LEVELS OF LYSOSOMAL HYDROLASES DURING GROWTH OF YOSHIDA ASCITES SARCOMA

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ABSTRACT

The profiles of six lysosomal acid hydrolases, *viz.*, acid phosphatase, acid ribonuclease, β-glucuronidase, arylsulfatases A and B, and cathepsin D were studied during the progression of the fast growing Yoshida ascites sarcoma. The initial high activities of the enzymes declined with tumour age during the period of active cell multiplication. However, they showed marked increase during the terminal phase when there was increased cell loss due to cell destruction and autolysis. An increase in the proportion of free activity to bound levels was also found during the terminal phase. This shift towards the "free" state may reflect intra-cellular release of the enzymes in the older tumour cells.

INTRODUCTION

LYSOSOMAL enzyme activities are known to increase during processes involving tissue degradation, as in regression of tumours, either spontaneous or induced by irradiation[1] or hormonal deprivation[2] or treatment with drugs[3]. Studies on solid tumours indicated that hydrolase levels remain low in regions of active growth, but are high in necrotic or senescent areas[4]. There is scanty information about the relationship of lysosomal enzyme levels to growth of ascites tumours where there is no demarcation between growing and senescent regions. However, in an ascites tumour like Yoshida ascites sarcoma (YAS), after rapid proliferation during the first few days, involution as well as loss of a certain population of cells from the proliferating fraction occur. Therefore the involvement of lysosomes in this process was studied and quantitated and the results are presented below.

MATERIALS AND METHODS

Tumour.—Yoshida ascites sarcoma was maintained in Wistar A/Iisc rats by serial intraperitoneal transplantation of 30–40 million cells every 4th day. On various days after transplantation, the tumour cells were collected in 10-fold excess of physiological saline from the peritoneal cavity after sacrificing the animal by cervical dislocation. The washings of the peritoneal cavity were pooled with the original sample and the YAS cells were counted in a haemocytometer.

Preparation of cell-free extract.—All operations were carried out at 4° C unless stated otherwise. The ascites cells in saline suspension were freed from contaminating erythrocytes by centrifuging and washing with hypotonic saline and finally centrifuged at 10,000 g to give a hard cell pellet. The cells were suspended in 0·25 M sucrose (10% w/v suspension) and sonicated for 5′. The sonicate was then centrifuged at 10,000 g for 20′. The supernatant (cell-free extract) was used immediately to assay the total enzyme activities.

Preparation of subcellular fractions.—The hard cell pellet obtained as above was ground in an all-glass mortar for 10′; phase contrast microscopy at this stage revealed more than 95% cell breakage with little damage to the particles. Differential centrifugation was used to separate nuclei and cell debris (1,200 g pellet), particulate or lysosomal fraction (15,000 g pellet), microsomes (105,000 g pellet) and cytosol (105,000 g supernatant). The lysosomal fraction was suspended in 0·25 M sucrose.

Assay of enzymes.—Acid phosphatase[5] and β-glucuronidase[6] were assayed by the hydrolysis of their respective *p*-nitrophenyl esters under optimal conditions of temperature and pH. Arylsulfatases A[7] and B[8] were assayed with *p*-nitrocatechol sulfate as substrate and cathepsin D[9] by following the hydrolysis of acid-denatured bovine haemoglobin at pH 4·0. Acid ribonuclease[10] was assayed by the release of acid soluble nucleotides from purified yeast RNA. In the case of the lysosomal fraction, the enzymes were assayed in the presence of 0·1% Triton X-100.

All activities were expressed as μ moles of product formed/hr/mg protein except acid ribonuclease, where the activity was expressed as units/mg protein. One unit of activity was that amount of enzyme which caused an OD change of 1·0 at 260 nm at 37° C in 30 min.

