STUDIES ON THE SIALIC ACID IN TUMOR AND NORMAL CELLS USING CISPLATIN AS A PROBE

S. B. PRASAD
Department of Zoology, School of Life Sciences, North-Eastern Hill University, Shillong 793014, India.

ABSTRACT

In the present work, biochemical studies have been made to compare sialic acid in Dalton's lymphoma and normal spleen lymphocytes treated with or without cisplatin. The effect of cisplatin on the cell-surface proteins of DL and NL-cells are also studied.

INTRODUCTION

CISPLATIN, a potent antitumor platinum compound, has been successfully used against various malignant tumors in different experimental animals[1-3] and in a variety of human malignancies[4]. Cisplatin has a definite effect on the surface of tumor and normal cells, and brings about changes in the lectin agglutinability of the cells[5] and in the topographical pattern of lectin-binding sites on the cell surface[6].

Sialic acid occurs at the terminals of cell-surface oligosaccharide chains and plays a key role in important interactions associated with cell periphery[7, 8]. The influence of sialic acid on the oncogenicity of tumor cells has been studied by many investigators as (i) the main determinant of the cell-surface negative charge, electrophoretic mobility[9] and the loss of contact inhibition[7], (ii) antigen-masking agent[10, 11] and (iii) component of the cell-surface involved in the adherence of tumor cells to mesothelial membrane prior to their dissemination to form metastasis[12]. It has been reported that with the progress of tumor development there is an increase in sialic acid content of Yoshida ascites sarcoma cells[13].

In view of the importance of sialic acid in the changed biological properties of malignant cells and the earlier knowledge on the effect of cisplatin on cell surface of tumor and normal cells, the present biochemical studies were undertaken to compare the sialic acid in Dalton's lymphoma and normal spleen lymphocytes treated with or without cisplatin. The effect of cisplatin on the cell-surface proteins of DL and NL cells is also studied on the basis of elution profile of proteins by Sephadex G-50 column chromatography.

MATERIALS AND METHODS

Dalton's lymphoma obtained from the Cancer Research Institute, Bombay was maintained in Dba (dark brown albino) mice. Single cell suspensions of normal spleen lymphocytes (NL) and Dalton's Lymphoma (DL) ascites cells were prepared in phosphate-buffered saline (PBS) at the concentration of 4×10^7 cells/ml as described earlier[6]. Cisplatin was thoroughly mixed with 0.89 % NaCl in dark 10–15 min before use. Cell suspensions of NL and DL cells were incubated with and without cisplatin (25 μg/ml) for 30 min at 37°C. The cell suspensions were centrifuged at 1500 rev/min for 8–10 min and the pellets (weighed) were homogenized in 0.1 N H_2SO_4 (1.0 ml/10 mg tissue) and incubated in a water bath at 80°C for 1 hr with intermittent shaking. The resulting suspensions were centrifuged at 2000 rev/min for 8–10 min. The sialic acid in the supernatants was estimated by the method of Warren[14] and the total sialic acid content was calculated in μmol/g tissue wt. The supernatants obtained after cisplatin treatment were also used for sialic acid estimation to find any release of sialic acid after cisplatin treatment of the cells. In another set of experiment NL and DL cell suspensions (5×10^7 cells/ml in PBS) were incubated with cisplatin (25 μg/ml), neuraminidase (4 units/ml, Sigma Co. U.S.A.) and trypsin (20 μg/ml) for 30 min at 37°C with intermittent shaking. Control cells were incubated without cisplatin, neuraminidase or trypsin. After incubation, the cell suspensions were centrifuged at 3000 rev/min for 8–10 min and the supernatants were used for Sephadex G-50 column chromatography. A column of 90 × 1 cm was prepared and equilibrated with running buffer (0.1 M tris-acetate buffer, pH 9.0 containing 0.1 % SDS and 0.01 % ethylene diamine tetra acetic acid). The supernatant (2 ml) was mixed with 1 mg of blue dextran and 0.05 ml phenol red and loaded on the top of the column. The flow rate was adjusted to 1 ml/5 min and each fraction (1 ml) was used for protein estimation by the method of Lowry *et al*[15]. The graph was plotted and the differences in peak patterns were compared for different batches of cells.

