chloride (IV). It was also demonstrated by the results of paper chromatography; the appearance of (IV) in urine could be detected 20 min. after the injection began and its quantity ratio to (III) increased gradually with lapse of time.

The authors are indebted to Drs. Y. Sakurai and H. Satoh of Iatrochemical Institute of Pharmacological Research Foundation for many valuable advices and the bioassay in this experiment, and also express their gratitude to Mr. S. Kawai for preliminary experiment.

Summary

N-Methyl[^{14}C]-bis(2-chloroethyl)amine N-oxide (^{14}C-labeled nitrogen mustard N-oxide) was synthesized for pharmacological and biochemical experiments using methyl-[^{14}C] iodide. Its specific activity was determined as 6,500 counts/min./μmole by Q-gas flow counter.

The labeled compound was given to a dog intravenously and the urine was taken out from the opened bladder. Concentration of the compound in the urine was quantitatively determined by radioactivity measurement.

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16. Morizo Ishidate, Yoshio Sakurai, and Eiichi Matsui : Studies on Carcinostatic Substances. XXIII.*1 Correlation between Anti-tumor and Leucopenia-inducing Effects of Alkylating Agents.

(*Iatrochemical Institute of Pharmacological Research Foundation**2)

Recently, there have been found a few derivatives of nitrogen mustard, which have been recognized as available anti-tumor agents, at least against such human tumors as Hodgkin's disease, reticulosarcoma, seminoma, and some kinds of lymphosarcoma. During the course of the treatment, however, hematopoietic tissues of the host were affected and, very frequently, a serious leucopenia was induced.

It has been a matter of particular interest to know whether or not the two actions of nitrogen mustard, the one against tumor and the other against bone marrow, could not be inclined to the former by modifying chemical structure of the molecule.

At the stage of animal experiment, a ratio of LD_{50} to MED (minimum effective dose) was usually noticed as a chemotherapeutic index, but, in most clinical treatment, the unfavorable side-effect of the alkylating agents, requiring discontinuation of the medication, was not the acute toxicity which led rats to death in animal experiment, but the unavoidably accompanying leucopenia of the patient. It was deemed important, therefore, to evaluate the clinical effectiveness of the agents by comparing the rate of one action to the other in animal experiment.

This paper deals with the experiment, in which 10 derivatives of nitrogen mustard, including some N-oxides having characteristic properties or structures, were given to rats bearing Yoshida sarcoma, and their percentage survival diagrams for 30 days and the decrease rate of leucocyte number were checked.

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*1 Part XXII : This Bulletin, 7, 873(1959).

*2 26 Nishigahara 1-chome, Kita-ku, Tokyo (石館守三, 桜井欽夫, 松井英一).

Experimental and Results

Rats (hybrid albino) of 80～100 g. in body weight were fed for 2 weeks with a fixed diet and in a constant condition, and the individual in abnormal physical condition if any was removed from the valid total number of experimental animals. The leucocytes were counted one day before inoculation and only the rats were collected, which were determined to have the leucocyte number more than 8,000 but less than 12,000. Twelve of the rats, having passed the above examination, were inoculated intraperitoneally with Yoshida sarcoma ascites (ca. 50,000,000 cells).

Two days after inoculation, the leucocyte number was again counted and the rats showing a deviation of more than ±2,000 in number were rejected from the batch. On the next day, after confirming the establishment of maximum growth of the tumor, a single dose of the test compound was injected into the peritoneal cavity of each animal. Since Iwata, *et al.*[1] had found that a single

TABLE I. Experimental Procedure

Days	
-	Counting of leucocyte number (12 rats)
- 1	Tumor transplantation
- 2	
- 4	Counting of leucocyte number
- 3	Injection (dose : 5/16 of LD_{50}, intraperitoneal)
- 5 – - 12 (5, 6, 7, 8, 9, 10, 11, 12)	Counting of leucocyte number
- 13	
- 29	
- 30	

