observed block in ribosome formation is likely to reflect subtle alterations in the structure of preribosomes [18] making impossible the action of processing enzymes, rather than being the consequence of a specific base substitution in the pre-rRNA chain.

Summary. The effect of a pyrimidine (5-fluorouridine) and a purine (toyocamycin) analogue on the synthesis and maturation of precursors to rRNA is studied. Both 5-fluorouridine and toyocamycin do not alter appreciably the synthesis and processing of mRNA and tRNA. The transcription of rRNA genes is also unaltered at concentrations below 3 μg/ml. The processing of 45 S pre-rRNA is still possible, with the prevalence of alternative routes leading to the formation of 36 S pre-rRNA. However, the formation of mature nucleolar 28 S and 18 S rRNA and the appearance of new ribosomes in the cytoplasm is blocked. The similarity in the site of action of these two different analogues suggests that the block in ribosome formation is due to alterations in the conformation of preribosomes making impossible the last steps of their processing.

Institute of Molecular Biology
Bulgarian Academy of Sciences
1113 *Sofia, Bulgaria*

KRASSIMIRA V. HADJIOLOVA
ZLATINA G. NAYDENOVA
ASEN A. HADJIOLOV

REFERENCES

1. R. J. Suhadolnik, *Nucleoside Antibiotics* Wiley-Interscience, New York (1970).
2. P. Langen, *Antimetabolites of Nucleic Acid Metabolism*. Gordon & Breach, New York (1975).
3. A. Cihak and H. C. Pitot, *FEBS Lett.* **6**, 206 (1970).
4. D. H. Wilkinson, A. Cihak and H. C. Pitot, *J. biol. Chem.* **246**, 6418 (1971).
5. K. V. Hadjiolova, E. V. Golovinski and A. A. Hadjiolov, *Biochim. biophys. Acta* **319**, 373 (1973).
6. A. A. Hadjiolov, K. V. Hadjiolova, R. Nikolova and I. Emanuilov, *Internat. J. Biochem.* **5**, 353 (1974).
7. S. N. Alam and T. K. Shires, *Biochem. biophys. Res. Commun.* **74**, 1441 (1977).
8. A. A. Hadjiolov and K. V. Hadjiolova, in *Antimetabolites in Biochemistry, Biology and Medicine* (Eds. J. Skoda and P. Langen) p. 77–85. Pergamon Press (1979).
9. A. A. Hadjiolov and N. Nikolaev, *Progress Biophys. Mol. Biol.* **31**, 95 (1976).
10. B. E. H. Maden, M. S. N. Khan, D. C. Hughes and J. P. Goddard, *Biochem. Soc. Symp.* **42**, 165 (1977).
11. G. P. Georgiev, A. P. Ryskov, C. Coutelle, V. L. Mantieva and E. R. Avakian, *Biochim. biophys. Acta* **259**, 259 (1972).
12. K. P. Dudov, M. D. Dabeva and A. A. Hadjiolov, *Analyt. Biochem.* **76**, 250 (1976).
13. A. A. Hadjiolov, M. D. Dabeva and V. V. Mackedonski, *Biochem. J.* **138**, 321 (1974).
14. M. D. Dabeva, K. P. Dudov, A. A. Hadjiolov, I. Emanuilov and B. N. Todorov, *Biochem. J.* **160**, 495 (1976).
15. I. Winicov, *J. Molec. Biol.* **100**, 141 (1976).
16. M. J. Purtell and D. D. Anthony, *Proc. natn. Acad. Sci. U.S.A.* **72**, 3315 (1975).
17. B. B. Stoyanova and A. A. Hadjiolov, *Eur. J. Biochem.* **96**, 349 (1979).
18. M.-A. Auger-Buendia, R. Hamelin and A. Tavitian, *Biochim. biophys. Acta* **521**, 241 (1978).

Biochemical Pharmacology, Vol. 30, No. 13, pp. 1863–1865, 1981.
Printed in Great Britain.

0006-2952/81/131863-03 $02.00/0

Active efflux common to vincristine and daunorubicin in vincristine-resistant P388 leukemia

(*Received* 8 *December* 1980; *accepted* 15 *January* 1981)

To date, cross-resistance between DNA intercalaters and vinca alkaloids has been observed with a variety of experimental tumor lines [1–7]. With regard to biochemical mechanism of resistance and cross-resistance in adriamycin(ADR)-resistant P388 leukemia, we proposed an increased active efflux of not only anthracyclines [8] but actinomycin-D (ACT) and vinca alkaloids [9]. Danø [10] and Skovsgaard [11] also reported the similar observation with daunorubicin-resistant Ehrlich carcinoma cells.

Since P388/ADR cells possess an efflux system common to DNA intercalaters and vinca alkaloids, it is most likely that vincristine (VCR)-resistant cells are also able to exclude anthracyclines as well as VCR using the similar transport system. On this subject, Skovsgaard [12] already reported that VCR-resistant Ehrlich carcinoma cells possess an energy-dependent drug extrusion common to VCR and daunorubin (DAU). Here we present evidence that P388/VCR cells are also endowed with enhanced capacity for outward transport of those different classes of drug.

