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EFFECT OF NEURAMINIDASE ON ELECTROPHORETIC MOBILITY AND IMMUNE CYTOLYSIS OF HUMAN UTERINE CERVIX CARCINOMA CELLS

by

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The electrophoretic mobility of human uterine cervix carcinoma cells at pH 7.2 was found to be 0.89 μ/sec/v/cm at 25° C, while normal human cervix cells had a mobility of 0.58 μ/sec/v/cm. Treatment of the carcinoma cells with neuraminidase for 30 min reduced the electrophoretic mobility to 0.46 μ/sec/v/cm. Neuraminidase liberated more N-acetyl-neuraminic acid from carcinoma cells than from normal cells. Immune cytolysis of carcinoma cells by lymphocytes was studied by means of ^{51}Cr-release technique. Autologous and homologous lymphocytes from carcinoma cervix patients had a lytic effect on carcinoma cervix cells; and the cytolysis was increased about two-fold when the malignant cells were treated with neuraminidase. Homologous lymphocytes from healthy donors had comparatively little cytolytic action on malignant cells. Autologous lymphocytes had virtually no effect on normal cervical cells and neuraminidase treatment did not affect their susceptibility to lymphocytes. Autologous and homologous serum from cancer patients had a cytolytic influence on malignant cells; but normal serum was without effect. Although calcium chloride reduced the negative surface charge on the malignant cells, as gauged by their electrophoretic mobilities, no increase in the cytolysis of carcinoma cells by autologous lymphocytes was observed in the presence of charge-reducing concentrations of calcium chloride.

These results suggest the possibility that in the case of cervical carcinoma, sialic-acid-containing moieties partially mask tumor-specific antigens on cell surface and prevent their recognition by immunologically competent cells of the host.

Currie and Bagshawe (1967) have advanced the hypothesis that sialic acid coating of cancer cells can mask cell surface antigens, inhibiting thereby the host's immunological rejection mechanisms. Partial removal of sialic acid from tumor cells by enzymatic treatment was observed to increase their immunogenicity. Studies on these lines have so far been done mostly on animal cancers such as TA3 (Sanford, 1967), Landschutz ascites cells (Currie, 1967; Currie and Bagshawe, 1968 *a,b*) L 1210 murine leukemic cells (Bagshawe and Currie, 1968) and methylcholanthrene-induced sarcoma in mice (Currie and Bagshawe, 1969). Similar observations are lacking on human malignant cells. We have studied human cervical carcinoma cells. Their electrophoretic mobilities have been determined before and after treatment with highly purified neuraminidase, and the cytolytic action of autologous and homologous lymphocytes on carcinoma cells has been studied in relation to the enzymatic removal of sialic acid from enzyme-accessible sites.

MATERIAL AND METHODS

Preparation of carcinoma cells

Punch biopsy specimens of human uterine exocervix carcinomas were collected in Eagle's

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Minimum Essential Medium. Only patients in clinical stages II and III were included in these studies. Histological confirmation of epidermoid carcinoma was obtained in all patients. The biopsy material, after being washed free of debris and blood clots, was chopped into fine pieces with scissors. The cells were teased out with needles and by mild trituration of the tissue with a glass manual homogenizer. The resulting suspension was filtered through a wire mesh gauze, centrifuged at $150 \times g$ for 10 min, and resuspended in Eagle's medium. The cell concentration was adjusted to 2×10^6 cells/ml.

Preparation of normal cervical cells

Non-cancerous cervical tissue was obtained from total hysterectomy specimens, where surgery was done for non-malignant conditions such as fibroma and dysfunctional uterine bleeding. Cell suspensions were prepared by the procedure described for the carcinoma cells.

Neuraminidase

Crystalline enzyme from *Clostridium perfringens* (Type V) (1 unit of enzyme liberates 1 μ mole of N-acetylneuraminic acid per min or 4,500 μg per 15 min) was obtained from Sigma Chemical Co., St. Louis, Mo. USA. The enzyme needs no calcium ions for activation (Cassidy *et al.*, 1965). One unit of the enzyme was dissolved in 10 ml of isotonic saline (0.85% NaC1) buffered with 50 mM acetate buffer of pH 5.5. The activity of *Cl. perfringens* neuraminidase decreases considerably at pH 7.2 (Cassidy *et al.*, 1965). The enzyme was free of proteolytic activity.

Neuraminidase treatment of cells

To 0.5 ml of carcinoma or normal cervical cell suspension in Eagle's medium, 0.5 ml of neuraminidase solution was added and the mixture incubated for 30 min at 37° C. The cells were then washed four times with Eagle's medium. The viability of cells was tested by Trypan Blue exclusion.

As a control, 0.5 ml of the original carcinoma cell suspension was incubated for 30 min at 37° C with an equal volume of acetate-buffered saline at pH 5.5. Since both enzyme-treated and untreated cells were processed in an identical manner, the effect of pH and suspending medium will be reflected in both control and test material.

Labelling of target cells

To 0.5 ml of cell suspension containing 1×10^6 cells, 30 μc of ^{51}Cr-sodium chromate (specific activity, 50-200 μc/μg of chromium (Bhabha Atomic Research Centre, Bombay, India) were added and the mixture incubated at 37° C for 1h, at which time plateau labelling was observed (Fig. 1A). After centrifugation, the pellet was washed five times with Eagle's medium containing nonradioactive sodium chromate. The final cell suspension was adjusted to 2×10^5 viable cells/ml of Medium-199.

