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Characteristics of 6-Mercaptopurine-Resistant L1210 Cells Newly Developed by *in vivo* and *in vitro* Treatment with 6-Mercaptopurine

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Summary. **6-Mercaptopurine-resistant sublines of L1210 cell were obtained by *in vivo* and *in vitro* treatment with 6-mercaptopurine. These 27 sublines were classified into high- and low-resistant sublines. The MAC level of the high-resistance group was 0.3 μg/ml, and that of the low-resistance group was 0.3-1 μg/ml. The sensitive parental cells showed a MAC of 0.003 μg/ml. Most of the resistant sublines kept their MAC levels stable even after 9 months of serial subcultures in a drug-free medium, though some sublines changed their MAC levels.**

The level of [^{14}C] 6-MP incorporation by high-resistant cells was the lowest among three types of subline, that of the sensitive cells was the highest, and that of the low resistant cells was intermediate.

The thioinosinate level of the sublines was estimated by Dowex-1 column chromatography. More thioinosinate was found in cell-free lysate of the resistant subline than in the sensitive subline. Activity of hypoxanthine guanine phosphorybosyl-transferase in cell-free lysate was also estimated. These sublines showed the same level of enzyme activity in contrast to the difference in the thioinosinate level.

Both the 6-MP-resistant and cytosine-D-arabinofuranocide (ara-C)-resistant cells were examined reciprocally for incorporation of 6-MP and ara-C. Neither resistant sublines incorporated the corresponding drug, thus indicating that the 6-MP resistance mechanism is specific for purines.

We conclude that the 6-MP resistance of the L1210 cell is due to a defective situation of cell membrane permeability or to an active exclusion system of metabolite.

Cancer cells resistant to carcinostatic agents are suspected to be obstructive factors in chemotherapy. In experimental studies, many kinds of drug-resistant sublines have been obtained in cultured cell lines, and efforts have been made to elucidate the mechanisms of drug resistance of these cultured cell lines. Kessel et al. reported that actinomycin D-resistant (12) and daunomycin-resistant (13) L1210 and P388 cells manifested drug retention levels. Methotrexate-resistant L1210 cells were found to have a reduced drug incorporation level (15), and the lower influx of the drug into daunomycin-resistant Ehrlich ascites tumor cells rather than into the drug-sensitive parental cells has been reported (21). A 6-mercaptopurine (6-MP)-resistant Ehrlich ascites tumor cell line was found to exhibit reduced purine ribonucleoside kinase (3), and 6-MP-resistant L1210 cells had no permeability barrier to the entry of this agent (7, 8).

We obtained two types of 6-MP-resistant murine L1210 cell lines by chemotherapeutic treatment of animals and by *in vitro* exposure of cells to drugs. We now report the biological and biochemical characteristics of these 6-MP-resistant sublines and discuss possible mechanisms of the resistance.

MATERIALS AND METHODS

Animals. DBA/2 mice were supplied by the Animal Laboratory of Gunma University. Both sexes of mice were used with a body weight of 20±1 grams.

Tumor cell line. Murine leukemia cells, L1210v/c, were kindly supplied by Dr. M. Shimoyama, National Cancer Center Hospital, Tokyo. The cells were highly tumorigenic in DBA/2 mice; an intraperitoneal (i.p.) injection of 10^6 cells resulted in death by day 7 or 8 after injection.

Drugs. 6-Mercaptopurine (6-MP) was purchased from Kojin Co., Ltd., Tokyo; and [^{14}C] 6-MP (New

England Nuclear Corp., 6.03 mCi/mmole), [^{14}C] adenine (Amersham Radiochemical Centre, 58 mCi/mmole), and [^{3}H] cytosine-D-arabinofuranoside (ara-C, the same centre, 24 Ci/mmole) were purchased from the Japan Isotope Association.

Medium and culture. The standard medium for the floating culture consisted of 20% calf serum (Flow Lab. Inc.), 80% RPMI1640, (Nissui Pharmaceutical Co., Ltd., Tokyo), and antibiotics (50 μg/ml streptomycin and 50 unit/ml penicillin). This medium was used for the maintenance of the L1210 line and its sublines.

Agar cloning technique. L1210 cells (10^2) were inoculated onto soft agar plates which were prepared by the method of Kuroki (14), by using 0.5% agar (Noble agar, Difco) in the above medium. The cultures were incubated for 2 weeks at 37℃ in a humidified atmosphere (5% CO_2, 95% air), and a single clone or a cell colony was obtained. The cell plating efficiency was 89±7.7% at the late exponential stage. To estimate the level of 6-MP-resistance, cell growth was examined on soft agar plates containing various concentrations of 6-MP.