RESULTS AND DISCUSSION

The growth curve of YAS is shown in Fig. 1. There was rapid growth during the first three days. The cell population reached a maximum on the fourth day and thereafter declined. The mean survival period of the tumour bearing rats was six days. The mean growth rate showed continuous deceleration even during the period of rapid growth, and fitted well with a Gompertzian function[11]. The retardation of growth in the later stages may reflect a situation similar to that in Ehrlich ascites tumour[11] where a growth retardation results from

a prolongation of cell cycle time and a moderate decline in the proliferating fraction with a slow increase in the rate of cell loss.

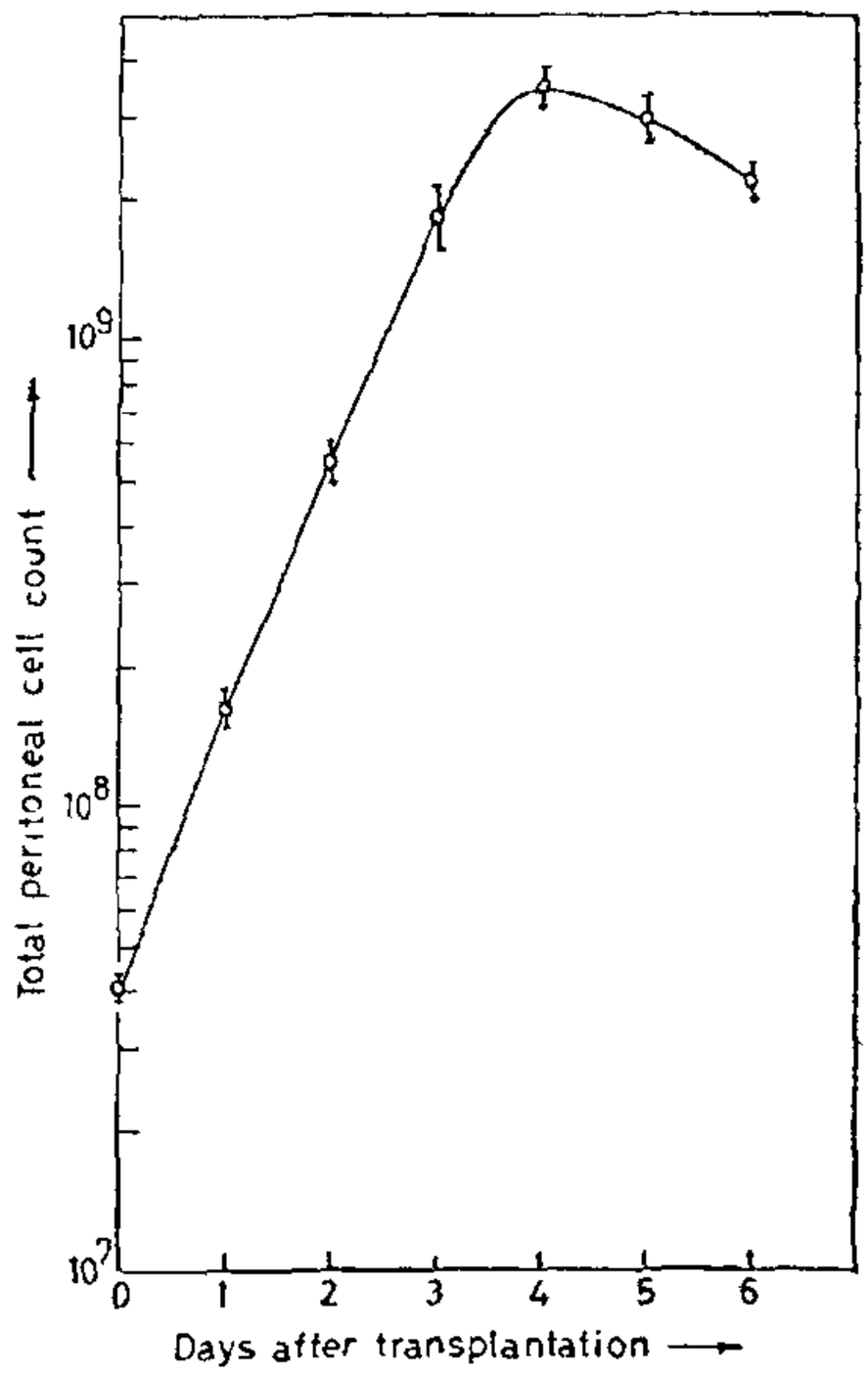

FIG. 1. Growth curve of Yoshida ascites sarcoma *in* Wistar A/Iisc rats. Each point represents mean ± S.E. of 5 experiments.

