gen stream. The initial substrate concentration was 1.66×10^{-4}—2.62×10^{-3}M and enzyme concentration was 3.75×10^{-6}M. Observed initial velocities were plotted according to Lineweaver and Burk in order to determine K_m and k_{cat}.[14] In the case of *p*-nitrophenyl ester **1b**, the rate assay was carried out by observing the formation of *p*-nitrophenol at 405 or 320 mμ. The concentration of *p*-nitrophenol produced was calculated taking experimental ε value 17600 (405 mμ) at pH 8.2 or 8200 (320 mμ) at pH 5.4. Three ml of buffer solution (0.05M Tris, pH 8.2 or 0.1M acetate, pH 5.4) and 50 μl of substrate solution (in DMF) were pipetted in a 1 cm quartz cuvette placed in thermostated cuvette holder at 25°. The enzymatic hydrolysis was then initiated by introduction of 50 μl of enzyme solution; $[S]$: 1.02×10^{-5}M—1.02×10^{-3}M, $[E]$: 1.00×10^{-5}M. The normality of trypsin was determined by extrapolating the photometric trace of the steady-state back to zero time and measuring initial burst.[1,4]

Acknowledgement We are grateful to Daiichi Seiyaku Co. Ltd. for supplying *t*-AMCHA.

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Adverse Effect of Antitumor Drugs on the Prevention of Metastasis in Mice

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In 1952 Agosin, *et al.*[2] have found an increase in the incidence of metastases by the treatment of cortisone, although local tumoral growth was found to be diminished. Other investigators[3–7] also have reported that cortisone or hydrocortisone provoke disseminated metastases in the hosts when tumor cells were transplanted intravenously to the mice. The experiment reported here shows a stimulative effect of some antitumor drugs on blood-borne metastasis in mice.

The effect of hydrocortisone treatment 24 hours before, after or simultaneously with the tumor cell inoculation (10^6 and 3×10^6 cells per mouse) is shown in Fig. 1. Similarly, an adverse effect of chemotherapy on the tumor metastasis was observed in all cases so far tested for the treatment of the antitumor drugs (Fig. 2). Twenty four hours previous or simultaneous dosage of alkylating agent, antibiotic, plant-alkaloid, folic acid antagonist, antipyrimidine and antipurine were given to the mice inoculated with 3×10^6 tumor cells. The drugs were given with a dose of about 1/4 LD_{50} in all groups. When the drugs were injected prior to the inoculation of tumor cells, the survival time of most mice were shorter than those of the control. However, the life span of the host animals which had been treated simultaneously with cyclophosphamide or methotrexate was more prolonged than others. Particularly, the

1) Location: *a) Ukima 1-3-32, Kita-ku, Tokyo, 115, Japan; b) Hongo 7-3-1, Bunkyo-ku, Tokyo, 113, Japan.*

2) M. Agosin, R. Christen, O. Badinez, G. Gasic, A. Neghme, O. Pizarro and A. Jarpa, *Proc. Soc. Exp. Biol. Med.*, **80**, 128 (1952).
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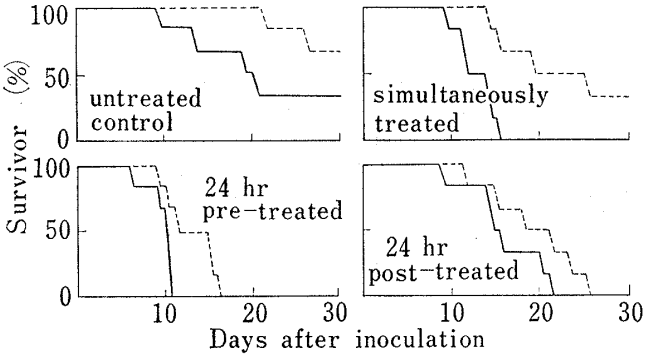

Fig. 1. Mice Receiving Ehrlich Carcinoma Cells intravenously and treated with 2.5 mg of Hydrocortisone Acetate Subcutaneously

———: inoculated with 3×10^6 cells
- - - - - - -: inoculated with 10^6 cells

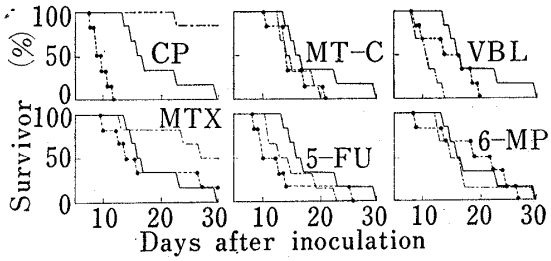

Fig. 2. Mice Receiving Ehrlich Carcinoma Cells intravenously and treated with Antitumor Agents Intraperitoneally