Techniques for isolating 6-MP resistant cells. Five-to-six week old mice were i.p. inoculated with 10^4 L1210 cells. Starting with the fifth post-inoculating day, they were i.p. injected with 6-MP dissolved in physiological saline every 3 or 4 days. By the 2-to-3 post-inoculating week (the ascites looked bloated enough to cause death due to the growth of L1210), ascites was withdrawn with a syringe, and 10^4 cells were i.p. transferred to another mouse in order to continue the 6-MP therapy. The 6-MP dose was increased after a transfer of the proliferated L1210 cells. The dose range was 1 to 20 mg/kg body weight. 6-MP-resistant sublines were isolated from mouse ascites after one or several i.p. transfers of the L1210 cells. A portion of the peritoneal ascites cells was suspended in a culture medium and adjusted to a concentration of 10^7 cells/ml, 0.1 ml of a cell suspension, diluted 10-fold, and inoculated onto the soft agar plates containing various concentration of 6-MP. 6-MP-resistant cells were obtained by plating 10^3-10^6 L1210 cells which passed through the above mouse peritoneal cavity for each plate. After 2 weeks of cultivation at 37℃, 6-MP-resistant colonies were picked up from the agar plates. The level of resistance was expressed in terms of MAC, i.e., the maximum 6-MP concentration in the agar plate which still allowed the growth of L1210 cells as a visible colony.

Sublines obtained through in vivo and in vitro experiments. The high-resistance sublines (MAC≧30 μg/ml) obtained by *in vivo* treatment of L1210-bearing mice were R_2, R_8, R_{10}, R_{13}, R_{17}, A_{10-1}, A_{10-4}, $B_{0.3-2}$, $B_{0.3-5}$, B_{3-2}. Highly resistant sublines obtained by inoculating L1210 cells onto 6-MP containing agar plates were D_9 and D_{11}. The low-resistance sublines (MAC=1.0−0.3 μg/ml) obtained by the *in vivo* experiments were R_1, R_{12}, R_{16}, R_{18}, K, B_1, C_1, C_2, C_6, C_7, and C_8; those obtained by the *in vitro* experiments were D_1, D_2, D_3, D_4, and D_6. Sensitive cells (MAC < 0.03 μg/ml), N, S, and Natsu were maintained in different subcultures of the original L1210 cells; E_1, E_2, E_3, E_4, and E_5 were sensitive cells obtained through drug-free subcultures of the R_{16}, low-resistance sublines.

Preservation of sublines. Original and newly obtained sublines were maintained by subcultures in a drug-free medium or in a frozen state (−80℃) in a medium containing 12.5% dimethylsulfoxide.

Incorporation of drugs. For incorporation experiments, the cells were adjusted to $2.5\times10^6-1\times10^7$ per ml, a half of the cell suspension was centrifuged, and precipitated cells were suspended in a 0.5 ml medium containing 1 μCi/ml [^{14}C] 6-MP. To assess the incorporation of adenine or ara-C, a medium containing either 0.1 μCi/ml [^{14}C] adenine or 0.25 μCi/ml [^{3}H] ara-C was used. After 0, 1/2, 1, 2, 3 h of incubation at 37℃, the cells were washed three times with 10 ml of chilled phosphate buffered saline (PBS, pH 7.2). Soluen 350 (Packard Instrument Co. Inc.) was added, and the cells were allowed to stand at room temperature for 18 hours. Radioactivity in the cells was counted on a scintillation counter (Packard, Model 3385 Tri-carb) after adding 10 ml of scintillator cocktail to the solubilized cells.

Assay of HGPRT activity. A crude extract of hypoxanthine guanine phosphoribosyltransferase (HGPRT) was obtained from the cells by a modification of the method of Roussos (18). Briefly, 10^8 to 10^9 cells from sublines grown in the peritoneal cavity of DAB/2 mice were homogenized in a glass homogenizer by 50 pestle strokes in the presence of a 0.05 M potassium phosphate buffer (pH 7.4) which was three times the weight of the cells and which contained 1 mM glutathione in reduced form and 10 μM EDTA. The homogenate was then centrifuged at 5,000×g for 30 min. The assay method of HGPRT activity was that described by Flaks (9). The reaction mixture consisted of 2.0 ml [^{14}C] guanine (0.2 μmole, specific activity; 51 mCi/mmole), 0.08 ml $MgCl_2$ (8 μmole), 0.08 ml Tris chloride buffer (pH 8.0, 80 μmole), 0.4 ml phosphoribosyl pyrophosphate (PRPP, 0.4 μmole), and 1.4 ml crude extract. This mixture was incubated for 20 min at 37℃, and the reaction