The viability of the cells was checked with trypan

blue before and after the cisplatin treatment it was around 85–90%.

RESULTS

Total sialic acid content of DL cells (1.4 μmol/g wet wt of tissue) is greater than that of NL cells (1 μmol/g wet wt of tissue) (figure 1). A decrease in the sialic acid content of NL as well as DL cells is noted after cisplatin treatment (figure 1). Sialic acid contents in the supernatants of cisplatin-treated cells show that the release of sialic acid is greater for DL cells than that of NL cells (figure 1). In the elution profile of the supernatants of control NL and DL cells about seven peaks (I to VII) are observed (figure 2A, 3A). In cisplatin-treated NL peak II and sub-peak IIA of control NL are probably co-eluted so that instead of two separate peaks only one prominent sharp peak II is noticed (figure 2b). Cisplatin treatment of DL cells reduced the height of peaks III, IV and V as compared to that of controls and peak VI becomes more pronounced (figure 3b). On the other hand, enzymatic effect of neuraminidase and trypsin on NL and DL cells shows significant changes in the patterns of almost all the peaks (figures 2, 3–C, D).

DISCUSSION

The cell-surface carbohydrate side chains composed of 2 to 15 monosaccharide units may consist of the sugars: N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, D-galactose, D-mannose, L-fucose and sialic acid[16]. Among these sugars sialic acid is the common constituent of cell surface carbohydrates[7,8]. The growth rate-dependent changes in the polysaccharide moieties of tumor cell surface were shown earlier[17]. In the present studies higher sialic acid content is noted for tumor (DL) cells than that of normal (NL) cells (figure 1) and the high sialic acid of tumor (DL) cells may be one of the necessities of the malignant cells helping malignancy. Rao and Sirsi[13] showed an increase in sialic acid content of Yoshida ascites sarcoma