Preparation of lymphocytes

20 ml of heparinized blood was collected from normal subjects or cervical cancer patients, and lymphocytes were prepared by the usual procedures (Holm *et al.*, 1964). Lymphocytes were suspended in Medium-199 to a concentration of 2×10^7 cells/ml.

Cytotoxicity test

The procedure was adapted from Brunner *et al.* (1968). 0.5 ml of the ^{51}Cr-labelled cells (enzyme-treated/untreated) was incubated with 0.5 ml of lymphocytes (of cancer patients/normal subjects) at 37° C for 12-15 h. The ratio of cancer/normal cells to lymphocytes was 1:100. At the end of the incubation, 1 ml of Medium-199 was added and the suspension shaken and centrifuged at $150 \times g$ for 10 min. One ml of the supernatant was removed and measured for radioactivity in a Nuclear Chicago well-type sodium iodide scintillation counter. The counts were multiplied by two to correct for the total volume of the mixture taken. The percentage of chromium release, which is the measure for percentage of cytolysis of malignant cells, was calculated as follows:

$$\frac{\text{Counts per min (CPM) of } ^{51}\text{Cr released in the supernatant}}{\text{CPM of total } ^{51}\text{Cr incorporated into the cells}} \times 100$$

Cytolysis as measured by chromium release gives results parallel to those obtained with dye-exclusion tests (Sanderson, 1965). Brunner (1968), in his studies on mastocytoma, showed that immune cytolysis was completed within 9 h. In our experiments, the ^{51}Cr release attained almost a plateau level of 9 h; but the incubation period was fixed at 12 h (Fig. 1B). Cytolytic ability of lymphocytes is dependent on the target cell-lymphocyte ratio.

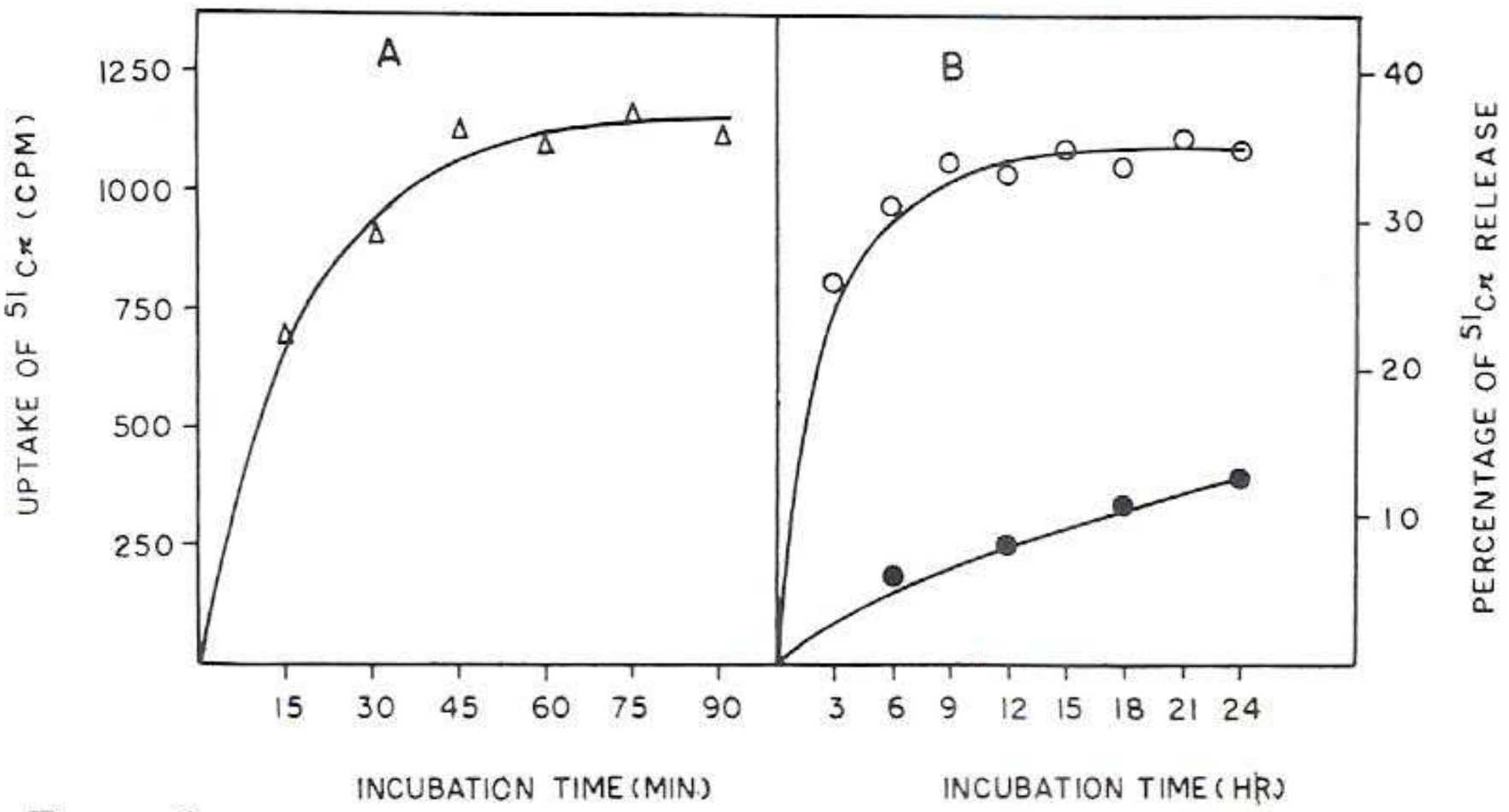

FIGURE 1

A. Time curve of chromium uptake by cervical carcinoma cells when 1×10^6 cells were incubated with 30 μC of ^{51}Cr at 37° C.

