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Autoradiographic Study on the Distribution of Cyclocytidine in Mice[1]

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The distribution of cyclocytidine was investigated by means of whole-body autoradiographic technique following intravenous injection in mice. Significant difference was found in the distribution pattern of radioactivity between cyclocytidine and other cytidine analogues such as aracytidine. Distribution of cyclocytidine was more localized in some organs than that of aracytidine, and was analogous to that of cytidine and not deoxycytidine. Cyclocytidine and aracytidine disappeared almost completely from the body within 24 hr. These two compounds were scarcely incorporated in acid-insoluble components in tissues within 10 min, whereas cytidine and deoxycytidine was rapidly incorporated into nucleic acids and the latter two compounds retained over 24 hr.

Cyclocytidine (2,2′-O-cyclocytidine) was markedly active against various mouse tumors with very low toxicity.[3] This compound exhibited clinically antileukemic activities similar to aracytidine (1-β-D-arabinofuranosyl cytosine: Ara-C) but with less gastrointestinal toxicity in a phase I study.[4] Investigation of the mechanism of action of cyclocytidine indicated that the compound inhibits primarily deoxyribonucleic acid (DNA) biosynthesis[5] through inhibition of DNA polymerase.[6] In the present paper, the distribution of cyclocytidine in mice after intravenous injection was examined by whole-body autoradiographic technique. The distribution of aracytidine, cytidine and deoxycytidine was also investigated for comparison.

Material and Method

Labeled Compounds——2-^{14}C-Cyclocytidine and 2-^{14}C-aracytidine were prepared from 2-^{14}C-cytidine by the method of Kanai, *et al.*[7] U-^{14}C-cytidine and 2-^{14}C-deoxycytidine were purchased from Radiochemical Center, Amersham, England.

Whole-body Autoradiography in Mice——Female mice of ddN strain or BDF_1 (for L1210 bearing animals), weighing 20±1 g were each injected with 2.5 μCi/mouse of labeled compound from the tail vein. L1210 leukemia cells (1×10^7) were implanted subcutaneously in the mouse and they were used 4 days after implantation. Animals used were 2 mice in a group and they were killed 10 min or 24 hr after injection of labeled compounds. The experiments were performed by the following modification of Ullberg's method.[8]

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3) A. Hoshi, F. Kanzawa, K. Kuretani, M. Saneyoshi, and Y. Arai, *Gann*, **62**, 145 (1971); A. Hoshi, F. Kanzawa, and K. Kuretani, *ibid.*, **63**, 353 (1972); W. Nakahara and R. Tokuzen, *ibid.*, **63**, 379 (1972); J.M. Venditti, M.C. Baratta, N.H. Greenberg, B.J. Abbott, and I. Kline, *Cancer Chemother. Rep.*, **56**, 483 (1972).

4) Y. Sakai, C. Konda, M. Shimoyama, T. Kitahara, T. Sakano, and K. Kimura, *Japan. J. Clin. Oncol.*, **2**, 57 (1972).

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6) A. Hoshi, F. Kanzawa, K. Kuretani, T. Kanai, and M. Ichino, *Biochem. Pharmacol.*, **22**, 2829 (1973).

7) T. Kanai, T. Kojima, O. Maruyama, and M. Ichino, *Chem. Pharm. Bull.* (Tokyo), **18**, 2569 (1970).

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The animals were lightly anesthetized with ether and killed by immersion in acetone cooled with solid CO_2. The frozen animals were then transferred to a refrigerator at −20°. Segittal sections (40 μ) were taken at various levels of each animal. They were mounted to tapes and dried at −20° for 7 days. The dried sections were brought to contact with Sakura Type N X-ray film and exposed for 7 days. The films were developed in a Konidol-X developer for 5 min and fixed in Konifix for 10 min.

Autoradiography of Acid Insoluble Fraction——The dried sections that were used for ordinary whole-body autoradiography were immersed in 7% trichloroacetic acid for 3 min and in tap water for 5 min, then dried at room temperature. Autoradiograms were obtained by ordinary procedures as described in the previous section.

Result

Distribution of Cyclocytidine and Aracytidine

Ten minutes after intravenous injection of 2-^{14}C-cyclocytidine (Fig. 1A), the highest uptake of radioactivity was shown by the kidney and liver, followed by salivary gland, intestinal mucosa, brown fat, bone marrow, and peristeum. An appreciable uptake was observed in the lymph node, thymus, myocardium, lung, adrenal and skin. Relatively low concentration of radioactivity was shown by the skeletal muscles, spleen, pancreas and gastric mucosa. Almost no uptake of radioactivity was observed in the brain and spinal cord. Radioactivity in blood was very low in both heart and liver. Radioactivity was not observed in bile though the compound distributed in liver at highest level.

