Chao: Arch. Biochem. Biophys. *134,* 524 (1969). — 44 Old, L. J. and E. A. Boyce: Ann. Rev. Med. *15,* 167 (1964). — 45 Pardee, A. B.: Cell Division. Nat. Cancer Inst. (monograph) *14,* 7 (1964). — 46 Pasternak, G.: Serologic studies on cells of Graffi virus-induced myeloid leukaemia in mice. — 47 Perlmann, P. and C. Holm: Adv. Immunol. *11,* 117 (1969). — 48 Ruhenstroth-Bauer, G., W. Kübler, G. P. Fuhrmann und F. Rueff: Klin. Wschr. *39,* 764 (1961). — 49 Sachs, L.: In: Molekulare Biologie des malignen Wachstums, 242–253. Springer, New York 1966. — 50 Shevliaghyn, V. J. and N. V. Karazas: Int. J. Cancer *6,* 234 (1971). — 51 Simon-Reuss, I., G. M. W. Cook, G. V. F. Seaman and D. H. Heard: Cancer Res. *24,* 2038 (1964). — 52 Sjögren, H. O.: Progr. exp. Tumour Res. *6,* 289 (1965). — 53 Stoker, M.: Nature (Lond.) *218,* 234 (1968). — 54 Sutherland, R. M., A. Rothestein and R. I. Weed: J. Cell. comp. Physiol. *69,* 185 (1967). — 55 Thunold, S.: Transplantation *6,* 716 (1968). — 56 Wallach, D. F. H. and E. H. Eylar: Biochim. Biophys. Acta *52,* 594 (1961). — 57 Weiss, L.: The Cell Periphery, metastasis and other contact phenomena. North Holland, Amsterdam 1967. — 58 Weiss, L. and T. S. Hauschka: Int. J. Cancer *6,* 270 (1971). — References for Note added in proof. — 59 Kraemer, P.: J. Cell Biol. *33,* 197 (1967). — 60 Mehrishi, J. N.: Thrombos. diathes. haemorrh. *26,* 370 (1971). — 61 Mehrishi, J. N.: Int. Arch. Allergy *42,* 69 (1972a). — 62 Mehrishi, J. N.: Prog. Biophys. Mol. Biol. *25,* 1 (1972b). — 63 Richards, F. M. and J. R. Knowles: J. Mol. Biol. *37,* 231 (1968). — 64 Sachtleben, P.: Hämatol. und Bluttransf. *8,* 126 (1969). — 65 Sachtleben, P., R. Gsell and J. N. Mehrishi: In preparation (1972). — 66 Sachtleben, P. and G. Ruhenstroth-Bauer: Med. exp. *6,* 183 (1962a). — 67 Sachtleben, P. and G. Ruhenstroth-Bauer: Med. exp. *6,* 226 (1962b). — 68 Seaman, G. V. F. and G. Uhlenbruck: Arch. Biochem. Biophys. *100,* 493 (1963). — 69 Weiss, L. and R. Zeigel: J. theor. Biol. *34,* 21 (1972). — 70 Weiss, L., R. Zeigel, O. S. Jung and I. D. J. Bross: Exp. Cell Res. *70,* 57 (1972a). — 71 Weiss, L., O. S. Jung and R. Zeigel: Int. J. Cancer *9,* 48 (1972b).

Author's address: Dr. J. N. Mehrishi, Present address for correspondence *only,* and not for reprint requests: Max-Planck-Institut für Biochemie, 8 München 2, Goethestr. 31.

Reprint requests to be addressed to the Secretary Miss V. Bevis, Dept. of Radiotherapeutics, Hills Road, Cambridge (England).