B. Time curve of release of chromium from tagged carcinoma cells.

●———● Percentage of chromium released spontaneously from carcinoma cells when lymphocytes were absent from the medium.

○———○ Percentage of chromium released when tagged carcinoma cells were incubated with patients' own lymphocytes in the ratio of 1:100.

With a 1:100 ratio, good results have been obtained by various investigators (Brunner, 1968; Jamieson *et al.*, 1969).

Cytotoxicity of serum

0.5 ml of fresh serum from patients with cervical carcinoma or healthy volunteers was added to 0.5 ml of cell suspension (enzyme treated/untreated), instead of lymphocytes, and the ^{51}Cr release was measured as described above.

Treatment of cancer cells with calcium chloride, histone and protamine sulphate

0.5 ml of carcinoma cell suspension was incubated with an equal volume of the following reagents for 30 min at 37° C: (a) 0.1 M calcium chloride, (b) histone (calf thymus, Sigma Chemical Co.) 2% solution in normal saline; (c) protamine sulphate (Sigma Chemical Co.), 2% solution in normal saline. After the incubation period, cells were washed in normal saline and further experiments (electrophoretic mobility or cytotoxicity) were done.

NANA release by neuraminidase

4×10^8 cells (malignant/normal) in 1 ml of isotonic saline were treated with 1 ml (0.1 unit) of neuraminidase solution for different time intervals at 37° C. N-acetyl-neuraminic acid (NANA) liberated into the supernatant was quantitatively estimated by the resorcinol method of Svennerholm (Spiro, 1966). Crystalline NANA (Sigma Chemical Co.) was used as standard.

Electrophoretic mobility of cells

Electrophoretic mobilities of carcinoma and normal cells of cervix were measured in a cylindrical microelectrophoresis apparatus based on the design of Bangham *et al.* (1958) (Rank Bros., Bottisham, England). The suspending medium contained 0.145 M NaCl, adjusted to pH 7.2 ± 0.02 by means of 0.145 M NaOH or HCl. Mobilities were measured at 30° C, and converted to values at 25° C. The results are the means of 30 observations on each sample and are expressed in microns/sec/volt/cm.

The mobilities were converted to zeta potentials (in mV) by using the Smoluchowski equation ($\zeta = 12.85\ u_E$, where u_E is mobility in μ/sec/v/cm), from which the surface charge density in e.s.u./cm^2 was derived by using the Gouy-Chapman equation, $\sigma = 3.52 \times 10^4 \times I^{1/2} \times \sinh(\zeta/51.3)$, where σ is the charge density in e.s.u/cm^2 and I is the molar concentration of electrolyte. Since $4.8 \times 10\text{-}^{10}$ e.s.u. = 1 electron charge, the number of electron charges lost per cell and those lost from

4×10^8 cells were calculated after measuring the cell sizes. The theoretical release of NANA from the cells for the observed decrease in mobility was calculated, by assuming that one molecule of NANA (MW 309) released by the action of neuraminidase decreased one electron charge on the surface of the cell.

RESULTS

Action of neuraminidase on malignant cells

The extent of N-acetyl-neuraminic acid released from normal cervical cells and from cervical carcinoma cells by treatment with neuraminidase is given in Figure 2. NANA release from carcinoma cells after 60 min of treatment with the enzyme is about one and a half times more than the amount liberated from the same number of normal cervical cells. Viability of cells as measured by Trypan Blue exclusion test was 95% at 30 min of enzyme treatment. The mortality of cells rises considerably when the enzyme treatment is continued beyond this period (22% at 60 min). The incubation period for neuraminidase treatment of cells was therefore fixed at 30 min for subsequent experiments.