was terminated by exposing the mixture to 100℃ for 1 min. Denatured protein was removed by 10 min centrifugation at 3,000 rpm, and the supernatant fluid was passed through a 3×1 cm Dowex-1 column. Unreacted guanine was removed from the column by elution with 50 ml 0.002 N HCl, guanosin-5'-monophosphate (GMP) was eluted with 10 ml 0.1N HCl, and the aliquots were assayed for their radioactivity. The entire procedure was also performed by using blanks.

Thioinosinate assay. Following the procedures used in the 6-MP incorporation experiments, $5\times10^6-1\times10^7$ cells were incubated at 37℃ for 2 h with 0.5 μCi of 6-MP and destroyed with 0.5% Triton X-100. The supernatant was applied on a Dowex-1 column by using the same procedures as described for an HGPRT assay, except for cell washing and Soluen treatment.

RESULTS

Stability of 6-MP resistance during subculture. Twenty sublines of 6-MP-resistant L1210 cells obtained by *in vivo* 6-MP treatment of mice were isolated on soft agar plates. After we estimated the level of 6-MP-resistance, each colony was transferred to a testtube and cultured at 37℃ in 1 ml liquid medium. One-tenth of the cell suspension was harvested and transferred to a fresh medium every 4 days. The cells divided 8 times during one transfer generation. No remarkable change in the 6-MP resistance level was observed after 3 transfer generations. During further subcultures for 9 months in a drug-free medium, only the R_{11} subline exhibited a loss of resistance. This subline had initially been highly resistant to 6-MP; however, it became low-resistant after 4 months of subcultures. After 9 months of subculture, its resistance dropped to the level of sensitive cells. Table 1 shows the stability of 6-MP resistance during prolonged subculture in 10 of the resistant sublines tested.

[^{14}C] 6-MP uptake. The [^{14}C] 6-MP uptake of the high-resistant (R_{13}), low-resistant (R_{16}), and sensitive cells (N) was compared. A culture medium (0.5 ml) containing 1.0 μCi/ml [^{14}C] 6-MP was added to glass tubes containing 2.5×10^6 packed sensitive cells, and the [^{14}C] 6-MP uptake of 3 identical samples was counted every 2 h of incubation.

As shown in Fig. 1, incorporation by the high-resistant subline was lower than that of sensitive cells; the low-resistant subline was intermediate between the sensitive and high-resistant cells. The result of the dye exclusion test and colony-forming efficiency of sensitive cells treated for 4 h with a 6-MP concentration of 70 μg/ml, which corresponded to 1 μCi/ml [^{14}C] 6-MP, were the same as that of untreated control cells.

Table 1. Stability of 6-MP resistance of L1210 sublines during serial subculture in drug-free medium

Drug-resistant sublines	Number of colonies on 6-MP-containing soft agar plates (μg/ml)														
	at time 0					after 4 months of subculture					after 9 months of subculture				
	30	3	0.3	0.1	0.03	30	3	0.3	0.1	0.03	30	3	0.3	0.1	0.03
R_1 (l)	—	—	60	85		—	—	42	90		—	—	—	63	86
R_2 (h)	80	94				85	92				103				
R_7 (h)	75	82				81	90				82	93			
R_8 (l)	—	70	88			—	71	92			98	107			
R_{10} (h)	81	98				98					95				
R_{11} (h)	90					—	34	63	92		—	—	—	—	90
R_{12} (l)	—	26	77	91		—	15	61	88		—	12	25	31	98
R_{13} (h)	75	88				91					95				
R_{17} (h)	85	91				83	95				80	91			
R_{18} (l)	—	—	33	51	88	—	—	34	60	81	—	—	30	58	84

(l): low resistant at time 0
(h): high resistant at time 0
— : no colony was formed
Blank spaces: experiments were not done