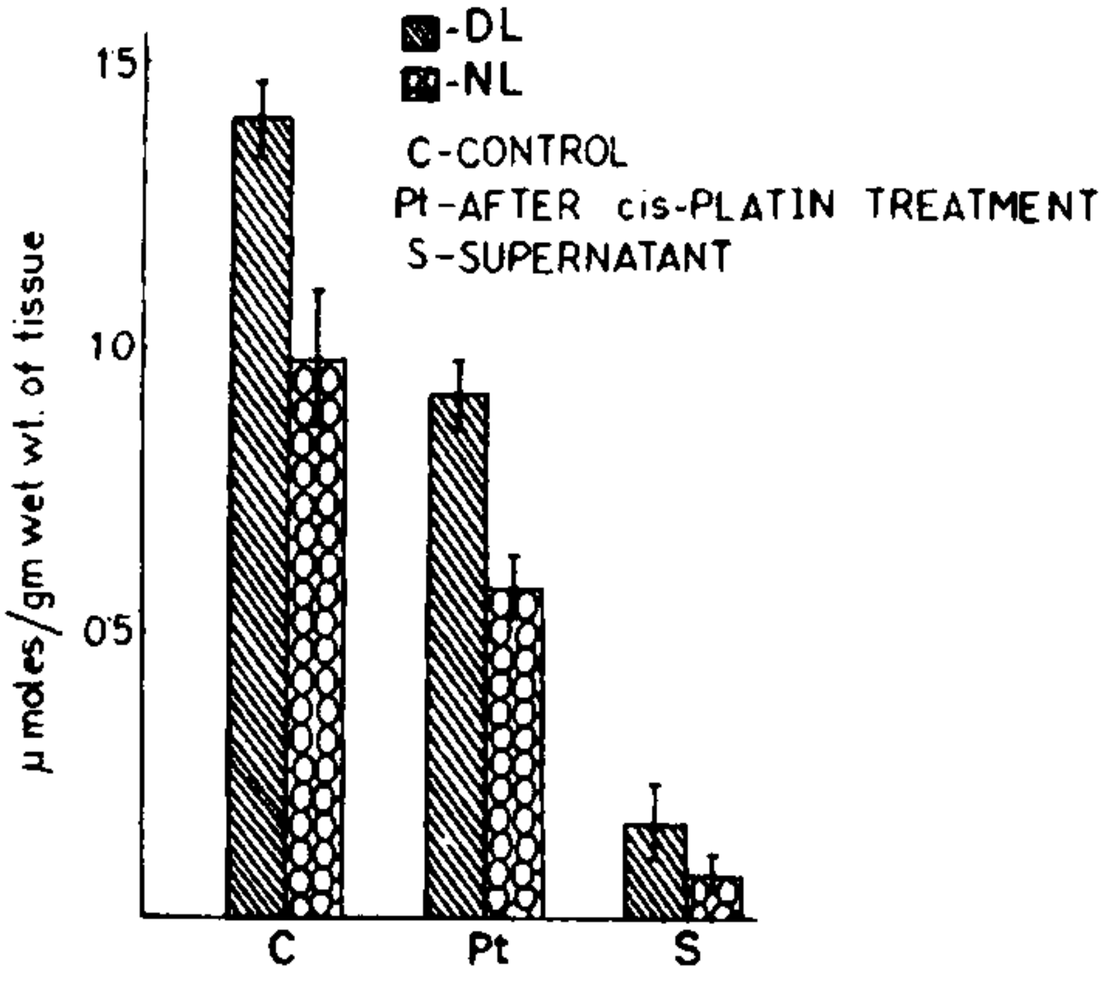

Figure 1. Histogram showing the total sialic acid contents of normal lymphocytes (NL) and Dalton's lymphoma (DL) cells incubated with or without cisplatin.