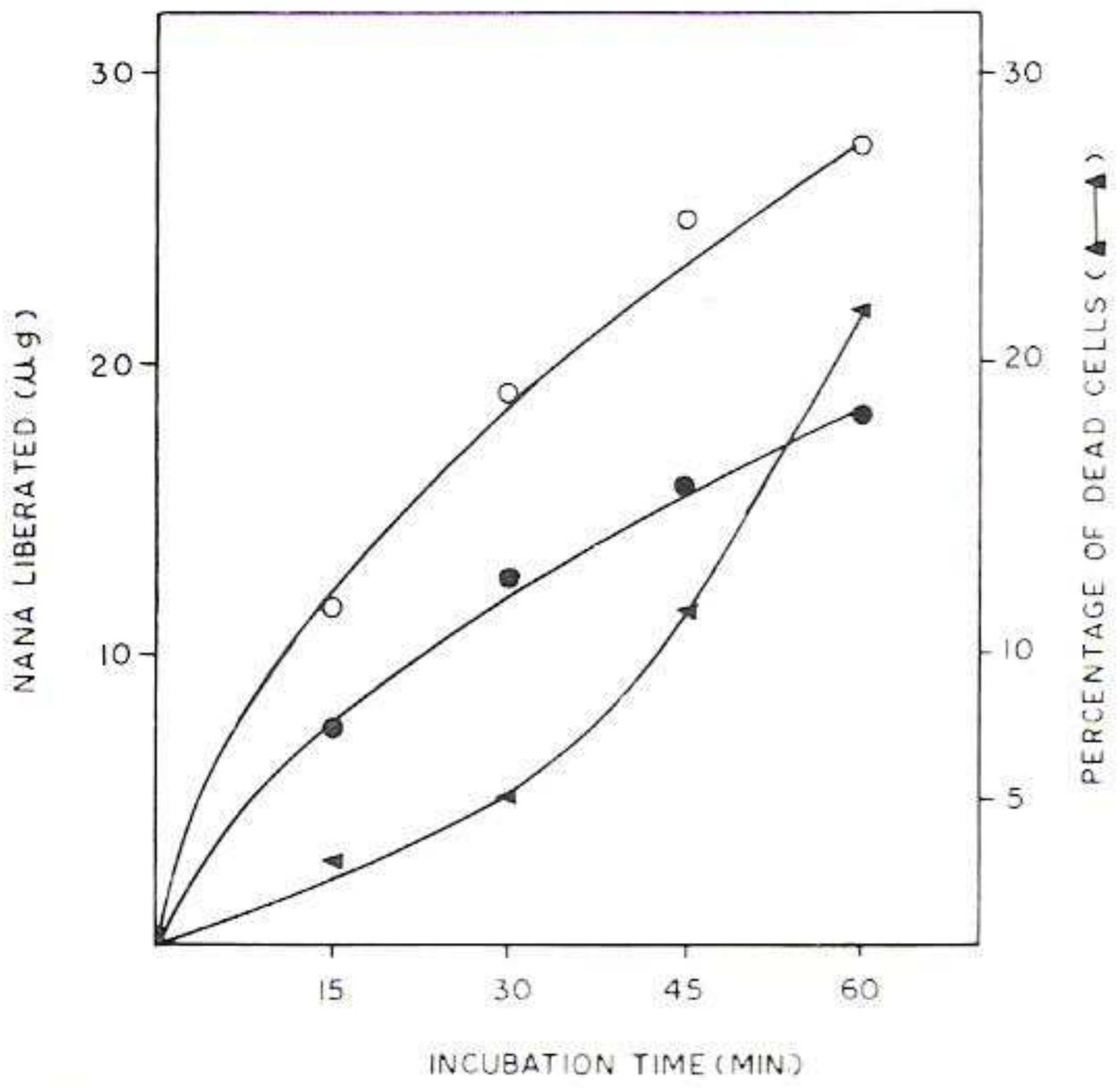

FIGURE 2

Effect of neuraminidase on normal and malignant cells of cervix.

○———○ N-Acetyl-Neuraminic Acid (NANA) liberated from 4×10^8 malignant cells when incubated with 0.1 unit of neuraminidase, at 37° C.

●———● NANA liberated from the same number of normal cells when incubated with same quantity of neuraminidase.

▲———▲ Percentage of death of malignant cells when incubated with neuraminidase for variable time periods at 37° C.

Electrophoretic mobilities

Figure 3 gives the electrophoretic mobilities of cells derived from cervical biopsies of normal subjects and from patients with carcinoma of the