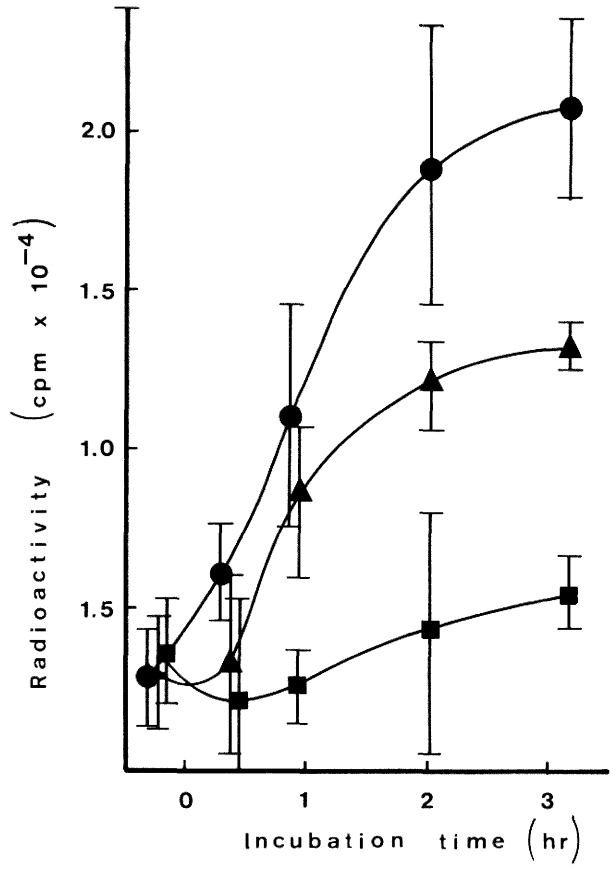

Fig. 1. Time course of 6-MP uptake.
Cells (2.5×10^6) were cultured at 37℃ in 0.5 ml of medium containing 0.5 μCi (correspond to 35 μg/ml) of [^{14}C] 6-MP. At indicated times, samples were washed three times with saline, and [^{14}C] 6-MP uptake was determined. Each point indicates the mean of 3 samples. Vertical bars indicate standard deviations; ●, 6-MP-sensitive cells (N); ▲, low-resistant cells (R_{16}); ■, high-resistant cells (R_{13}).

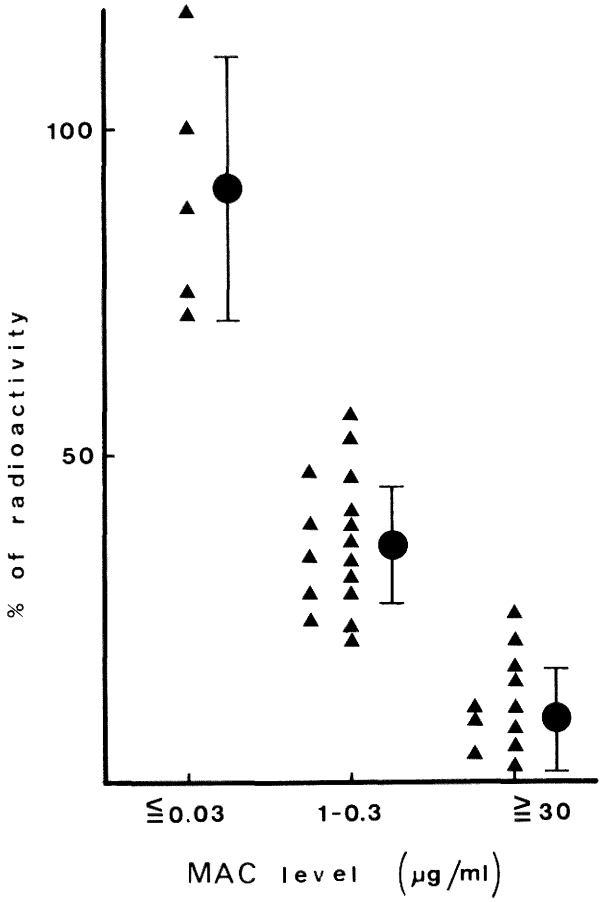

Fig. 2. Correlation between the level of 6-MP resistance and degree of [^{14}C] 6-MP incorporation.
6-MP-resistant sublines and -sensitive cells of L1210 were divided into three groups according to their level of 6-MP resistance. Cells were cultured at 37℃ in 0.5 ml of medium containing 0.5 μCi (corresponding to 35 μg/ml) of [^{14}C]6-MP for 2 hours. Data are shown in the terms of percent of [^{14}C] 6-MP uptake by sensitive cells. Open circles indicate the mean of[^{14}C]6-MP incorporation for each group; Vertical bars indicate the standard deviation.
MAC$<$0.03 μg/ml, sensitive cells, n=5
MAC=0.3−1.0 μg/ml, low-resistant sublines, n=16
MAC$\geqq$30 μg/ml, high resistant sublines, n=11

Resistance level and [^{14}C] 6-MP uptake. To assess the correlation between the degree of [^{14}C] 6-MP incorporation and the level of 6-MP-resistance, 5 sensitive, 11 high-resistant, and 15 low-resistant sublines were tested (Fig. 2). The mean±standard deviation incorporation of sensitive cells was 90.6±6.7% of parental L1210 cell (S), followed by the low-resistant (35.6±10.7%) and high-resistant (9.8±6.7%) sublines.