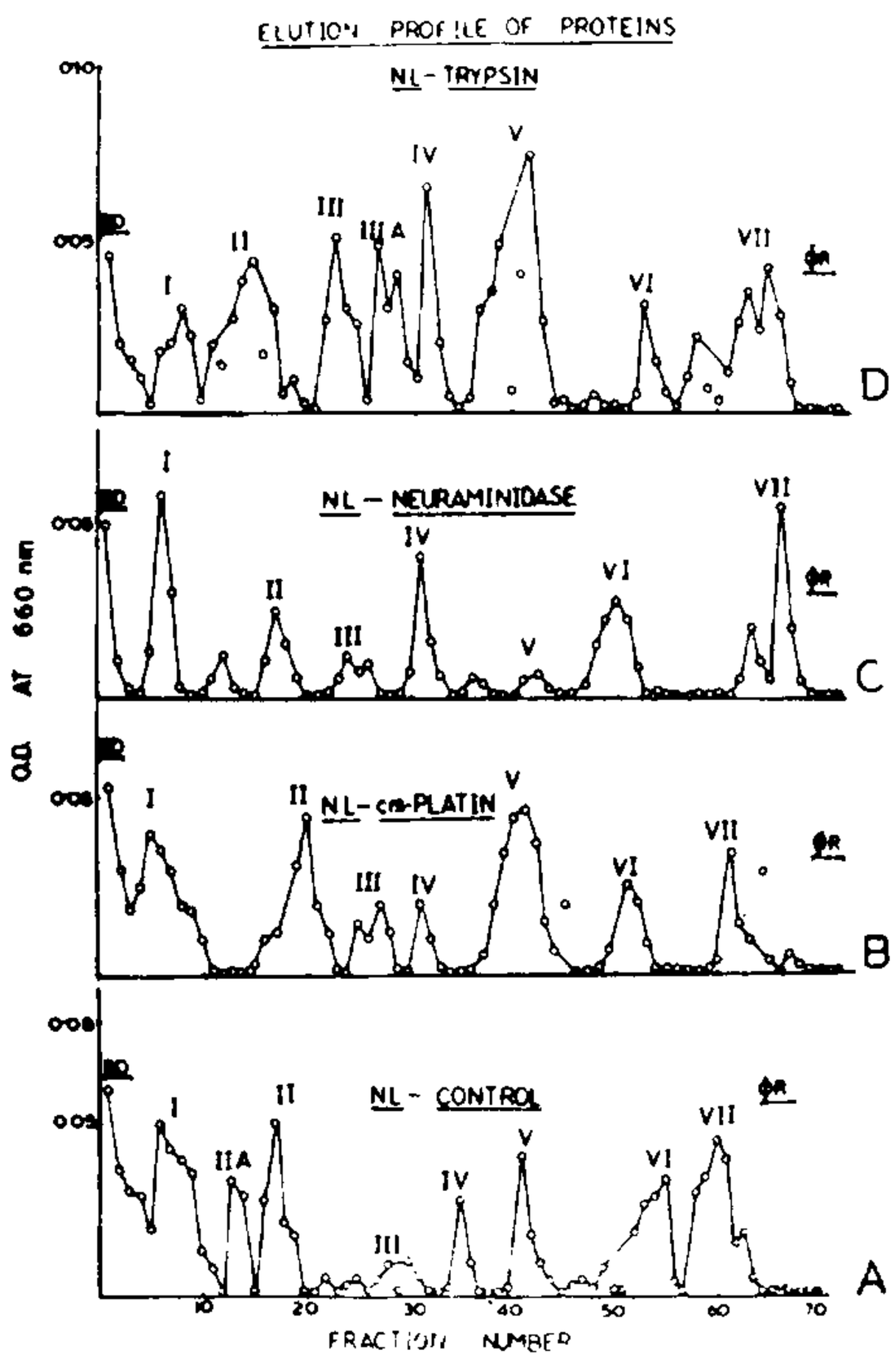

Figure 2. Sephadex G-50 column chromatography of proteins of the supernatant of NL under different experimental conditions. A, control, B-cisplatin treatment of NL.