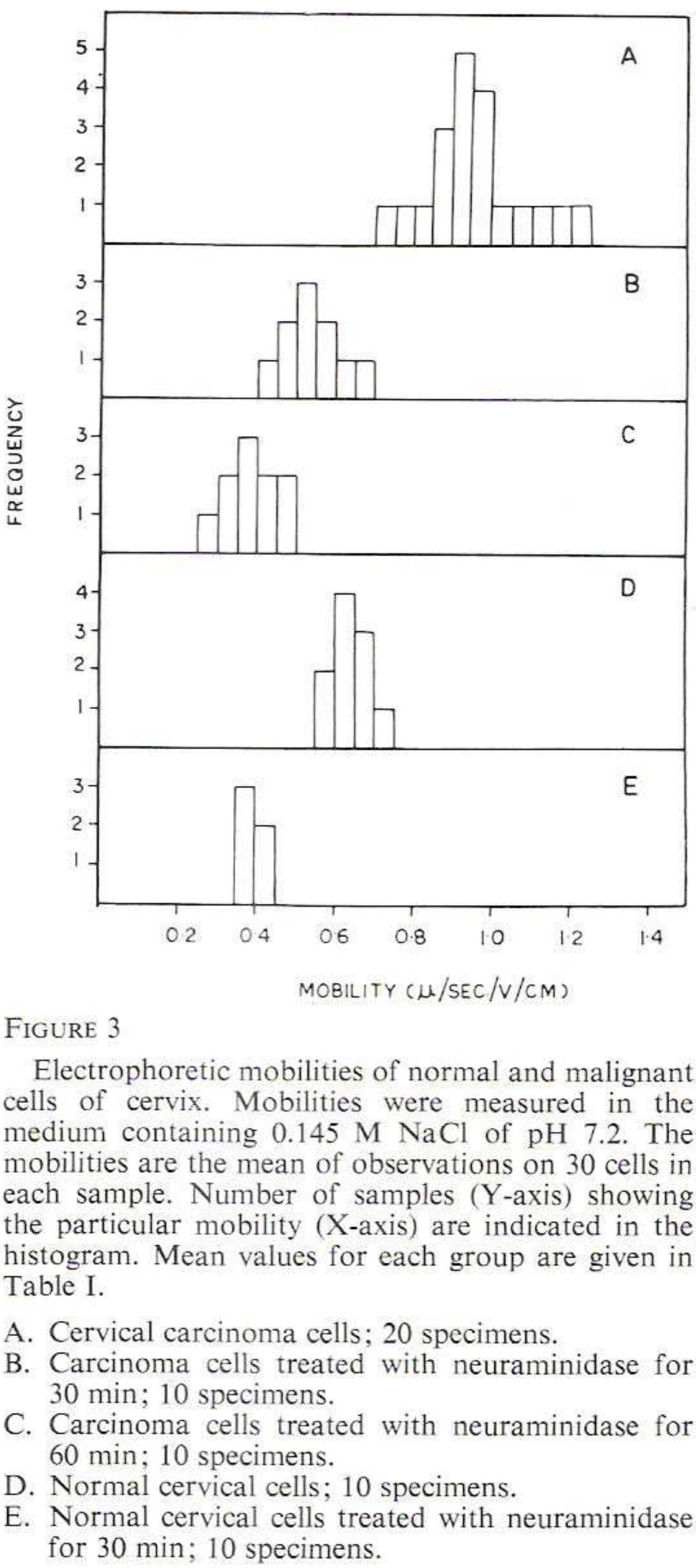

FIGURE 3

Electrophoretic mobilities of normal and malignant cells of cervix. Mobilities were measured in the medium containing 0.145 M NaCl of pH 7.2. The mobilities are the mean of observations on 30 cells in each sample. Number of samples (Y-axis) showing the particular mobility (X-axis) are indicated in the histogram. Mean values for each group are given in Table I.

A. Cervical carcinoma cells; 20 specimens.
B. Carcinoma cells treated with neuraminidase for 30 min; 10 specimens.
C. Carcinoma cells treated with neuraminidase for 60 min; 10 specimens.
D. Normal cervical cells; 10 specimens.
E. Normal cervical cells treated with neuraminidase for 30 min; 10 specimens.

cervix. The mean electrophoretic mobility of carcinoma cells from 20 patients examined was 0.89 ± 0.15 μ/sec/volt/cm at pH 7.2, a figure distinctly higher than the mobility of normal cervical cells (0.58 ± 0.04 μ/sec/v/cm), as determined from 10 biopsies. Treatment of carcinoma cells with neuraminidase for 30 min reduced the electrophoretic mobility to 0.46 ± 0.07 μ/sec/v/cm. Continued treatment with neuraminidase for 60 min led to even further lowering of the electrophoretic mobility (0.35 ± 0.08 μ/sec/v/cm). Neuraminidase-susceptible sialic acid moieties were present not only in the carcinoma cells, but also in normal cells, as indicated by the fact that electrophoretic mobility of normal cells after treatment with neuraminidase for 30 min was reduced to 0.36 ± 0.01 μ/sec/v/cm. (Table I).

Table II shows the reduction in charge densities in normal and carcinoma cells after neuraminidase treatment. The amount of sialic acid released by neuraminidase treatment was five to eight times higher than the calculated amount needed for the decrease in surface charge density.

Lymphocyte cytotoxicity tests

Autologous lymphocytes. To evaluate the cytotoxic effect of patients' own lymphocytes on the carcinoma cells *in vitro*, the cells before and after treatment with neuraminidase were incubated with lymphocytes (1:100 ratio). In the 10 cases studied, the mean percentage lysis of untreated carcinoma cells was 16% whereas the percentage of lysis of neuraminidase-treated carcinoma cells was 37% (Table III).

TABLE I

ELECTROPHORETIC MOBILITIES OF MALIGNANT AND NORMAL CELLS OF HUMAN UTERINE CERVIX

Type of cells investigated	Mode of treatment	No. of specimens studied	[1]Mobility μ/sec/v/cm	Standard deviation
Carcinoma cells	None	20	0.89	0.15
Carcinoma cells	Neuraminidase 3 min	5	0.86	0.13
Carcinoma cells	Neuraminidase 30 min	10	0.46	0.07
Carcinoma cells	Neuraminidase 60 min	10	0.35	0.08
Carcinoma cells	Calcium chloride 30 min	5	0.59	0.10
Normal cells	None	10	0.58	0.04
Normal cells	Neuraminidase 30 min	10	0.36	0.01

[1] All the experiments were done under identical conditions in the medium containing 0.145 M NaCl at pH 7.2. Results are the mean of observations on 30 cells in each specimen. Mobilities measured at 30° C have been converted to values at 25° C. All the mobility values carry a negative sign.