Incorporation of [^{3}H] ara-C and [^{14}C] adenine.[^{3}H]ara-C uptake was almost the same in the high- and low-6-MP-resistant sublines and in 6-MP sensitive cells, and very little uptake was shown by the ara-C-resistant cells (Fig. 3). The incorporation of [^{14}C] adenine by the high- and low-6-MP-resistant sublines was almost identical (not shown). These data suggest that the 6-MP resistance is specific for purines.

Change of 6-MP resistance level and its effect on 6-MP incorporation. Several sublines with altered 6-MP sensitivity were obtained through a 4-week subculture of R_{16} (6-MP low-resistant) cells. E_1, E_2, E_3, E_4, and E_5 were an R_{16} derived strain which showed the same 6-MP-resistance level as the sensitive parental line. [^{14}C] 6-MP uptake by these strains was almost the same as that of 6-MP-sensitive origi-

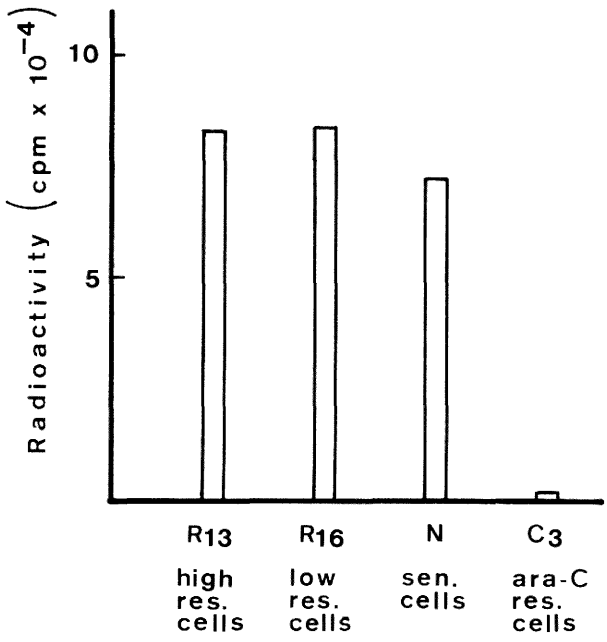

Fig. 3. Incorporation of[^{3}H] ara-C.
Cells (2.5×10^6) were cultured in 0.5 ml of medium containing 0.25 μCi/ml of [^{3}H] ara-C. For incubation and determination procedures, see the caption to Fig. 1.
R_{13}: 6-MP high-resistant subline
R_{16}: 6-MP low-resistant subline
N: 6-MP sensitive cells
C_3: 6-MP sensitive, ara-C resistant cells (ara-C MAC= 30 μg/ml).