———: untreated control (inoculated with 3×10^6 cells)
●- - - - -●: 24 hours pre-treated
—·—·—·—: simultaneously treated
dose: cyclophosphamide (CP); 100 mg/kg, mitomycin C (MT-C); 1 mg/kg, vinbrastin sulfate (VBL); 0.6 mg/kg, methotrexate (MTX); 20 mg/kg, 5-fluorouracil (5-FU); 40 mg/kg, 6-mercaptopurine (6-MP); 50 mg/kg

groups treated with cyclophosphamide showed typical patterns of their life prolongation curves. As shown in Table I the life span of the mice was observed for 30 days when a relatively small number of tumor cells was inoculated intravenously after the injection of cyclophosphamide. The survival time of the animals was not changed for 30 days among the groups treated and untreated with the drug, when the inoculum size was 10 to 10^4 cells. Antimetastasizing effect of cyclophosphamide was marked when given simultaneously with or after the inoculation of the cells in single administration but not so marked in the repeated treatments as described in the preceding report.[8] A single dosage of cyclophosphamide was reported to be very effective in prolonging the survival of homografts in the mouse if given 2 days after the grafting,[9] and the drug also suppresses antibody production most effectively if given after the antigen.[10] Since most antitumor drugs including those now employed belong to immunosuppressors, it is suggested that the stimulative effect of antitumor drugs on the progression of metastasis is ascribed to the immunosuppressive effect of these drugs.

Table I. Mice Receiving Ehrlich Carcinoma Cells of Various Inoculum Size intravenously and 24 Hours pre-treated with 100 mg/kg Cyclophosphamide intraperitoneally

Tumor cells in inoculum	Survivor for over 30 days per total treated	
	Control	Treated
10—10^4	6/6[a)]	6/6[a)]
10^5	6/6	4/6
10^6	3/6	0/6

a) All animals in each group were survived.

In the chemotherapy of tumor metastasis, it must be considered carefully for the schedule of the dosage of drugs, because it may possibly exert an adverse effect on the chemotherapy. The present experiment will provide a typical pattern of this adverse effect.

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Experimantal

The animals used in these studies were male ddY mice of about 5 weeks old, weighing 19—23 g. They were fed with standard diet CE-2 (CLEA Japan Inc., Tokyo) and with unlimited supply of water. The mice were supplied from an animal farm in Shizuoka Prefecture, Japan. Ehrlich ascites carcinoma cells used were the LP-12 cell line[11] which have been maintained by a serial intraperitoneal transplantation into ddY male mice. Tumorcells obtained from ddY mice 7 days after inoculation were employed as a material for the experiment. A 0.2 ml $NaHCO_3$-free Tyrode suspension of the LP-12 cells was transplanted into tail vein of the mice. Hydrocortisone acetate was injected subcutaneously and other antitumor drugs intraperitoneally. The treatment with the drugs performed 24 hours before, after or simultaneously with the transplatation of the tumor cells. Six mice in a group were employed for each trial.

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Factors Affecting Dissolution Rate of Cellulose Acetate Phthalate in Aqueous Solution[1,2]

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Various polyelectrolytes which are insoluble in acidic and soluble in neutral or weakly alkaline media have been available to enteric coating. However, precise dissolution studies of them have never been done, which should be important in discussing the drug availability or in finding the means useful to the quality control of enteric coated preparations.

In previous papers,[1,4] the dissolution of polyvinylpyrrolidone (PVP) was investigated in acetone-water system to make an approach to an understanding of general dissolution behaviors of synthetic polymers. Although generally PVP is not included in the category of polyelectrolytes, its dissolution rate was influenced by the addition of NaCl,[4] and from the viscosity measurement in water the low molecular weight fractions of PVP was considered to behave as a polyelectrolyte.[1] Accordingly, it seems significant to investigate the dissolution behaviors of intrinsic polyelectrolytes, analyzing the factors affecting the charges of solute molecules, such as pH and ionic strength of solution.

From the above points of view, in the present study, the dissolution of cellulose acetate phthalate J.P. VII Part II (CAP), which is popular and commercially available as an enteric coating material and is quantitatively determinable by ultraviolet (UV) absorption method, was investigated in various buffer solutions according to the rotating disk method.

Experimental

Materials——CAP obtained commercially was purified by a Soxhlet's extractor with ether, and the dried fraction passing through a 150 mesh (104 μ) sieve was used as the sample, containing less than 0.5% of free

1) This paper forms Part XV of "Physico-chemical approach to Biopharmaceutical Phenomena." Preceding paper, Part XIV: H, Nogami, T. Nagai, and A. Kondo, *Chem. Pharm. Bull.* (Tokyo), **18**, 2290 (1970).
2) This work is outlined in Abstracts of Papers, 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, July 1970, No. OB10-6.
3) Location: *Hongo, Bunkyo-ku, Tokyo.*
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