TABLE II

RELATIONSHIP BETWEEN RELEASE OF N-ACETYL NEURAMINIC ACID BY NEURAMINIDASE TREATMENT AND REDUCTION IN CHARGE DENSITY OF NORMAL AND CARCINOMA CELLS

	Mobility μ/sec/v/cm	Charge density σesu/cm^2	Reduction in charge density $\Delta\sigma$esu/cm^2	No. of electrons lost/4×10^8 cells	Theoretical yield of NANA/ 4×10^8 cells (μg)	Experimental yield of NANA/ 4×10^8 cells (μg)	Ratio of exptl/ theoretical yield of NANA
Carcinoma cells	0.89	3035					
Carcinoma cells treated with neuraminidase for 30 min . .	0.46	1542	1493	7.4×10^{15}	3.8	19.0	5.0
Carcinoma cells treated with neuraminidase for 60 min . .	0.35	1205	1830	9.0×10^{15}	4.6	27.6	6.6
Normal cervical cells	0.58	1946					
Normal cells treated with neuraminidase for 30 min . .	0.36	1250	696	2.6×10^{15}	1.4	11.1	8.0

TABLE III

EFFECT OF AUTOLOGOUS LYMPHOCYTES ON CERVICAL CARCINOMA CELLS BEFORE AND AFTER TREATMENT WITH NEURAMINIDASE

Patients		Percentage of lysis of						
		Untreated carcinoma cells			Neuraminidase-treated carcinoma cells			Increase in lysis after neuraminidase treatment
Initials	Age	Without lymphocytes	With autologous lymphocytes	Actual lysis	Without lymphocytes	With autologous lymphocytes	Actual lysis (7)—(6)	
S.K.	55	6	16	10	6	37	31	21
B.D.M.	50	7	42	36	8	89	81	45
L.W.	50	5	19	14	5	36	31	17
S.	65	3	34	31	5	48	43	12
H.D.	65	8	29	21	7	51	44	23
B.D.K.	60	6	19	13	3	28	25	12
B.W.	55	10	22	12	9	30	21	9
P.	44	6	20	14	7	36	29	15
A.K.	53	4	15	11	4	38	34	23
S.B.	60	8	10	2	5	34	29	27
Mean		6.3		16.4	5.9		36.8	24.7
S. error				3.0			5.0	3.9
p								<0.001

Spontaneous release of ^{51}Cr from the tagged cells was routinely evaluated in all cases studied and values subtracted from the observed figures. The pattern of spontaneous release of ^{51}Cr at different times of incubation of tagged cells is shown in Figure 1 B. Spontaneous release from enzyme-treated and untreated cells was almost identical (Table III).

Five specimens of normal cervical cells were tested in a parallel manner with autologous lymphocytes. There was a negligible cytolytic effect of lymphocytes in these cases and treatment with neuraminidase did not materially change the results (Table IV).

Homologous lymphocytes. In 10 cases studied, the mean value for lysis of untreated cancer cells by homologous lymphocytes from cancer patients was about 13%, while neuraminidase treatment increased the cytolysis to 31% (Table V).

Normal lymphocytes from healthy donors had little cytotoxic action on untreated (5%) or on neuraminidase-treated (6%) carcinoma cells (Table V).

Effect of other reagents on cytolysis of carcinoma cells by autologous lymphocytes

In order to see whether the high surface charge was a factor impeding the interaction of lymphocytes with carcinoma cells, a series of experiments was performed in which the net surface anionic charge density was reduced either by cations or by basic proteins. Carcinoma cells were incubated in 0.1 M calcium chloride solution for 30 min, washed in saline and resuspended in 0.145 M NaCl at pH 7.2. The electrophoretic mobility of carcinoma cells on treatment

TABLE IV

PERCENTAGE OF LYSIS OF NORMAL CERVICAL CELLS BEFORE AND AFTER TREATMENT WITH NEURAMINIDASE BY AUTOLOGOUS LYMPHOCYTES

Subjects		Percentage of lysis	
Initials	Age	Untreated normal cervical cells with autologous lymphocytes [1]	Neuraminidase-treated normal cervical cells with autologous lymphocytes [1]
M.K.	35	4	5
B.S.	40	1	2
P.K.	38	2	2
D.S.M.	43	1	3
D.W.K.	37	2	2
Mean	—	2	3

[1] Spontaneous release is subtracted.

TABLE V

EFFECT OF HOMOLOGOUS LYMPHOCYTES ON CERVICAL CARCINOMA CELLS BEFORE AND AFTER TREATMENT WITH NEURAMINIDASE

Carcinoma cells from patients		Percentage of lysis of carcinoma cervix cells				Increase in lysis after neuraminidase treatment	
		By homologous lymphocytes from patients with carcinoma cervix		By homologous lymphocytes from healthy donors			
Initials	Age	Before neuraminidase treatment [1]	After neuraminidase treatment [1]	Before neuraminidase treatment [1]	After neuraminidase treatment [1]	By homologous lymphocytes from patients with cervical carcinoma	By homologous lymphocytes from healthy donors
S.K.	55	13	32	6	8	19	2
B.D.M.	50	14	40	5	2	26	−3
L.W.	50	11	29	3	4	18	1
S.	65	25	36	8	6	11	−2
H.D.	65	15	48	2	4	33	2
B.D.K.	60	10	25	5	6	15	1
B.W.	55	12	32	6	7	20	1
P.	44	8	25	4	10	17	6
A.K.	53	5	16	4	5	11	1
S.B.	60	13	23	3	5	10	2
Mean		12.6	30.6	4.6	5.7	19.0	1.1
S. error		1.6	2.7	0.6	0.7	1.3	0.7
p						<0.001	N.S.[2]