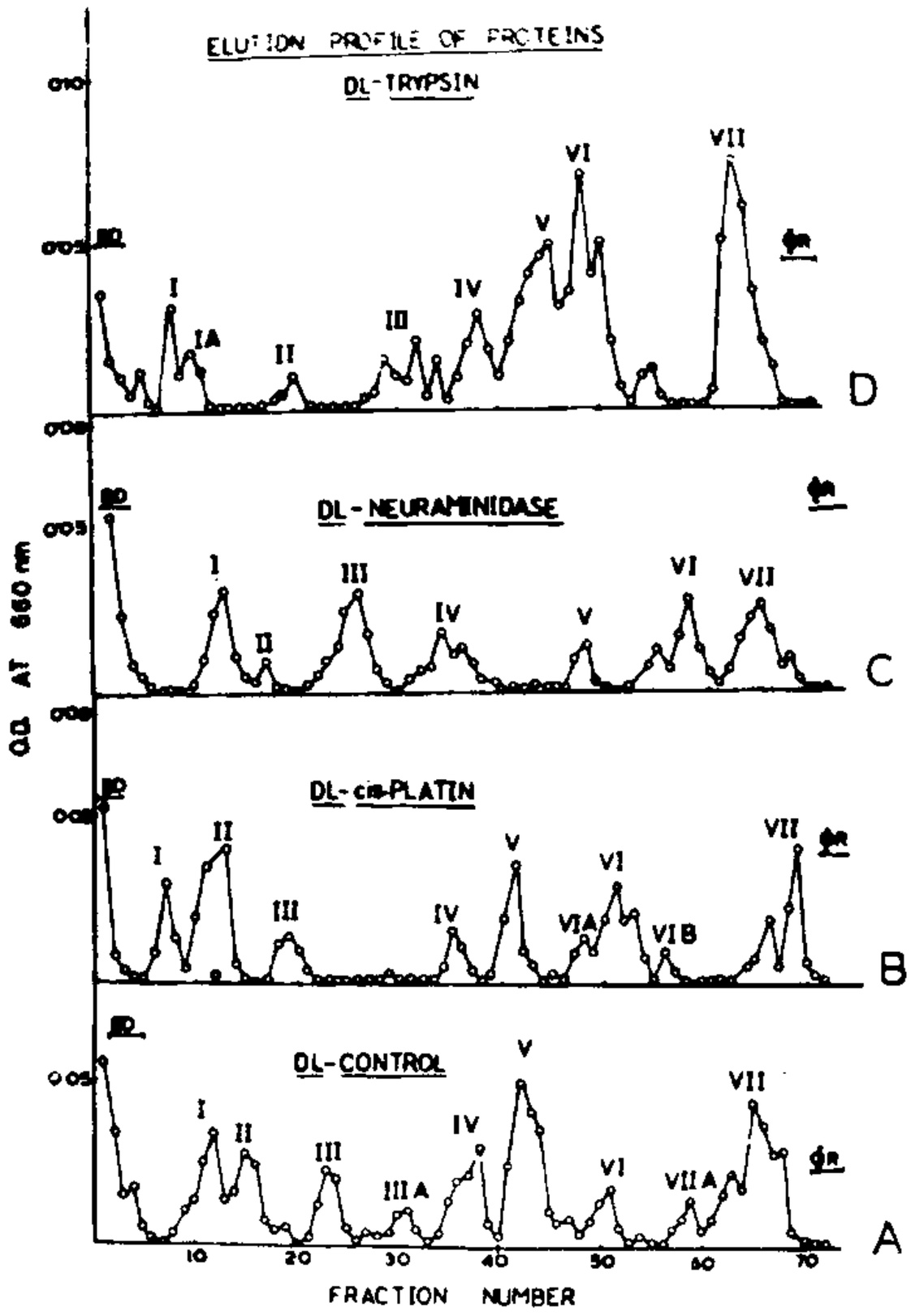

Figure 3. Sephadex G-50 column chromatography of proteins of the supernatants of DL cells incubated as such (control, A), with cisplatin (B), neuraminidase (C), trypsin (D).

cells with the progression of tumor development. Ultrastructural studies by colloidal iron labelling showed a thicker surface coat on DL cells than that of NL[6]. After cisplatin treatment of NL and DL cells a decrease in sialic acid content is observed for NL and DL cells, and at the same time some sialic acid appears in the supernatants of cisplatin-treated cells (figure 1) suggesting the removal of sialic acid moieties from the cell surface after cisplatin treatment. This proposition is supported by our earlier finding of the gradual decrease in cell surface coat thickness for NL and DL cells after cisplatin treatment[6]. The gradual removal of ruthenium red staining substances (mucopolysaccharides) from the fibrosarcoma cell surfaces has also been reported[18].

Sephadex G-50 column chromatographic patterns of proteins of the supernatants of NL and DL cells treated with or without cisplatin (control) show that elution profile, position and number of most of the peaks remain almost the same after cisplatin treatment (figures 2, 3–A, B). Only very slight shift in the fraction or change (increase or decrease) in the heights of some peaks (like peaks IIIA and VI for DL cells, figure 3b) of proteins are observed suggesting that little alteration occurs in the cell membrane proteins after cisplatin treatment of the cells. On the other hand, as expected, enzymatic (particularly trypsin) effect shows some major changes in the peak patterns of NL and DL cells (figures 2, 3–C, D). Earlier studies with lactoperoxidase catalyzed iodination of cell surface proteins of NL and DL cells and cisplatin treatments showed no difference in the banding patterns on polyacrylamide gels for controls and cisplatin-treated cells[6]. Thus it is possible that cisplatin treatment of the cells does not bring about any significant specific change/release of proteins except the release of cell surface sialic acid/mucopolysaccharide residues.

