during this period. Addition of GA causes the lag phase to be shortened or to disappear completely. The final number of cells in the culture containing GA is doubled.

We calculated generation time from the slope of the logarithmic phase of the growth curve. Cell numbers were determined with a Coulter counter which also provided a histogram of cell sizes. The mean generation time of *G. breve* is 6.6 days. The mean generation time and cell size (Fig. 2) of experimental and control are not significantly different. Thus the main effect of GA appears to be elimination of the lag period and increase in the final yield of cells. The optimum concentration of GA causing this effect was 10^{-7} mole/liter, whereas higher concentrations (above 5×10^{-7} mole/liter) markedly inhibited the effect.

The use of *G. breve* as a homogenous unicellular cell population for studies on the mode of hormone action has several advantages over use of complicated multicellular plant systems with extensive morphological differentiation.

In nature *Gymnodinium breve* blooms periodically during the year, but blooms in the laboratory have been elusive. If GA can be used to effect algae blooms in the laboratory, we would suggest that the level of GA in seawater may be one of the causes for the bloom. A possible source of GA could be debris of higher plants washed to sea and marine algae, both of which contain GA in their tissues (*12*). The possibility exists that the effect of GA on *Gymnodinium breve* results from the same mechanism as that in higher plants (*13*); however, the mode of action is unknown.

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Cell Surface Coatings and Membrane Potentials of Malignant and Nonmalignant Cells

Abstract. *A positive surface potential indicating a cell coating is common to malignant cells, lymphocytes, and normal and malignant trophoblastic cells. This characteristic was not generally found for other normal cell types tested by microelectrode penetration.*

Studies of electromobility show that malignant cells and trophoblastic cells of the placenta possess a highly charged surface constituent (*1*). This predominate surface negativity is attributed primarily to sialic acid moieties of mucopolysaccharides on the cell exterior. Treatment of the sialic acid groups with neuraminidase causes reduction of the characteristically high electromobility rates of these cells to near the rates for normal cells (*2*). Lymphocytes also possess a high negative surface charge (*1*, *3*). The distortion of normal cell interaction due to a similar surface charge on malignant cells, trophoblast, and lymphocytes may mediate the lack of immune rejection of trophoblastic cells during implantation in pregnancy and of malignant cells in cancer. On the other hand, the lack of such a coating and charge characteristic on transplanted cells of normal organs would permit the expected cellular reaction and rejection.

We have studied the electrophysiological properties of representative malignant cells and compared them to those of normal cells. The finding of an electrically charged sialomucin cell coating (similar to placental fibrinoid) on the surface of cancer cells and normal and malignant trophoblastic cells suggested an investigation to determine whether these surface characteristics could be detected by penetration of single cells and bioelectrical recordings (*4*).

The various types of cells under investigation were cultured on glass cover slips and studied by microscopic observation in special holding chambers used to maintain the test cells during experimentation. The cells were immersed in tissue culture medium kept at 36°C and adjusted to the appropriate *p*H with a variable mixture of air and carbon dioxide above the medium. Microelectrodes were used to impale single cells for electrical recordings (*5*) and were found acceptable when impedance was 20 megohms or above, a value indicating an electrode tip diameter under 0.5 μm (*6*).

We first studied transmembrane potential of the malignant trophoblastic cell (BeWo) in vitro (Fig. 1). This cell line, derived from a malignant tumor of the placenta following normal pregnancy, was transplanted and maintained in the hamster cheek pouch (*7*) and later established in continuous cell culture (*8*). It performs all of the normal functions of the placental trophoblast thus far tested (*9*).

Impalement of the malignant trophoblast revealed potentials which demonstrated a distinct positive deflection preceding the normal transmembrane potential (prepotential) (Fig. 1, right). A surface constituent producing this positive deflection was therefore implicated. The magnitude of the positive prepotential ranged between 1 and 8 mv and was dependent on several factors, most notably *p*H. The higher magnitudes (5 to 8 mv) were recorded at *p*H 8, lower values (1 to 2 mv) between *p*H 7 and *p*H 6. All malignant cells studied—cervical cancer (HeLa), human laryngeal carcinoma (HEp2 line), choriocarcinoma (BeWo line), rat sarcoma (256 and L cells)—uniformly demonstrated this characteristic. These properties were also found in normal human trophoblast and lymphocytes, whereas normal kidney, embryonic heart, and lung cells rarely gave prepotential records (Table 1).