[1] Spontaneous release is subtracted.
[2] Not significant.

with $CaCl_2$ was reduced from 0.89 ± 0.15 μ/sec/v/cm to 0.59 ± 0.10 μ/sec/v/cm (Table I). Treatment of malignant cells with calcium chloride, histone or protamine sulphate did not increase the cytolytic efficiency of autologous lymphocytes (Table VI).

TABLE VI

EFFECT OF AUTOLOGOUS LYMPHOCYTES ON CERVICAL CARCINOMA CELLS BEFORE AND AFTER TREATMENT WITH DIFFERENT REAGENTS [1]

Mode of treatment of carcinoma cells	Number of samples tested	Percentage of lysis of carcinoma cells when incubated with autologous lymphocytes (Mean values)	
		Untreated carcinoma cells [2]	Treated carcinoma cells [2]
Calcium chloride	3	23	26
Histone	3	25	29
Protamine sulphate	3	28	23
Neuraminidase	10	16	37

[1] The concentration of reagents employed and mode of treatment has been described in the " Material and Methods " section.
[2] Spontaneous release is subtracted from both columns.

Serum effects

Autologous serum. The cytolytic effect is not restricted to lymphocytes. The presence of cytolytic factors in autologous sera of carcinoma patients is indicated by the data in Table VII. Neuraminidase treatment of malignant cells increased their susceptibility to lysis by these factors from 24% to 50%.

Homologous sera. It was seen that the mean value of cytolysis of carcinoma cells by homologous cancer sera was increased from 17% to 37% when the malignant cells were pretreated with neuraminidase (Table VII).

Normal sera had a negligible cytolytic effect on carcinoma cells (7%) as well as on neuraminidase-treated carcinoma cells (8%) (Table VII).

DISCUSSION

Many types of malignant cells have been shown to carry a high net negative surface charge as evidenced by their electrophoretic mobility (Ambrose *et al.*, 1956, Forrester *et al.*, 1962;

TABLE VII

EFFECT OF SERUM FACTORS ON CERVICAL CARCINOMA CELLS BEFORE AND AFTER TREATMENT WITH NEURAMINIDASE

No. of experiment	Percentage of lysis of cervical carcinoma cells						Increase in lysis after neuraminidase treatment		
	By autologous serum		By homologous serum from patients with cervical carcinoma		By homologous serum from normal healthy donors				
	Before neuraminidase treatment [1]	After neuraminidase treatment [1]	Before neuraminidase treatment [1]	After neuraminidase treatment [1]	Before neuraminidase treatment [1]	After neuraminidase treatment [1]	By autologous serum	By homologous serum from patients with cervical cancer	By homologous serum from healthy donors
1	8	68	16	26	3	5	60	10	2
2	36	58	16	35	8	15	22	19	7
3	30	41	10	28	15	10	11	18	−5
4	35	67	19	41	4	6	32	22	2
5	17	45	26	54	10	7	28	28	−3
6	28	51	17	38	3	4	23	21	1
7	11	31			7	13	20		6
8	26	34			5	6	8		1
9	37	64			12	14	27		2
10	11	39			6	5	28		−1
Mean	23.9	49.8	17.3	37.0	7.3	8.5	25.9	19.7	1.2
S. error	3.3	4.1	1.9	3.7	1.2	1.2	4.2	2.2	1.1
p							<0.001	<0.001	N.S.[2]

[1] Spontaneous release is subtracted.
[2] Not significant.

Fuhrmann, 1965). This may not, however, be a universal trait of malignant cells. Vassar (1963*a* and *b*) did not find any significant difference in the electrophoretic mobilities of normal and malignant epithelial cells of human origin.

In our studies, human cervical carcinoma cells were observed to have almost one and half times the mobility of normal cervical cells (Fig. 3). Electrophoretic mobility was reduced to normal levels by treatment of malignant cells with neuraminidase for 30 min suggesting the contribution of sialic-acid-containing moieties to the surface charge of the malignant cells. The change in electrophoretic mobility consequent on treatment with neuraminidase is not due to the non-specific adsorption of the enzyme on the cell surface, as short (3 min) exposure of cells to neuraminidase does not cause a reduction in their mobility (Table I). Spontaneous release of ^{51}Cr did not differ significantly in neuraminidase-treated or untreated cells, thereby indicating that the viability of the cells remained unaffected by the neuraminidase treatment. Kraemer (1968) found that *Cl. perfringens* neuraminidase contained phospholipase activity and was cytotoxic to erythrocytes as gauged by trypan blue exclusion. The possible contribution of such contaminants to the lability of cervix cells cannot be fully excluded. However, in our experiments, the treatment with neuraminidase was restricted to a time period at which 95% of cells remained viable as gauged by trypan blue exclusion test (Fig. 2). Moreover, treatment with neuraminidase did not increase the cytolysis of the cervical carcinoma cells by lymphocytes from healthy donors (Table III), showing that treatment with neuraminidase does not *per se* increase the cytolysis of the cells.