Figures 2 and 3 show the profiles of the six lysosomal hydrolases during tumour progression. All of them followed, in general, the same pattern of a decline *in* activity during the period of rapid growth. Following transplantation, except the stem-line cells, most of the mature YAS cells which form the major population in the inoculum undergo degeneration. This could account for the high activities of the lysosomal enzymes on the first day, since they are involved in tissue degradation. Thereafter the stem-line cells start rapid multiplication until the fourth day, when the proportion of cells undergoing degeneration and lysis again increases. Associated with this process there is a rise in lysosomal activity also.

The data in Table I indicate that the percentage of free activity was the highest when the tumour was in the senescent or regressing stage. This would indicate an internal labilization of the lysosomal membrane coupled with elevated activities of the lysosomal enzymes. This would explain the cytological changes reported[12]. Most of the cells of old YAS tumours undergo vacuolation of cytoplasm, karyolysis and chromosomal breakages and autolysis, leaving a population of dormant "stem-line cells" which proliferate when transplanted to

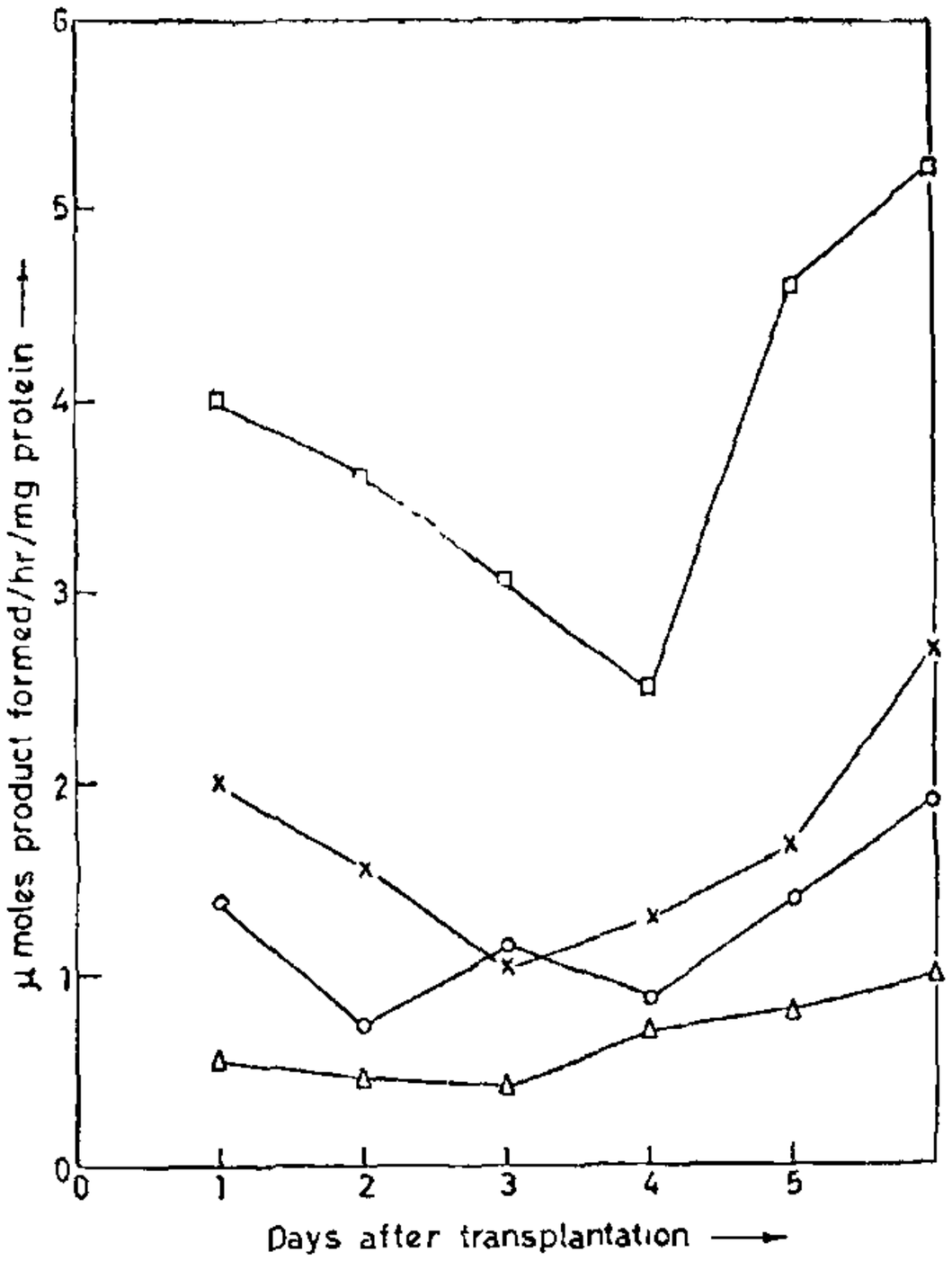

FIG. 2. Levels of four acid hydrolases (free *plus* bound) in YAS during tumour progression. Data are mean of three experiments.
□—□ acid phosphatase
△—△ β-glucuronidase
○—○ arylsulfatase A
×—× arylsulfatase B