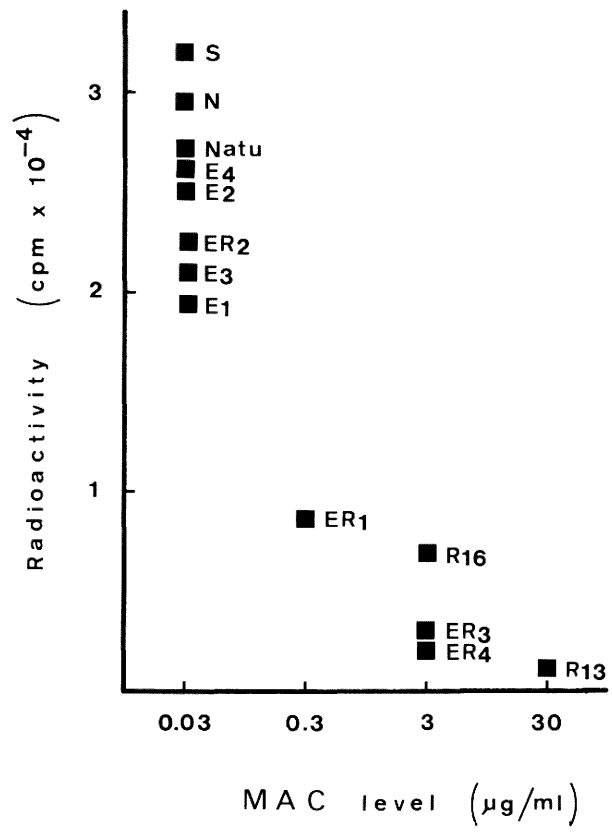

Fig. 4. Correlation between resistance level to 6-MP and incorporation of[^{14}] 6-MP among various resistant and sensitive cells.
Cells (5×10^6 /0.5 ml) were cultured for 2 hours at 37℃ in medium containing 0.5 μCi of [^{14}C] 6-MP.
S, N, Natsu: 6-MP sensitive cells.
R_{16}: low-resistant subline.
R_{13}: high-resistant subline.
E_1, E_2, E_3, E_4, E_5: R_{16}-derived sensitive cells.
ER_1, ER_2, ER_3, ER_4: R_{16}-derived restored cells after temporary abolishment of 6-MP resistance.

nal cells (N). Substrain ER_1, ER_2, ER_3, and ER_4 regained their resistance to 6-MP after a temporary loss. They incorporated 6-MP, almost the same as in R_{16} cells (Fig. 4).

Thioinosinate synthesis by 6-MP-resistant and -sensitive cells. We examined whether the amount of thioinosinate synthesized in proportion to 6-MP uptake and blocked nucleic acid metabolism might be related to the expression of 6-MP-resistance. After incorporation of [^{14}C] 6-MP, cell lysates were prepared with Triton-X, and the amounts of thioinosinate synthesized by 6-MP-sensitive and -resistant cells was compared, by using Dowex-1 column chromatography (Fig. 5). The first broad peak eluted with 0.002 N HCl coincided with 6-MP; the second peak eluted with 0.1N HCl represented a 6-MP ribotide. Sensitive cells manifested remarkably high radioactivity of 6-MP ribotide and a broad radioactive peak of 6-MP. No remarkable peak was recognized in the resistant cells by elution with 0.1N HCl; only a small amount of radioactivity, corresponding to 6-MP ribotide, was observed. These findings confirmed that the resistant cells synthesized thioinosinate at a reduced level as a result of decreased 6-MP incorporation.

Comparison of the HGPRT level among sublines. As shown in Fig. 6, 6-MP-sensitive (N) and low- (R_{16}) and high- (R_{13}) resistant sublines showed common peaks due to GMP during elution with 0.1N HCl. When the enzyme-free reaction mixture was eluted, a peak by guanine was obtained during elution with 0.002N HCl. It is indicated that the cells exhibiting reduced incorporation also possess the enzyme at the same level.

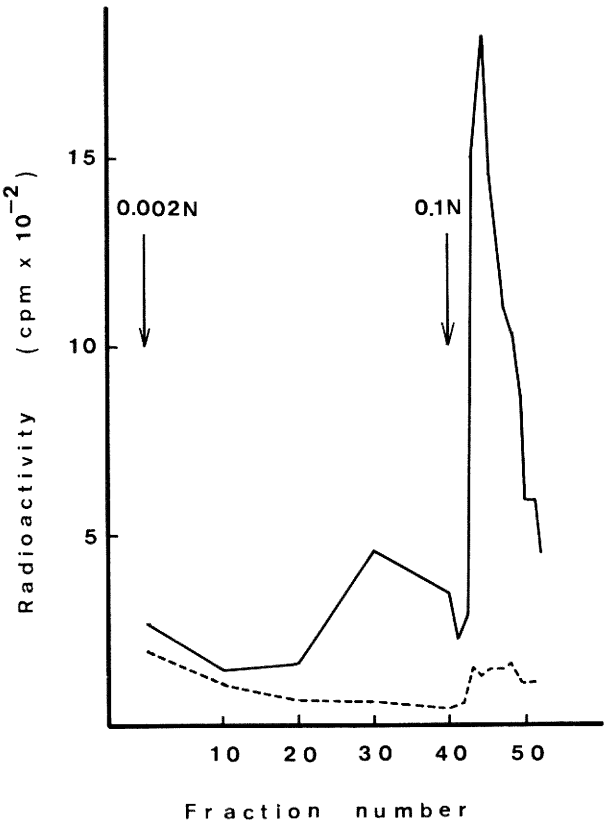

Fig. 5. [^{14}C] thioinocinate in 6-MP low-resistant and sensitive cells.