Materials and methods. A vincristine-resistant P388 subline (P388/VCR) was established by in vivo procedure, as reported previously [13]. In brief, the resistant subline cells were selected by daily treatment (day 1–9) with 0.25 mg/kg of VCR only on the first transplant generation. *In vitro* sensitivity was determined by the primary suspension culture technique, which was described in the previous paper [8].

For the measurement of drug uptake, cells were harvested 7 days after transplantation of 10^6 cells of each leukemia line into CD2F1 mice, and washed 2 to 3 times and then suspended in Hanks' balanced salt solution (HBSS). Final cell density was adjusted to be 10^6 cells/ml.

Table 1. *In vivo* sensitivity of vincristine-sensitive and -resistant P388 leukemia to vincristine, adriamycin and actinomycin-D

Drugs	Dose (mg/kg)	P388/S		P388/VCR	
		MeST (range)*	ILS†	MeST (range)	ILS
Vincristine	0.8	20.0 (19–22)	76	12.3 (11–13)	4
	0.4	19.0 (16–19)	68	12.0 (11–12)	1
	0.2	19.0 (16–19)	68	11.3 (11–12)	−5
Adriamycin	4	19.3 (19–25)	70	12.3 (12–14)	4
	2	19.5 (19–22)	72	12.3 (12–13)	4
	1	19.5 (16–23)	72	12.5 (12–13)	5
Actinomycin-D	0.25	19.0 (13–21)	68	11.5 (11–14)	−3
	0.125	19.5 (19–21)	72	13.5 (12–19)	14
Control		11.3 (11–13)	0	11.8 (11–12)	0

* Median survival days.
† Increase in life-span (%).
Cells (10^6) were inoculated intraperitoneally into female CD2F1 mice. Drugs were injected also i.p. on days 1 and 5.

When drug uptake was measured in the presence of a metabolic inhibitor, glucose-free HBSS was employed. Cells were incubated with either tritium-labeled DAU or VCR after 10 min warming-up pre-incubation. At an appropriate interval, triplicate 1-ml aliquots were withdrawn and added to centrifuge tubes with 4 ml of cold 0.9% NaCl solution, followed by 2 washes with the same solution and centrifugation (600 *g*, 5 min, 4°). After the tubes were placed upside down on the filter paper for 3 to 5 hr to remove the washing solution, cells were solubilized in 0.5 ml of Protosol (New England Nuclear, Boston, U.S.A.) and 15 ml of liquid scintillation cocktail (Econofluor; NEN) were added. The radioactivity was determined in a Beckman Model LS-355 liquid scintillation counter with 52% counting efficiency for tritium.

[^{3}H]-DAU (sp. act. 15 mCi/mmole) was kindly donated by Farmitalia (Milan, Italy) through Meiji Seika Kaisha, Ltd. (Tokyo, Japan). [^{3}H]VCR (sp. act. 4.2 Ci/mmole) was purchased from The Radiochemical Center, Amersham, England.

Results and discussion. Table 1 presents *in vivo* sensitivity of P388/VCR cells to VCR, ADR and ACT, compared to that of the parent P388 leukemia cells. As demonstrated here, P388/VCR was resistant to not only VCR but DNA intercalaters such as ADR and ACT. We also measured 50 per cent growth-inhibitory concentration (IC_{50}) of drugs in cell culture system of these cell lines, and obtained the degree of cellular resistance by the ratio of IC_{50} for P388/S to those for P388/VCR. P388/VCR was 820-fold, 820-fold and 320-fold resistant to VCR, ADR and ACT, respectively. This cellular resistance was quite stable even after serial transplantation free from drug treatment.

VCR uptake by P388 leukemia was completely temperature-dependent and presented an unsaturable process, because intracellular level of VCR was linearly increased and the ratio of intracellular/extracellular VCR concentration at the steady state level was constant at the range of 2.5×10^{-5} mM to 2.5×10^{-3} mM of extracellular VCR concentration.

Uptake levels of VCR by the sensitive and resistant P388 cells were 0.062 and 0.015 pmoles/10^6 cells, respectively, when cells were incubated in HBSS with 5×10^{-6} mM VCR for 20 min. Figures 1 and 2 show significantly enhanced uptake of VCR and DAU by both cell lines, particularly by the resistant cells, in the presence of 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation,

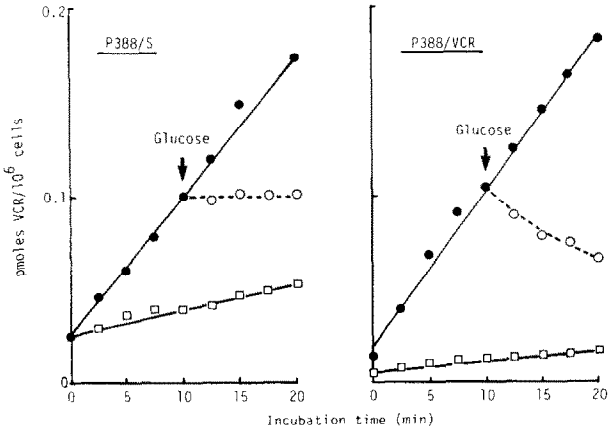

Fig. 1. Uptake of [^{3}H]vincristine by vincristine-sensitive and -resistant P388 leukemia cells. Cells were pre-incubated in HBSS (□—□) or glucose-free HBSS with 1 mM 2,4-dinitrophenol (●—●) for 10 min at 37° and then [^{3}H]vincristine (final concn, 3.5×10^{-6} mM) was added. ○—○, uptake level after glucose (final concn, 1 mg/ml) was added.