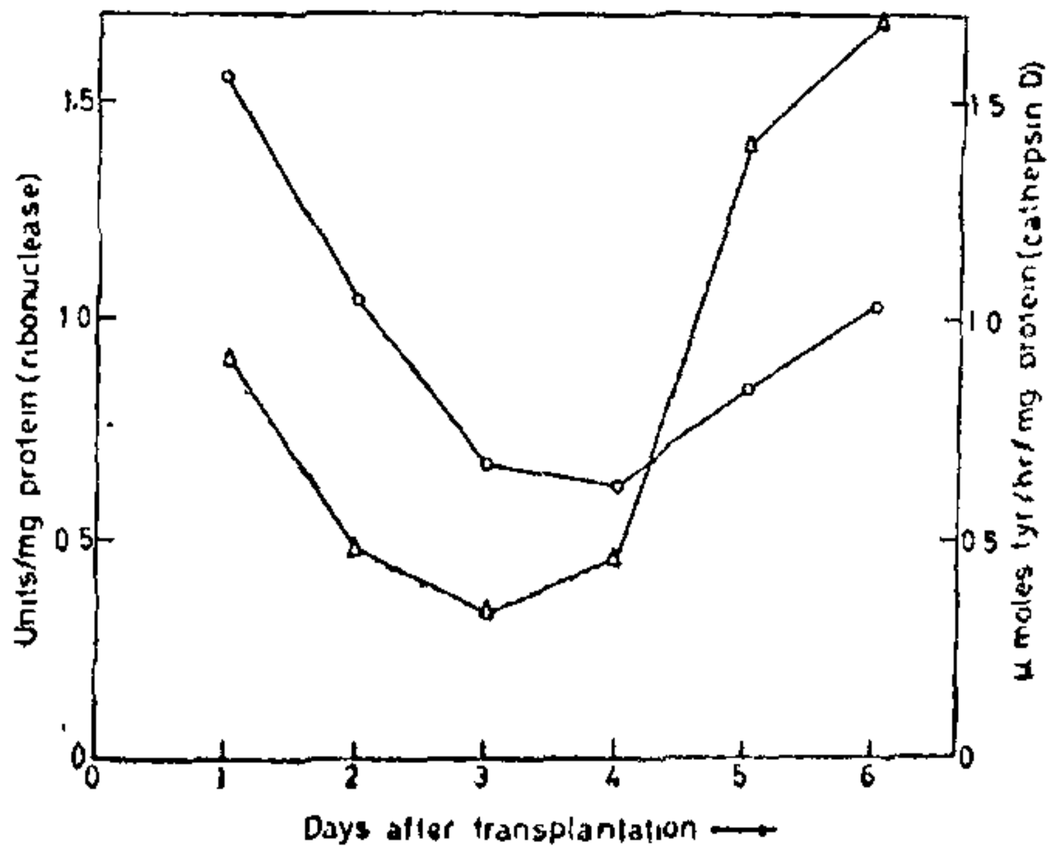

FIG. 3. Levels of acid ribonuclease and cathepsin D (free *plus* bound) in YAS during tumour progression. Data are mean of three experiments.
○—○ acid ribonuclease
△—△ cathepsin D

TABLE I

Percentage free activity of lysosomal enzymes during growth of Yoshida ascites sarcoma*

Enzymes	% Free activity				
	Days				
	1	2	3	4	5
Acid phosphatase ..	12·00	8·20	6·00	7·80	20·00
Acid ribonuclease ..	6·00	5·28	4·32	8·91	10·00
β-Glucuronidase ..	6·50	6·41	3·78	4·52	7·83
Arylsulfatase A ..	3·00	0 80	0·93	1·26	3·29
do. B ..	2·87	0·72	0·83	1·73	4·21
Cathepsin D ..	20·09	18·28	11·00	22·61	29·20

Data are mean of three experiments.

* Free activity was calculated by the formula $F/B+F \times 100$, where 'F' is free activity in cytosol, and 'B' is the bound activity in the lysosomal fraction.

new hosts. Parry and Ghadially[13] reported an increase in lysosomes during tumour growth and their rupture during the terminal phase.

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CYTOLOGICAL STUDIES IN NORMAL AND MUTAGEN TREATED STRAINS OF TRITICALE (*TRITICALE HEXAPLOIDE*, LART)

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CYTOGENETIC investigation in many strains of 6 x triticales have demonstrated significant differences in meiotic instabilities, frequency of aneuploids and kernel fertility. Sporadic attempts have been made to increase the fertility level and quality of 6 x triticale, which is superior in seed and fertility characteristics to the 8 x forms but still not as good as wheat[1-3]. Ruebenbaver and Nalepa[4] have, however, demonstrated