Cells (5×10^6) were cultured with 0.5 μCi (35 μg) of [^{14}C] 6-MP for 2 hr at 37℃ and then destroyed with Triton X-100. The supernatant was chromatographed through a Dowex-1 column. Untreated [^{14}C] guanine was removed by elution with 0.002N HCl; [^{14}C] GMP was eluted with 0.1N HCl.

——— 6-MP sensitive cells (N).
..... low-resistant subline (R_{16}).

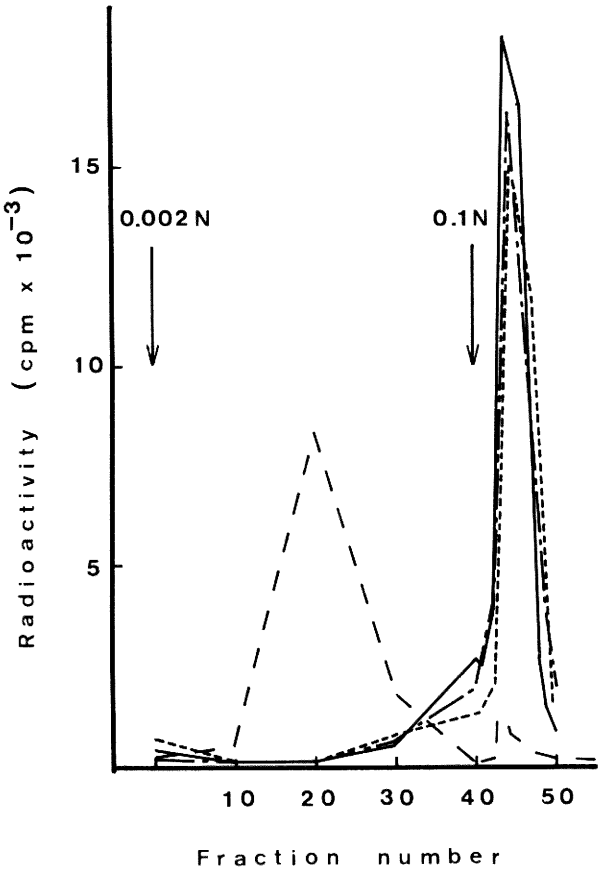

Fig. 6. HGPRT activity of 6-MP sensitive cells and low- and high-resistant sublines.

Cells (5×10^8) were homogenized and centrifuged: The supernatant was reacted for 20 min at 37℃ in the presence of [^{14}C] guanine (0.002 μmole, specific activity, 51 mCi/mmole) and centrifuged. For elution conditions, see caption of Fig. 5.

——— 6-MP sensitive cells (N).
..... low-resistant subline (R_{16}).
-------- high-resistant subline (R_{13}).
---------- enzyme-free control.

DISCUSSION

Of 20 6-MP-resistant sublines obtained by treating L1210 bearing mice with 6-MP, 19 were stable in their resistance, although one sublines (R_{11}) lost its resistance during subsequent cultures in a drug-free medium. The stability in resistance of the 19 6-MP-resistant cell lines suggests that their resistance is not a temporal change in cell physiology. The unstable sublines may suggest the existence of extra-chromosomal genes for 6-MP resistance. While some workers have reported the existence of cytoplasmic genes for drug resistance in cultured cell lines (1, 2, 23, 24), it is presently unknown whether our variant possesses this cytoplasmic gene.

For the experiments on [^{14}C] 6-MP incorporation, we chose a concentration of 1 μg/ml because the incorporation level paralleled the dose of the added labeled drug. The 1 μCi/ml concentration, corresponding to 70 μg/ml, was considered to be relatively high for the present cells (the MAC of high resistant cell is 30 μg/ml). Both the 6-MP resistant sublines and the sensitive cells were treated with this high concentration of cold 6-MP for a short time and

checked for cell viability. None of the tested sublines were killed, nor was their colony-forming ability suppressed, thus indicating that incorporation may not be affected by possible cell damage due to the high 6-MP dose.

Under the above conditions, the incorporation level of the cells reached a plateau at around 2 h of incubation. The incorporation levels were inversely correlated with the degree of resistance; 6-MP-sensitive cells showed a high, low-resistant cells showed an intermediate, and high-resistant cells showed a low level of 6-MP incorporation (Fig. 1). About 30 sublines of various degrees of resistance were tested for [^{14}C] 6-MP incorporation (Fig. 4), and our results support the suggestion that there exists a relation between the incorporation level and the degree of resistance.