Thus the prepotential appears to be

Table 1. Comparative cell penetrations in vitro. BeWo, trophoblastic cell (choriocarcinoma); HeLa, cervical cancer; HEp2, larynx cancer; Sarcoma 256, rat sarcoma; WI-38, normal human lung.

Cell type	Prepotential (mv)	Average transmembrane potential (mv)
BeWo	+5 – +8	−35
HeLa	+2 – +7	−25
HEp2	+3 – +5	−23
Sarcoma 256	+2 – +4	−20
Normal human lymphocyte	+4 – +8	−12
Normal human embryonic heart	0	−35
Normal human embryonic kidney	0	−20
WI-38		−22
Monkey kidney fibroblasts		−27

associated with a surface constituent encountered by the electrode tip before membrane impalement. The finding of such a potential suggests the presence of a cell surface coating of an ionic nature. A surface coating was identified in the trophoblastic cells by colloidal ion staining (*10*). The positive polarity of the prepotential is of interest in comparison to the negative surface charge of these cells noted in electrophoretic studies by others (*2*).

One may visualize the malignant cell with a mucopolysaccharide coating containing terminal groups of negatively charged sialic acid moieties (*11*) (Fig. 2) as follows. The typical cell membrane forms an ionic double layer of positive and negative ions at its outer surface which establishes a potential continuum with the surrounding electrolytes. The sialomucin coating alters the ionic distribution at the surface, possibly by displacing the normal bilayer of surface ions to the outer margin of the sialomucin layer.

Similar to the normal cell surface, a reference potential is thus established between the negative terminal groups of sialic acid and the external electrolytes. With respect to this reference potential, an induced positive potential is recorded within the coating layer due to the positively charged outer bilipid cell membrane. A diagram suggesting the evolution of the pattern observed during penetration of the coated cell membrane is shown (Fig. 2). At position 1, the electrode records the base-line potential established in the external medium by the indifferent electrode (ground symbol). Position 2 shows the electrode at the membrane surface inside the coating layer. In this position, the electrode tip detects the positive potential of the outer bilipid membrane within the coating material. Position 3 shows the classical negative transmembrane potential as the electrode tip experiences the high concentration of negative charge within the cell. Withdrawal of the electrode returns the trace to base line. Occasionally a positive deflection is also seen on withdrawal; possibly it is dependent on the extent of damage produced during impalement. Accordingly, the traces of Fig. 1 are consistent with a sialomucin surface coating of a cell as diagramed in the model of Fig. 2.

Terminal groups of sialic acid significantly contribute to the surface negativity determined on malignant cells by electromobility studies (*12*). The positive prepotential recorded in our study may be more closely associated with the mucopolysaccharide substrate which takes a positive polarity when referenced to the potential continuum between its charged terminal groups and the external electrolytes. This surface layer is apparently of substantial thickness since the surface potential is detected by a microelectrode which has a tip diameter estimated at somewhat under 0.5 μm in comparison to the approximate 100-Å thickness of the bilipid cell membrane. Electron microscopic studies have shown that malignant cells have an increased surface thickness of mucopolysaccharide material identified by ruthenium red staining techniques (*13*); others have not found the thickness to be greater than normal (*14*).

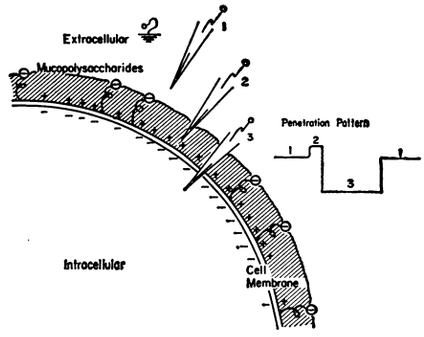

Fig. 2. Diagram of the bilipid cell membrane with a mucopolysaccharide surface coating. The potential pattern resulting from electrode penetration of the cell membrane and coating is shown.