Distribution of cyclocytidine in tumor tissues (L1210 leukemia) was also determined. The compound distributed moderately in the tumor and the concentration was equivalent

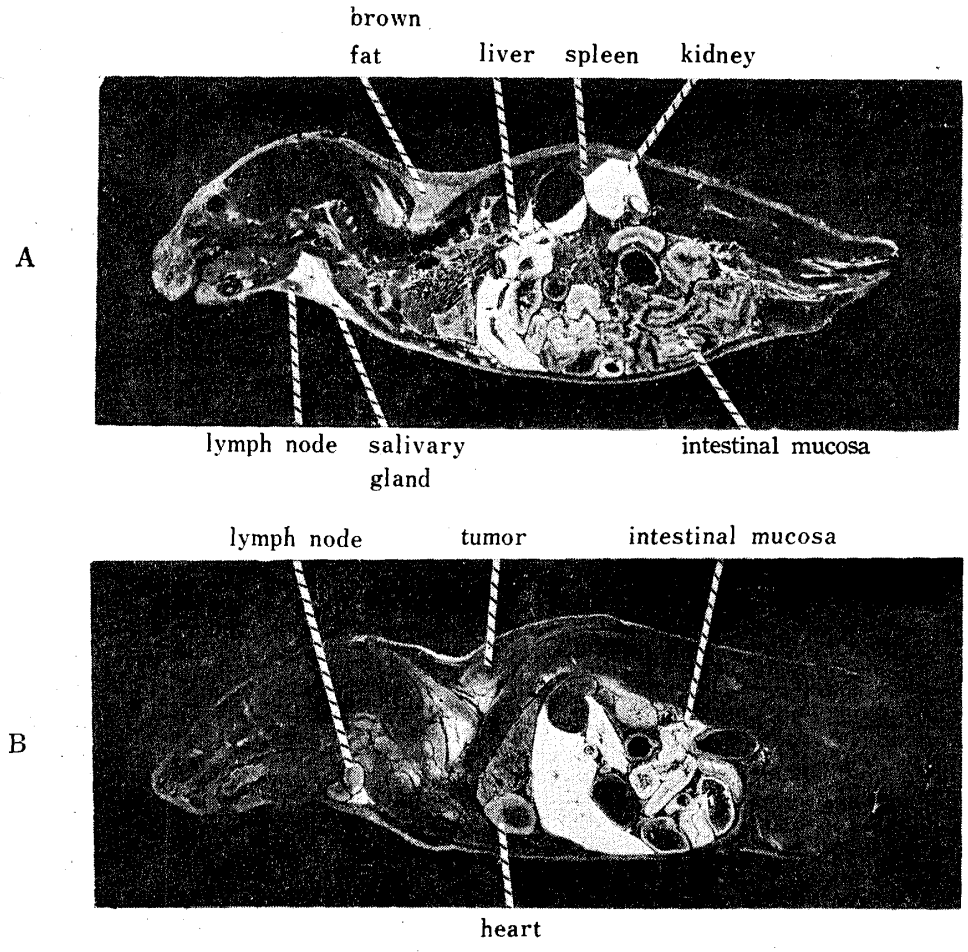

Fig. 1. Autoradiograms of Cyclocytidine in Normal (A) and Tumor-bearing (B) Mice 10 min after Intravenous Injection

to that of spleen and lymph node. Some uptake of radioactivity was observed unexpectedly in brain of tumor bearing animals (Fig. 1B). Difference of distribution in tissues of radioactivity between normal and tumor bearing animals was only this evidence.

Distribution of aracytidine was determined as a reference to cyclocytidine. After injection of 2-^{14}C-aracytidine, a much higher uptake of radioactivity was shown generally by most of organs and tissues. Ten minutes after injection (Fig. 2), the highest uptake of radioactivity was shown by the kidney, followed by thymus and myocardium. A high radioactivity