Whether the incorporated 6-MP was converted to 6-thioinosinate both in the resistant and sensitive cells deserves attention. The levels of thioinosinate generated by cell-free lysate of 6-MP sensitive and low resistant cells were compared (Fig. 5). In the 6-MP-sensitive cells (N), most of the incorporated 6-MP was converted to the ribotide form. We hypothesized that inosinate generated in the 6-MP-sensitive cells entered the metabolic pathway and suppressed their nucleic acid metabolism. In the low-resistant subline (R_{16}), a small amount of thioinosinate was shown possibly corresponding to the reduced 6-MP incorporation by these cells.

Reduced enzyme activity in the salvage pathway has been reported in a study on 6-MP-resistant L1210 (7, 8). However, column chromatography revealed the same level of HGPRT activity in the 6-MP-sensitive and the low- and high-resistant sublines (Fig. 6). Since the unreacted guanine showed a peak during 0.002N HCl elution, a finding also made with respect to the enzyme-free control, almost all of the labeled guanine might be converted to GMP in all the sublines examined. The observation that there was no difference in the HGPRT level among the 6-MP-sensitive cells and the high- and low-resistant sublines at the termination time of reaction supports the finding that all these cells possess enough enzyme activity to convert the added guanine to GMP.

Our data suggest that the development of resistant variants is due to a change in cell membranes which is related to drug permeation or to an acquisition of an active exclusion system of metabolite.

While Dano et al. observed a small difference in the *in vitro* uptake of daunorubicin by resistant and sensitive Ehrlich ascites tumor cells, other workers reported remarkable differences in the drug uptake level between drug-resistant and sensitive cells (5, 6, 20). Kessel and his collaborators explained this phenomenon in terms of retention and efflux of the incorporated drug in resistant and sensitive sublines of L1210 and P388 cells (12, 13). Retention and efflux were also emphasized in daunomycin-resistant and -sensitive Ehrlich ascites tumor and emetine-resistant and -sensitive P388 cells (4, 6, 21). In "polykaryocyte" formation experiments, Goldstein and Zeleny observed that actinomycin D entered the drug-resistant HeLa cells (10).

The contribution of altered cell membrane permeability to drug resistance was reported in Chinese hamster cells treated *in vitro* with daunomycin and actinomycin D (17) and in Ehrlich ascites tumor cells treated *in vitro* with daunomycin, adriamycin, and rubidazone (16). The methotrexate-resistance of L1210 cprresponded to decreased drug incorporation (15).

Davidson reported that 6-MP-resistant and -sensitive L1210 cells incorporated labeled 6-MP equally, and that there was no permeability barrier to the entry of 6-MP in the resistant cells (7, 8). Our finding does not coincide with his report, possibly because of the different sources of the resistant cells.

Whether the change in the cell membrane is limited to its permeability by 6-MP or whether it also includes permeability by other drugs is of interest. We found that both the highly 6-MP-resistant and -sensitive cells incorporated [^{14}C] adenine to almost the same degree. The difference in the [^{3}H] ara-C incorporated by the 6-MP-sensitive cells and the high- and low-resistant sublines which were not ara-C resistant was not remarkable. This observation suggests that the resistance mechanism is specifically restricted to 6-MP. Cells which lost their 6-MP resistance during subcultures returned to a [^{14}C] 6-MP incorporation level which was as high as that of the parental sensitive cells. This finding suggests a strong relation between drug resistance and incorporation level.

An active exclusion of drugs by the resistant cells may be included in the resistance mechanism. If the exclusion system exists in the resistant cells, it must be specific for drugs as indicated by the results of incorporation experiments.

Some of these cells lost their resistance relatively easily, suggesting the possible involvement of extrachromosomal genes in the resistance. Some bacteria contain small circular DNAs as drug-resistant plasmids, and extrachromosomal DNA has been found in cultured mammalian cells (22). Gene amplification for drug resistance has also been reported (16, 19).

We counted chromosome numbers of 100 metaphase figures of 10 high- and 10 low-resistant sublines and failed to find any differences among them. Furthermore, a morphological study of the chromosomes showed, with one exception, no distinguishable changes in the karyotypic characteristics in the high- and low-resistant sublines and the parental L1210 cells. We have reported elsewhere the finding of double-minute chromosomes in the low-resistant subline (R_{16}) (11). Though the double-minute chromosomes may contribute to express the drug resistance in the above subline, this report may shed some light on the source of the genetic information for the 6-MP-resistance of L1210 cells.

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