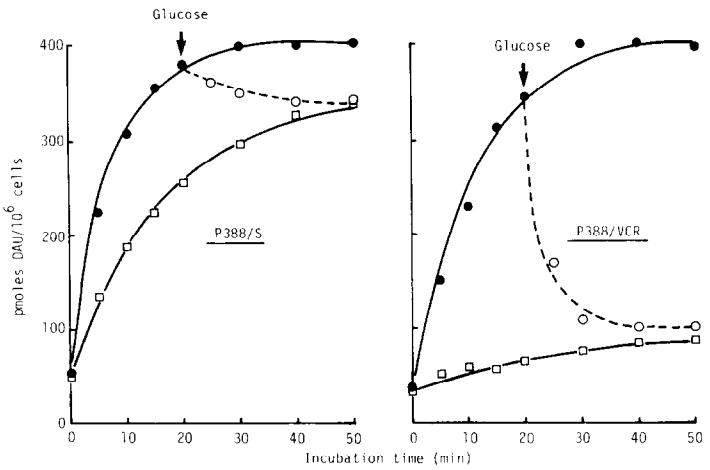

Fig. 2. Uptake of [^{3}H]daunorubicin by vincristine-sensitive and -resistant P388 leukemia cells. Cells were pre-incubated in HBSS (□—□) or glucose-free HBSS with 1 mM 2,4-dinitrophenol (●—●) for 10 min at 37° and then [^{3}H]daunorubicin (final concn, 1.8×10^{-3} mM) was added. ○—○, uptake level after glucose (final concn, 1 mg/ml) was added.

and in the absence of glucose. The addition of glucose (final 1 mg/ml) at 10 or 20 min induced net efflux of VCR from the resistant cells, and DAU from both cell lines, even though drug was still present in the medium. Intracellular drug level after the addition of glucose looks to be approaching to that by the cells which were incubated in the normal medium.

These results strongly suggest the existence of energy-dependent efflux system for anthracyclines as well as vinca alkaloids in VCR-sensitive and -resistant P388 leukemia cells. In addition, P388/VCR seems to possess higher efflux activity compared to the sensitive one, as P388/ADR does [8, 9]. Thus, the apparent lower uptake of drugs by the resistant cells seems to be caused by this outward transport. As to mechanism of VCR resistance in P388 leukemia, Bleyer *et al.* [14] already reported impaired accumulation and binding of VCR within the cell. However, they did not clarify whether alteration in efflux activity was correlated with reduced accumulation or not in their resistant subline.

Studies on efflux system itself for these drugs are presently in progress.

Acknowledgements—This work was supported in part by Grants-in-Aid for Cancer Research from the ministry of Education, Science and Culture, Japan and in part an award of Society of Promotion of Cancer Research, Japan.

Cancer Chemotherapy Center
Japanese Foundation for Cancer Research
Kami-Ikebukuro 1–37–1
Toshima-ku, Tokyo 170
Japan

MAKOTO INABA
REIKO FUJIKURA
YOSHIO SAKURAI

REFERENCES

1. J. L. Biedler and H. Riehm, *Cancer Res.* **30**, 1174 (1970).
2. K. Danø, *Cancer Chemother. Rep. (Part I)* **56**, 701 (1972).
3. N. T. Bech-Hansen, J. E. Till and V. Ling, *J. Cell Physiol.* **88**, 23 (1976).
4. R. K. Johnson, M. P. Chitnis, W. M. Embrey, and E. B. Gregory, *Cancer Treat. Rep.* **62**, 1535 (1978).
5. L. J. Wilkoff and E. A. Dulmadge, *J. natn. Cancer Inst.* **61**, 1521 (1978).
6. C. D. Aldrich, *J. natn. Cancer Inst.* **63**, 751 (1979).
7. S. B. Kaye and J. A. Boden, *Biochem. Pharmac.* **29**, 1081 (1980).
8. M. Inaba, H. Kobayashi, Y. Sakurai and R. K. Johnson, *Cancer Res.* **39**, 2200 (1979).
9. M. Inaba and Y. Sakurai, *Cancer Lett.* **8**, 111 (1979).
10. K. Danø, *Biochim. biophys. Acta* **323**, 466 (1973).
11. T. Skovsgaard, *Cancer Res.* **38**, 1785 (1978).
12. T. Skovsgaard, *Cancer Res.* **38**, 4722 (1978).
13. M. Inaba, R. Fujikura and Y. Sakurai, *GANN* **70**, 607 (1979).
14. W. A. Bleyer, S. A. Frisby and V. T. Oliverio, *Biochem. Pharmac.* **24**, 633 (1975).