EFFECT OF CERTAIN SALTS ON THE ALKALINE PHOSPHATASE ACTIVITY OF VAGINAL EXFOLIATED CELLS FROM NORMAL & CANCER CERVIX

by

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Cations are known to play fundamental roles in diverse biological functions and often impairment of cation balance has been observed in neoplastic conditions (Prasad *et al* 1969; Mirand *et al* 1968; deLong *et al* 1950. Fisher and Fisher, 1968; Humphrey, 1963; Bresciani and Auricchio, 1962). Administration of certain trace elements have been reported to have caused suppression of spontaneous tumours in mice (Schroeder *et al,* 1963; Schroeder *et al,* 1966; Kanisawa and Schroeder, 1967, 1969). In our previous report, necrosis of fibrosarcoma cells in mice have been shown with therapy of specific salts of which K^+ plays an important role in combination with lactose (Chowdhury and Roy Chowdhury). The specificity of such action of salts in malignant condition necessitates scrutiny in respect of the influence of ions in the biological expression of malignancy.

Enzymes are the functional phenotypes of cells and the nature of enzyme reactions are seen to be profoundly altered by the nature and concentrations of ions present (Whitaker and Tappel, 1962). Alkaline phosphatase is an important enzyme participating in growth, differentiation and dedifferentiation (Moog, 1952; McWhinnie and Saunders, 1966).

Characterization of this enzyme in the exfoliated cells of cervix, both in normal and malignant conditions, have provided information with respect to its heterogenic behaviour under the influence of diverse cations (Roy Chowdhury *et al,* 1972; Roy Chowdhury *et al,* 1973). In the present communication, the influence of various salts of K^+ have been studied on the activity of alkaline phosphatase in the cervical exfoliated cells during normalcy and malignancy.

Material and Method

Cervical smear was obtained from patients of cancer cervix under premenopausal condition and the diagnosis of epidermoid carcinoma was confirmed by histopathological examinations. For comparative studies, smears were collected from premenopausal normal women at around 18th day of the menstrual cycle taking care to exclude from the series any case of gynaecological complications.

Alkaline phosphatase activity is demonstrated cyto-chemically by Gomori's technique (1952) using Na-ß-glycocero-phosphate as substrate. For elucidation of the effect of potassium salts on the enzyme activity, the different salts of potassium used are—chloride, acetate, metabisulphite, ferrocyanide, iodide and nitrate at

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concentration of 0.1M in the incubation mixture. Combination of lactose and potassium chloride at different molarity are similarly set up and the results are evaluated with respect to the control slides where the usual incubation mixture is used without any of the above salts. Subjective grading is made according to the intensity of staining reaction in the superficial, intermediate, parabasal cells and in malignant cells in case of cancer cervix.

Results and Discussion

The influence of different salts on alkaline phosphatase activity in the nuclear and cytoplasmic compartments of the different cell types reveal an interesting picture with respect to normal (Table I) and cancer cervix (Table II) cases. As evidenced from the Tables, potassium chloride has got marked activating influence on the enzyme in the cytoplasm of all the cell types of cancer cases, parti-

TABLE I

Activity of Alkaline Phosphatase under the Influence of Different Salts of Potassium in the Exfoliated Cells from Normal Cervix

Salts (.1M)	Superficial cells		Intermediate cells		Parabasal cells	
	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm
Control	+	+	+	+	+	+
KCl	+	+	+	+	++	+
CH_3COOK	+	+	+	+	+	+
$K_2S_2O_5$	−	+	−	+	−	+
$K_3Fe.(CN)_6$	+	+	+	+	+	+
KI	+	−	+	−	+	−
KNO_3	+++	++	+++	++	+++	++

TABLE II

Activity of Alkaline Phosphatase under the influence of Different Salts of Potassium in the Exfoliated Cells from Cancer Cervix

Salts (.1M)	Superficial cells		Intermediate cells		Parabasal cells		Malignant cells	
	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm
Control	+	+	+	+	+	+	+	+
KCl	+	+++	+	+++	+	+++	+	++++
CH_3COOK	++	++	++	++	+	+	+	+
$K_2S_2O_5$	+	+	+	+	+	+	+	+
$K_3Fe.(CN)_6$	−	−	−	−	−	−	−	−
KI	+	−	+	−	+	−	+	−
KNO_3	++	++++	++	++++	++	++++	++	++++

cularly in the cancer cell itself. This effect is not manifested in normal cells except for some amount of nuclear stimulation of parabasal cells. The acetate salt of potassium, however, evoked stimulation of enzyme activity in superficial and intermediate cells only in cancer cervix cases contrary to no effect in the normal cases. While potassium metabisulphite caused nuclear inhibition of enzyme activity in normal cells only, potassium ferrocyanide inhibition of both nuclear and cytoplasmic enzyme activity in all the cell types from cancer cervix. Potassium iodide and potassium nitrate showed identical influence in the normal and cancer cases of inhibition with iodide and activation with nitrate.

Tables III and IV reveal the effect of combinations of lactose and potassium chloride of different molarity on the activity of alkaline phosphatase in the exfoliated cells from normal and cancer cervix cases. Strangely enough the activating influence of KC1 seen with 0.1M concentration in cells from cancer cervix is maintained at 0.01M concentration in combination with lactose. On further lowering of the dose of salt this effect is lost, except for some amount of stimulation in the cytoplasm of malignant cells with 0.001M concentration. Lactose itself does not have any effect on the enzyme activity. The normal cells do not exhibit any differential activity of this enzyme with such combination.

There are evidences that alkaline phosphatase activity is seen to be activated or inhibited under the influence of different ions (Hinsch, 1960; Roy Chowdhury *et al*, 1973) and also that the metallic ions demarcate alkaline phosphatase of different tissues (Fishman *et al*, 1962). The present result distinctly reveals that variations of anions with respect to same cation produce differential behaviour of alkaline phosphatase activity which discriminates normal from cancer cervix cases. This is of significance in the light of molecular heterogeneity of this enzyme

TABLE III

Influence of Potassium Chloride of Varying Molarity and Lactose on Alkaline Phosphatase Activity of Exfoliated Cells from Normal Cervix

Different molarity of KCl and lactose	Superficial cells		Intermediate cells		Parabasal cells	
	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm
Control	+	+	+	+	+	+
KCl—.1M	+	+	+	+	++	+
KCl—.01M and lactose	+	+	+	+	+	+
KCl—.001M and lactose	+	+	+	+	+	+
KCl—.0001M and lactose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+

TABLE IV

Influence of Potassium Chloride of Varying Molarity and Lactose on Alkaline Phosphatase Activity of Exfoliated Cells from Cancer Cervix

Different molarity of KCl and lactose	Superficial cells		Intermediate cells		Parabasal cells		Malignant cells	
	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm	Nucleus	Cyto-plasm
Control	++++	++	++++	++	++++	++	++++	++
KCl—.1M	++++	++	++++	++	++++	++	++++	++
KCl—.01M and lactose	++++	++	++++	++	++++	++	++++	++
KCl—.001M and lactose	++++	++	++++	++	++++	++	++++	++
KCl—.0001M and lactose	++++	++	++++	++	++++	++	++++	++
Lactose	++++	++	++++	++	++++	++	++++	++

at the level of ionic homoestasis and its alteration in malignancy.

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