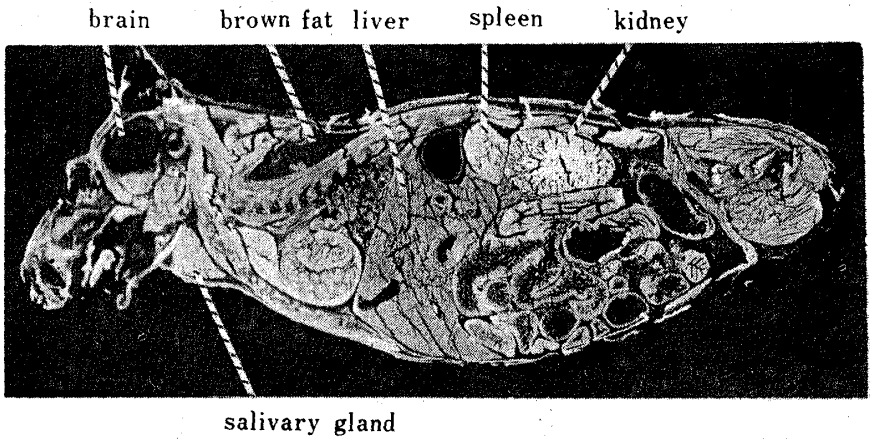

Fig. 2. Autoradiogram of Aracytidine 10 min after Intravenous Injection

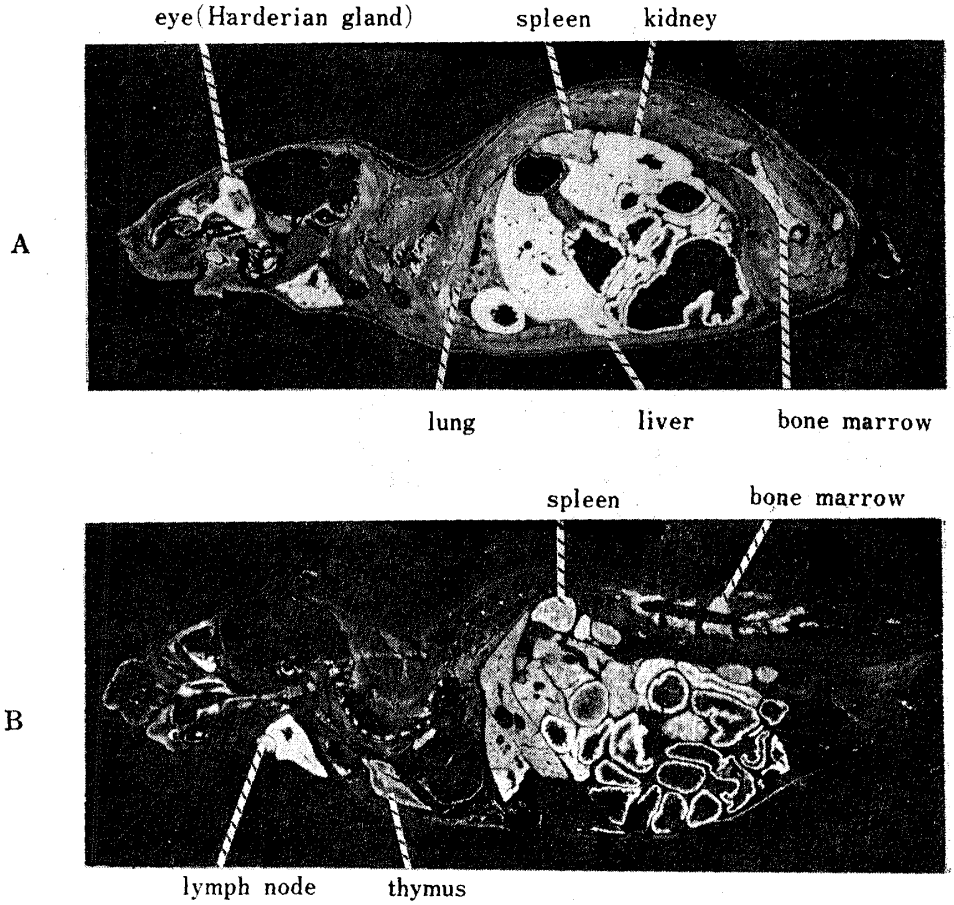

Fig. 3. Autoradiograms of Cytidine 10 min (A) and 24 hr (B) after Intravenous Injection

was shown by most of the organs. In the brain, a low concentration of radioactivity was distributed, while no uptake of radioactivity was observed in the case of cyclocytidine.

Twenty four hours after injection of 2-^{14}C-cyclocytidine or 2-^{14}C-aracytidine, the radioactivity disappeared almost completely from the body, however, retention of some radioactivity was observed in the salivary gland for cyclocytidine.