The magnitude of the prepotentials recorded depended on the *p*H. All cells tested were examined at *p*H 6 to *p*H 8. Typically the prepotential measured 0 to 3 mv for *p*H 6 to *p*H 7.2 and rose sharply between *p*H 7.5 and *p*H 8. The transmembrane potential, on the other hand, showed small dependence on *p*H, with the largest records near *p*H 7 and approximately a 10 percent decline at extremes of *p*H. The cell types with highest prepotential values when maintained at high *p*H showed markedly increased survival as indicated by maintenance of a consistent transmembrane potential.

The results of multiple penetrations of various cell types are shown in Table 1. The prepotential values reported are those measured at *p*H 7.5 to *p*H 8.0. The transmembrane potentials were average values of multiple penetrations of given cell types recorded between *p*H 6 and *p*H 8. Notably, normal human lymphocytes demonstrated potentials before penetration similar to those found in the malignant cells. This suggests a

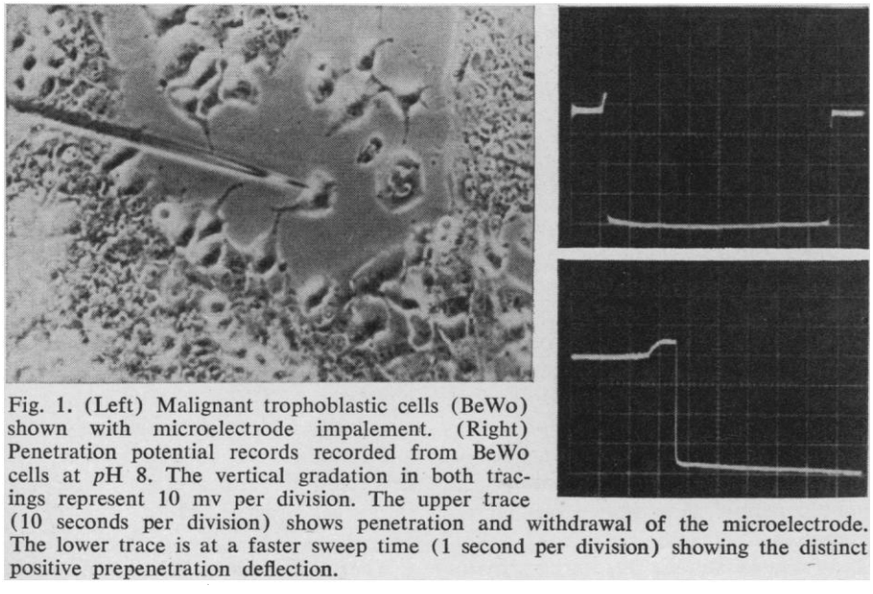

Fig. 1. (Left) Malignant trophoblastic cells (BeWo) shown with microelectrode impalement. (Right) Penetration potential records recorded from BeWo cells at *p*H 8. The vertical gradation in both tracings represent 10 mv per division. The upper trace (10 seconds per division) shows penetration and withdrawal of the microelectrode. The lower trace is at a faster sweep time (1 second per division) showing the distinct positive prepenetration deflection.

similar surface constituent. The malignant cell lines HeLa (cervical cancer), HEp2 (laryngeal carcinoma), and 256 (rat sarcoma) were investigated under comparable conditions and demonstrated similar prepotential records. On the other hand, cultures of normal human embryonic heart and embryonic kidney rarely showed detectable prepotentials; normal monkey kidney and normal human lung cells (WI-38) seldom gave prepotential records except in cases in which the cells were rounded just prior to mitosis. This may have reflected an increase in cell surface chemicals during the G_2 phase of the cell cycle and the early mitotic process (*15*). Investigation of normal trophoblast and benign molar tumors of the trophoblast, as well as the malignant trophoblast, revealed prepotentials similar to those found in the malignant cell group. This reversion of malignant cell surface characteristics to that of a primitive trophoblast suggested a common biologic denominator between normal reproduction and cancer.