The involvement of sialic acid in the masking of antigens in tumor cells has been suggested[10, 11]. Bekesi *et al*[19] noted that neuraminidase treatment of 6C3 HED lymphosarcoma cells results in a significant increase in the immunogenicity of the tumor cells. They attributed this effect to the enzymatic release of N-acetyl neuraminic acid moieties from the cells. Brazil and McLaughlin[20] showed that *Vibrio cholerae* neuraminidase-treated Landschutz ascites tumor cells, when transplanted, caused considerable reduction in tumor cell number. They suggested the possibility that neuraminidase treatment resulted in the exposure of new antigenic sites on the tumor cell membrane with which cytotoxic factors possibly 'natural antibodies' could react resulting in *in vivo* destruction of the majority of the cells. In the present studies total survival time of the mice after i.p. transplantation of tumor cells (0.5 ml of ascites tumor) is 8 to 10 days. When tumor cells are first incubated with cisplatin (25, 50 μg/ml) *in vitro* before transplantation or cisplatin is injected *in vivo* (8 mg/kg body weight of mice) with tumor cells, there is longer survival of the mice upto 5–6 weeks or no development of tumor. When cisplatin (10 mg/kg body weight of mice) is injected in tumored mice, there is gradual regression of the tumor. On the basis of the release of sialic acid after cisplatin treatment of tumor cells a similar mechanism is suggested in the regression of Dalton's lymphoma and other malignant tumors in general after cisplatin treatment.

14 August 1985; Revised 28 February 1986

1. Rosenberg, B., Van Camp, L., Trosko, J. E. and Mansour, V. H., *Nature (London)*, 1969, **222,** 385.
2. Kociba, R. J., Sleight, S. D. and Rosenberg, B., *Cancer Chemother. Rep.*, 1970, **54,** 325.
3. Sodhi, A. and Aggarwal, S. K., *J. Natl. Cancer Inst.*, 1974, **53,** 85.
4. Prestayko, A. W., D'Aoust, J. C., Issell, B. F. and Cooke, S. T., *Cancer Treat. Rev.*, 1979, **6,** 17.
5. Prasad, S. B. and Sodhi, A., *Chem. Biol. Interact.*, 1981, **36,** 355.
6. Prasad, S. B. and Sodhi, A., *Indian J. Exp. Biol.*, 1982, **20,** 559.
7. Abercrombie, A. and Ambrose, E. J., *Cancer Res.*, 1962, **22,** 525.
8. Ashwell, G., In: *Mammalian cell membranes*, Butterworths, London, Vol. 4, p. 57.
9. Bohn, B., Thies, C. and Brossmer, R., *Eur. J. Cancer*, 1977, **13,** 1145.
10. Currie, G. A. and Bagshawe, K. D., *Br. J. Cancer*, 1968, **22,** 588.
11. Currie, G. A. and Bagshawe, K. D., *Br. J. Cancer*, 1969, **23,** 141.
12. Cormack, D., *Cancer*, 1970, **30,** 1459.
13. Srinivasa Rao, V. and Sirsi, M., *Indian J. Biochem. Biophys.*, 1973, **10,** 37.
14. Warren, L., *J. Biol. Chem.*, 1959, **234,** 1971.
15. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193,** 265.
16. Wagner, R., In: *Introduction to biological membranes*, John Wiley, New York, 1980, p. 343.
17. Tanino, T. and Egawa, K., *Int. J. Cancer*, 1976, **17,** 730.
18. Sodhi, A. and Prasad, S. B., *Indian J. Exp. Biol.*, 1981, **19,** 328.
19. Bekesi, J. G., Roboz, J. P. and Holland, J. F., Israel *J. Med. Sci.*, 1976, **12,** 288.
20. Brazil, J. and McLaughlin, H., *Eur. J. Cancer*, 1978, **14,** 757.

ANNOUNCEMENT

FOURTH ALL INDIA SYMPOSIUM ON ESTUARINE BIOLOGY

It is proposed to conduct the Fourth All India Symposium on Estuarine Biology at the Centre of Advanced Study in Marine Biology during September 1986. Papers are invited bearing on the following aspects of Estuarine Biology: 1. Physical and chemical aspects of estuaries; 2. Biology of estuarine organisms; 3. Embryological studies of estuarine organisms; 4. Physiology and biochemistry of estuarine organisms; 5 Estuarine microbiology; 6. Estuarine ecology and aquaculture; 7. Utilization of estuarine resources and 8. Contributions of estuaries to sea.

Abstracts not exceeding 250 words are invited for the Symposium. For further details contact: K. Krishnamurthy, Convener, CAS in Marine Biology, Annamalai University, Parangipettai 608 502, India.