Distribution of Cytidine and Deoxycytidine

The distribution of cytidine and deoxycytidine which are normal components of nucleic acids was investigated for comparison. Ten minutes after intravenous injection of U-^{14}C-cytidine (Fig. 3A), the highest uptake of radioactivity was shown by the various organs and tissues such as kidney, adrenal, myocardium, eye, salivary gland, liver, pancreas, intestinal mucosa and bone marrow. Relatively low concentration of radioactivity was shown by skeletal muscles and skin.

After 24 hr, the concentration of radioactivity still remain in the most of all tissues, however, radioactivity accumulated in the lymph node, thymus and spleen but it disappeared from the eye (Fig. 3B).

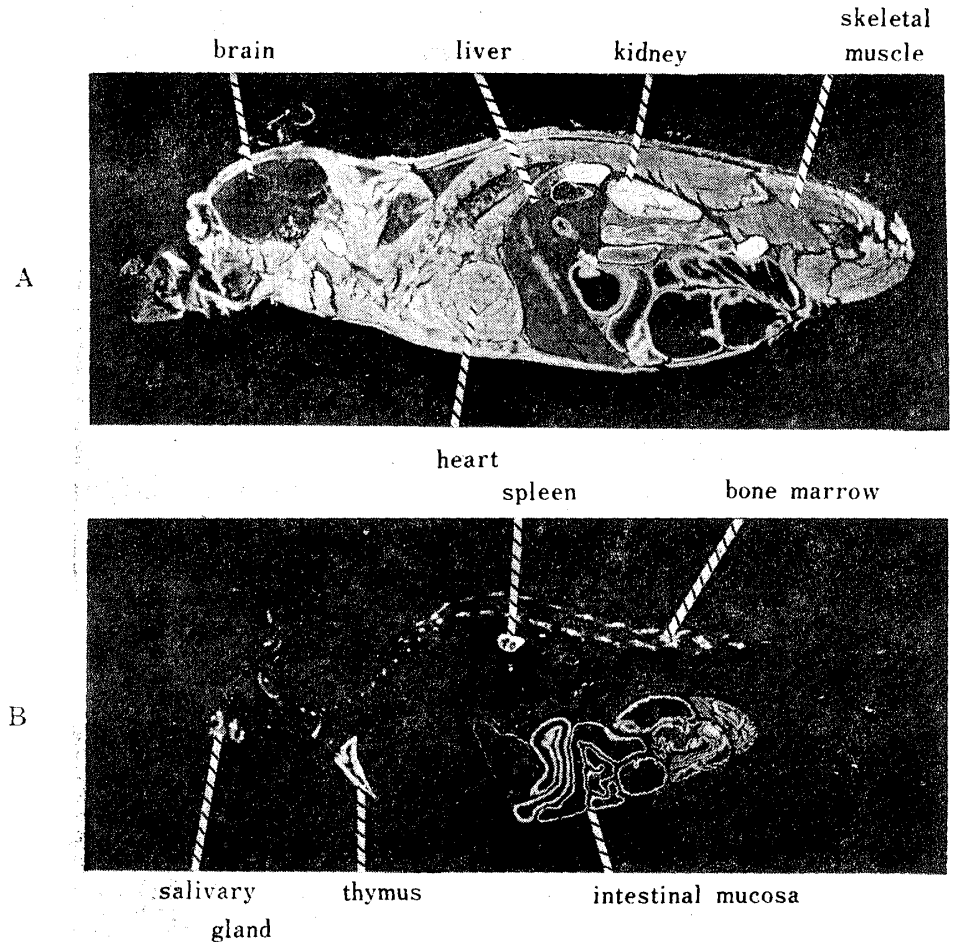

Fig. 4. Autoradiograms of Deoxycytidine 10 min (A) and 24 hr (B) after Intravenous Injection

On the other hand, 10 min after injection of 2-^{14}C-deoxycytidine (Fig. 4A), the highest uptake of radioactivity was shown by the kidney, spleen, salivary gland and lymph node, followed by eye, thymus, myocardium and gastro-intstinal mucosa. Some radioactivity was observed in the brain.