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Pulmonary Surfactant and Evolution of the Lungs

Abstract. *Pulmonary surfactant has been looked for and found in 11 representatives of four vertebrate classes. The amount of surfactant, estimated by quantitative spreading as a surface film, correlates well with alveolar surface area and with the amount of saturated, mainly dipalmitoyl, phosphatidylcholine in the lung parenchyma. The quantities of other phospholipids do not correlate well with alveolar surface area.*

The area of the alveolar surface of the lungs differs greatly among vertebrate species and is related to the rate at which oxygen is demanded by the individual. This relationship, clearly shown for mammals (*1*), also obtains for some lower vertebrates, such as the frog, the turtle, and the chicken (*2*). About 4.5 m^2 of respiratory surface is apparently needed for each millimole of oxygen absorbed per minute, and the required area is provided in the more active animals by subdivision of the lung into very small air spaces. Stabilization of this structure is attributed to the presence of a phospholipid-rich surfactant which can lower alveolar surface tension nearly to zero (*3*). The purposes of this investigation (*4*) were to determine whether the quantity of surfactant present in various lungs is appropriate to their respiratory surface area and whether their lipid composition reflects such a relationship. Previous studies did not quantify the surfactant (*5*, *6*) and did not reveal functionally significant differences (see *7*, *8*) in percentage composition of the lipids.

The lungs were excised from adult animals under anesthesia (guinea pig, rabbit, mouse, dog, sea lion, rat, chicken, turtle, and frog) or obtained fresh from the abattoir (cow) and immediately chilled in crushed ice. Human lung tissue, obtained during thoracotomy for bronchiogenic carcinoma and examined for absence of pathologic changes, was similarly processed. Weighed samples of parenchyma containing as little airway tissue as possible were homogenized thoroughly with 30 times their weight of 0.9 percent sodium chloride and centrifuged at 500*g* for 5 minutes to sediment fragments of connective tissue and cartilage. A small sample of the supernatant was dispersed in twice its volume of isopropyl alcohol (*9*) for demonstration of surfactant by efficient spreading on the surface of 0.9 percent sodium chloride in a surface balance similar to that described by Harkins and Anderson (*10*). Area was 350 cm^2 initially and was decreased at a constant rate of 4.5 cm^2/sec. Measurements were made at room temperature (24° to 26°C). The area occupied by the surface film when compressed so that surface tension fell to 12 dyne/cm (*11*) was taken as a measure of the amount of surface active material (SAM) in the sample. Multiplying this area by the ratio of the volume of the homogenate to the volume of the sample and by the ratio of the weight of the lung to the weight of the tissue sample gave the area coverable by surfactant in the whole lung. Other tissues (heart, liver, kidney) did not contain the surfactant as determined

Table 1. Correlation of surfactant, pulmonary surface area, and phospholipid concentrations in vertebrate lungs. Symbols: N, number of replicate lipid analyses; PE, phosphatidylethanolamines; SPH, sphingomyelins; LPC, lysophosphatidylcholines; PC_u, phosphatidylcholines containing one or more ethylenic bonds; PC_s, phosphatidylcholines containing no ethylenic bonds; A_{sp}, specific area; SAM, surface active material; *r*, correlation coefficient; *P*, probability of correlation. Phospholipid concentrations in milligrams per gram of fresh tissue, mean values. Standard errors of means averaged 9 percent of the means. Specific area in square centimeters per gram of fresh tissue. Surface active material in square centimeters of surface coverable at 12 dyne/cm by extracted surfactant per gram of fresh tissue. Each correlation coefficient gives the correlation between the component listed above it and A_{sp}.

Animal	N	PE	SPH	LPC	PC_u	PC_s	A_{sp}	SAM
Mouse	6	5.80	2.82	0.34	10.50	6.55	2985	9500
Guinea pig	4	6.49	3.46	.68	11.20	7.95	2811	9500
Rat	12	5.23	2.76	.35	10.33	5.00	2186	6400
Rabbit	10	5.54	3.45	.39	9.60	5.20	2091	7700
Sea lion	4	2.83	2.08	.14	5.10	6.90	1600	7900
Chicken	6	5.16	2.67	.25	6.58	4.72	1590	4700
Dog	4	4.49	4.10	.37	9.90	5.20	1378	8600
Man	2	1.41	2.46	.50	3.20	3.90	1253	6700
Cow	4	5.65	4.18	.22	9.30	2.30	831	4600
Turtle	6	2.28	1.50	.14	4.59	0.31	214	440
Frog	3	3.24	1.74	.25	8.97	.63	117	190
r		.65	.39	.58	.50	.90		.87
P		$<.05$	$>.2$	$>.05$	$>.1$	$<.01$		$<.01$