In our experiments, more NANA is released from the neuraminidase treated cells than would be expected from the decrease in electrophoretic mobility or from the reduction in the charge density. Similar findings were reported previously by others (Cook *et al.*, 1961, 1962; Wallach and Eylar, 1961). There are several theoretical reasons for this discrepancy (Wallach and Esandi, 1964); an important one being that perhaps NANA is also released from the less superficial layers of the cell wall whereas loss from the super-

ficial layers only (approximately 10 Å in our experimental conditions) will contribute to the electrokinetic behavior. Moreover it has been demonstrated that neuraminidase enters cells and releases NANA from the intracellular compartments (Nordling and Mayhew, 1966).

Autologous and homologous lymphocytes from carcinoma patients were able to lyse the malignant cells, and this capacity was increased two-fold after neuraminidase treatment (Tables III and V). On the other hand, the lysis of normal cervical cells by the subject's own lymphocytes was negligible and neuraminidase treatment did not appreciably change the lytic activity (Table IV). Similarly, lymphocytes from healthy donors were not effective against the malignant cells (Table V). These observations indicate that some determinants on the surface of malignant cells are different from those of the normal cells. Furthermore, these determinants may be " foreign " to the organism and may act as antigens to provoke immune response. Our findings are consistent with those of Hellström *et al.* (1968) and Bubeník *et al.* (1970 *a*, *b*) who showed that autologous and homologous lymphocytes from patients with tumors have a cytotoxic effect on the respective tumor cells.

Reduction in net negative surface charge was taking place when carcinoma cells were treated with calcium chloride, as shown by the decrease in electrophoretic mobility after calcium chloride treatment (Table I). However, treatment of malignant cells with calcium chloride, histone or protamine sulphate did not change their susceptibility to lysis by autologous lymphocytes (Table VI).

In our studies, neuraminidase-treated carcinoma cells were also found to be more susceptible to the lytic effect of factors present in the autologous sera as well as in homologous sera from patients suffering from cervical carcinoma, but not to sera from healthy persons (Table VII). This would indicate the possible presence of common tumor-specific antigens in human cervical carcinoma cases, a finding consistent with other observations on human carcinomata (Hellström *et al.*, 1968; Bubeník *et al.*, 1970 *a*, *b*). The carcinoma cells would appear to be more amenable to cytolytic action by serum factors when treated with neuraminidase. The action of serum factors against tumor cells is not very clear. Sera from different types of cancer patients had a cytotoxic effect on HeLa cells (Arpels and Southam, 1969). Sera from some patients were cytotoxic *in vitro* (Hellström *et al.*, 1968; Bubeník *et al.*, 1970*b*), but sera from some patients blocked the cytolytic effect of lymphocytes (Hellström *et al.*, 1969; Bubeník *et al.*, 1970*b*). It may be stated that lysis in the presence of serum factors may be due to entirely different mechanisms than the lysis studied in the presence of autologous lymphocytes.

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EFFET DE LA NEURAMINIDASE SUR LA MOBILITÉ ELECTROPHORÉTIQUE ET LA CYTOLYSE IMMUNITAIRE DES CELLULES DE CARCINOME CERVICAL HUMAIN

La mobilité électrophorétique des cellules de carcinome cervical humain à pH 7.2 est de 0.89 μ/sec/v/cm à 25°C alors que, pour les cellules cervicales normales, elle est de 0.58 μ/sec/v/cm. Le traitement des cellules de carcinome à la neuraminidase pendant 30 minutes a réduit la mobilité électrophorétique à 0.46 μ/sec/v/cm. La neuraminidase a libéré plus d'acide N-acétyl-neuraminique dans les cellules de carcinome que dans les cellules normales. La cytolyse immunitaire des cellules de carcinome par les lymphocytes a été étudiée au moyen de la technique de libération du ^{51}Cr. Les lymphocytes autologues et homologues de sujets atteints de carcinomes du col de

l'utérus ont un effet lytique sur les cellules de ces tumeurs; l'intensité de la cytolyse a presque doublé lorsque les cellules malignes ont été traitées à la neuraminidase. Les lymphocytes homologues de donneurs sains ont un effet cytolytique relativement faible sur les cellules malignes. Les lymphocytes autologues n'ont virtuellement aucun effet sur les cellules cervicales normales et le traitement à la neuraminidase n'a pas affecté leur sensibilité aux lymphocytes. Le sérum autologue ou homologue de cancéreux a une influence cytolytique sur les cellules malignes, mais le sérum normal n'a aucun effet. Bien que le chlorure de calcium réduise la charge négative à la surface des cellules malignes, comme le montre leur mobilité électrophorétique, aucune augmentation de la cytolyse des cellules de carcinome par les lymphocytes autologues n'a été observée en présence de concentrations de chlorure de calcium capables de réduire la charge.

Ces résultats montrent qu'il est possible que, dans les cas de carcinome cervical, les fractions contenant de l'acide sialique masquent partiellement les antigènes spécifiques de la tumeur à la surface des cellules et empêchent leur identification par les cellules immunologiquement antagonistes de l'hôte.

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