Table I. Radioactivity Appeared on Autoradiograms

Organs	Cyclocytidine		Aracytidine		Cytidine		Deoxycytidine	
	10 min	24 hr	10 min	24 hr	10 min	24 hr	10 min	24 hr
Brain	−	−	±	−	−	−	±	−
Spinal cord	−	−	−	−	−	−	−	−
Eye	+	−	+	−	###	−	##	−
Salivary gland	###	+	##	−	###	###	###	##
Lymph node	+	−	±	−	##	###	###	−
Brown fat	##	±	±	−	+	+	±	−
Thymus	+	−	##	−	##	###	###	###
Myocardium	+	−	##	−	###	##	##	−
Heart blood	±	−	##	−	±	−	##	−
Hepatic blood	±	−	##	−	±	−	##	−
Lung	+	−	##	−	+	+	##	−
Liver	###	−	##	−	###	###	+	−
Gall bladder	−	−	−	−	−	−	−	−
Pancreas	±	−	+	−	###	###	+	−
Spleen	±	−	##	−	+	###	###	###
Gastric mucosa	±	−	+	−	###	###	##	+
Small intestinal mucosa	###	−	##	−	###	###	##	##
Large intestinal mucosa	+	−	##	−	###	###	##	##
Adrenal	+	−	+	−	###	###	+	−
Kidney	###	−	###	−	###	###	##	−
Urinary bladder	###	−	###	−	###	−	###	−
Bone marrow	##	±	+	−	###	##	+	##
Skeletal muscle	−	−	##	−	±	+	+	−
Skin	+	−	##	−	−	+	##	−

###: highest radioactivity, ##: higher activity, +: moderate activity, ±: low activity and −: no activity

After 24 hr, the radioactivity except in some organs such as thymus, spleen, bone marrow, salivary gland and gastro-intestinal mucosa, disappeared almost completely from the body (Fig. 4B). The results obtained by the autoradiographic studies are qualitatively summarized in Table I.

Forms of Radioactive Compounds in Tissues

Since distribution of radioactivity in organs and tissues were different among nucleosides tested, radioactive compounds in tissues were further examined qualitatively. It might be expected that cytidine and deoxycytidine are incorporated in nucleic acids as soon as its distribution. However, incorporation of cyclocytidine in cell components was not clear, therefore, the incorporated form in tissues of cyclocytidine was examined.

Radioactivity in segittal sections 10 min after injection of cyclocytidine was decreased by washing with cold trichloroacetic acid. Concentration of aracytidine was also decreased by the same treatments. However, radioactivity was not lowered in some organs after injection of cytidine (Fig. 5A). Distribution pattern of radioactivity in the washed sections 10 min after injection of cytidine or deoxycytidine was similar to the pattern of radioactivity in the sections 24 hr after injection of the corresponding compound. As a result, cyclocytidine was seemed to be not incorporated in organs and tissues as an acid-insoluble compounds, whereas, cytidine and deoxycytidine was rapidly incorporated in the acid-insoluble fraction presumably nucleic acids.

Discussion

Mechanism of action of cyclocytidine was previously examined in intact cells[5] and cell-free system.[6] Cyclocytidine was found as a water soluble "transport form" of aracytidine