that exposure to ionizing radiation can lead to the isolation of mutants with higher seed fertility and increased winter hardiness in triticales. Our main objective in the present study was to develop some 6 x triticale lines which will have meiotic stability, pollen viability and kernel fertility comparable to wheat.

We treated three strains of 6 x triticales, *viz.*, Arm. 130, PC 186 and BC 245 with gamma-rays (5, 10, 15 Kr), EMS (0·15%, 0·30% and 0·45%) and gamma irradiation *plus* EMS in combination (5 Kr + 0·15% EMS, 10 Kr + 0·15% EMS, and 15 Kr + 0·15% EMS) to see whether mutagen treatment is helpful in improving the reproductive behaviour. Normal and mutagen treated seeds were grown in the field in *Rabi*, 1972. Meiosis was studied in both normal and mutagen treated M_1 populations. The young spikelets were fixed in a freshly prepared mixture of absolute alcohol and acetic acid (3 : 1) for 24 hr and finally stored in 70% alcohol. The anthers were then squashed in acetocarmine and meiosis was studied in detail. The frequency of univalents was observed at first metaphase, frequency of lagging chromosome and of chromatin bridges at first anaphase and frequency of micronuclei in tetrads. Pollen fertility was recorded as count of fertile pollen seen as completely filled, round and deeply stained with acetocarmine under microscope. Percent kernel setting was estimated for each treatment after counting the number of spikelets per spike. One hundred kernel weight in gm was also recorded.

The two strains Arm 130 and B C 245 were found sensitive to 5, 10, 15 Krs gamma irradia-