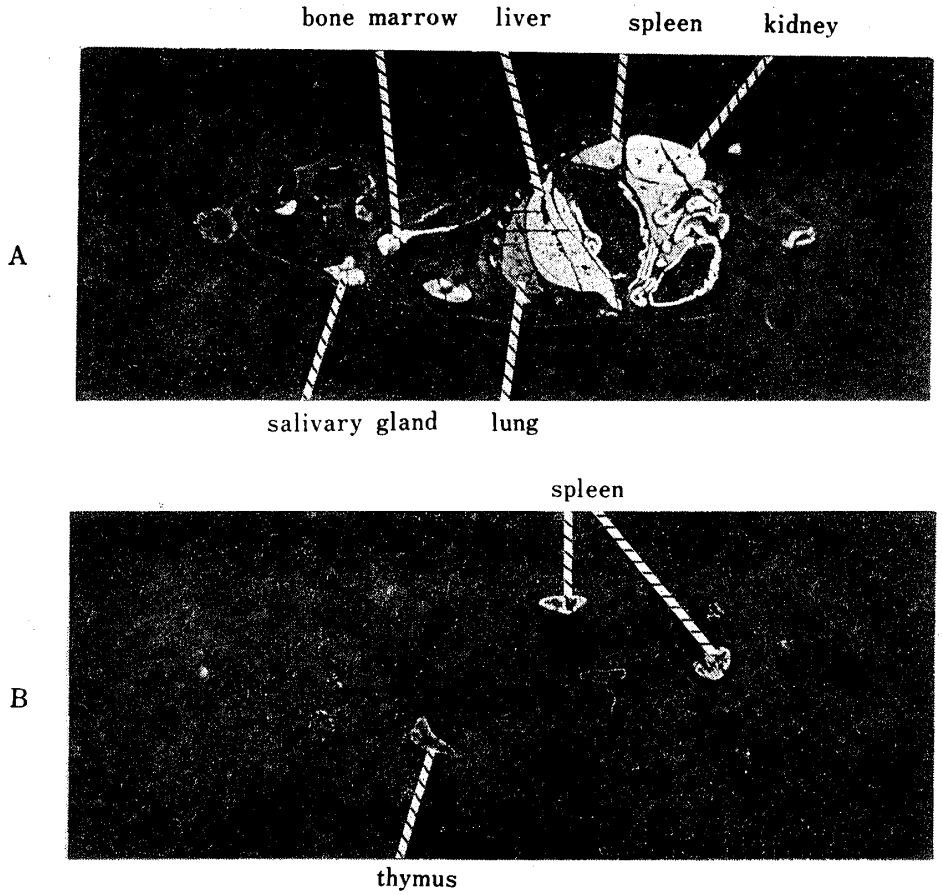

Fig. 5. Autoradiograms Cytidine (A) and Deoxycytidine (B) after Treatment with Trichloroacetic Acid

in biochemical or chemical levels. Pharmacokinetic nature concerning absorption, distribution and excretion of the compound is the important subject for studies on mechanism of action *in vivo*. The present results revealed that the distribution of cyclocytidine was more localized than that of aracytidine, the latter was distributed homogeneously in organs and tissues after intravenous injection. Cyclocytidine was especially distributed in liver and kidney and was excreted in urine soon after injection. Concentration of this compound in blood was lower than that in most of the tissues at 10 minutes. It means that cyclocytidine is incorporated into tissues in higher level from blood stream. On the other hand, concentration of aracytidine in blood was equivalent to that in other tissues. Significant differences in the distribution pattern between cyclocytidine and aracytidine were this higher incorporation into some organs and localization in tissues and organs of the former compound. Lower toxicity of cyclocytidine in mice may be due to the lower distribution in target organs.

Distribution of cyclocytidine in tumor tissues (L1210) was examined. Cyclocytidine was distributed moderately in the tumor tissues but the concentration of the compound was not high though the compound was markedly active against the leukemia. Cyclocytidine was distributed unexpectedly in brain of tumor bearing mice. The cause of this distribution was considered to be due to the distribution into leukemia cells in brain and not into brain tissue itself. Because L1210 leukemia cells were proliferated after invasion into brain tissue.

Distribution pattern of cyclocytidine was also compared with that of normal nucleosides such as cytidine and deoxycytidine. Distribution of cyclocytidine had a resemblance to that of cytidine and not to that of deoxycytidine, though the chemical conformation of sugar moiety of cyclocytidine was more analogous to deoxycytidine or aracytidine than cytidine. On the other hand, distribution of aracytidine had a resemblance to that of not cytidine but

deoxycytidine. Furthermore, it is an interesting evidence that the distribution of cytidine was different from that of deoxycytidine, though chemical natures such as solubility of the two nucleosides were similar in each other. Distribution of nucleosides in tissues is considered to be controlled by only the direction of hydroxy group or exsistence of the group at 2′ position of sugar moiety. Further, distribution of cyclonucleoside is considered to be controlled by cyclo- or tricyclic-structure in the molecule.

Ability of incorporation of cyclocytidine into acid-insoluble fraction of tissues was examined and it was revealed that cyclocytidine and aracytidine were not incorporated into the acid-insoluble fraction. It is a different nature of arabinoside or cyclonucleoside from both riboside and deoxyriboside. Cytidine and deoxycytidine were incorporated soon after administration into acid-insoluble fractions in tissues presumably ribonucleic acid and deoxyribonucleic acid, respectively.

Though the relationship between toxicity and distribution pattern was not enough understanding, some possibilities were suggested that cyclocytidine was distributed in gastro-intestinal mucosa and bone marrow. These are considered to be the caused of gastro-intestinal and hematological toxicity. Further, higher distribution in salivary gland may be the cause of parotis pain in clinical treatment.

In conclusion, significant difference was found in the distribution pattern of radioactivity between cyclocytidine and aracytidine. Distribution of cyclocytidine in tissues was more localized than that of aracytidine and was analogous to that of cytidine and not deoxycytidine. Cyclocytidine was distributed moderately in tumor tissues as well as in spleen and lymph node. Cyclocytidine was disappeared almost completely from the body within 24 hr. These two compounds were scarcely incorporated in acid-insoluble fraction in tissues. Quantitative examination of distribution in tissues is now in progress.