TABLE II. Biological Data of the Test Compounds

No.	Compound	LD_{50} (mg./kg.)	Dose (mg./kg.)	MED (mg./kg.)	$\frac{LD_{50}}{MED}$
27	$CH_3-\underset{\downarrow \atop O}{N}\langle{CH_2-CH_2-Cl \atop CH_2-CH_2-Cl}\cdot HCl$	80.0	25.0 15.0	1.0	80
24	$CH_3-N\langle{CH_2-CH_2-Cl \atop CH_2-CH_2-Cl}\cdot HCl$	1.6	0.5	0.05	32
183	$C_6H_5-CH_2-\underset{\downarrow \atop O}{N}\langle{CH_2-CH_2-Cl \atop CH_2-CH_2-Cl}\cdot HCl$	33.3	10.4	5.0	7
204	$C_2H_5-O-C_2H_4-N\langle{CH_2-CH_2-Cl \atop CH_2-CH_2-Cl}\cdot HCl$	36.7	11.5	1.0	37
243	$HOOC-\underset{| \atop CH_3}{CH}-N\langle{CH_2-CH_2-Cl \atop CH_2-CH_2-Cl}\cdot HCl$	12.7	4.0	0.05	254
471	$C_6H_5-O-C_2H_4-N\langle{CH_2-CH_2-Cl \atop CH_2-CH_2-Cl}\cdot HCl$	19.1	6.0	0.05	382
483	${C_2H_5 \atop C_2H_5}\rangle N-(CH_2)_3-N\langle{CH_2-CH_2-Cl \atop CH_2-CH_2-Cl}\cdot 2\,HCl$	2.0	0.6	0.05	40
484	${C_2H_5 \atop C_2H_5}\rangle \underset{\downarrow \atop O}{N}-(CH_2)_3-\underset{\downarrow \atop O}{N}\langle{CH_2-CH_2-Cl \atop CH_2-CH_2-Cl}\cdot 2\,HCl$	316.7	98.6	10.0	32
513	$N-(CH_2-\underset{| \atop Cl}{CH}-CH_2)_3\cdot HCl$	15.3	4.8	0.1	153
524	${Cl-CH_2-CH_2 \atop Cl-CH_2-CH_2}\rangle \overset{+}{N}(-CH_2-CH_2-CH_2-O-)\cdot I^-$	5.5	1.7	0.1	55

LD_{50} by the Behrens-Kärber method

1) H. Iwata, *et al.* : Nippon Yakurigaku Zasshi, **50**, 169(1954).

dose of 25 mg./kg. of N-methyl-bis(2-chloroethyl)amine N-oxide (HN_2-O), just 5/16 of the LD_{50}, was enough to induce a constant rate (ca. 80%) of leucocyte depression in a rat, a dose of the same rate to the LD_{50} was used in the present work in all the compounds tested.

Blood count was begun on the day following the injection and continued until leucocyte number recovered to the initial count. Different to the preceding experiment,[2] blood was withdrawn alternately from both vena supra orbitalis of all animals, because it was proved to be easier in technique and also supplied larger quantity of blood than from the tail vein. Moreover, the leucocyte number of 6 untreated rats, counted by this method, was nearly constant through 15 days in spite of repeated puncture. The procedure of experiment is graphically demonstrated in Table I.

The percentage survival diagrams for 30 days were prepared with regard to all test compounds. A list of the compounds and the result of experiments are shown in Table II and in Figs. 1~11.

The Percentage Survival Diagram with the Curves indicating the Depression of Leucocyte Number

—— Untreated Yoshida Sarcoma rats (Control)
—— Treated with the test compounds (i. p.)
o—o Leucocyte count during treatment of the test compounds
↑ Injection

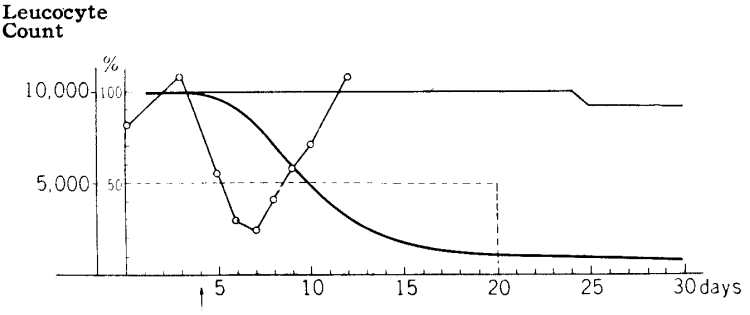
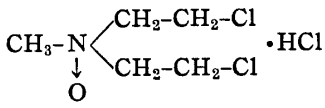


Fig. 1.



No. 27 25 mg./kg.

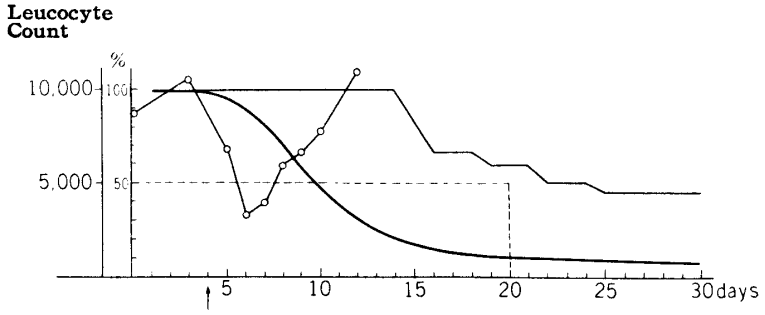
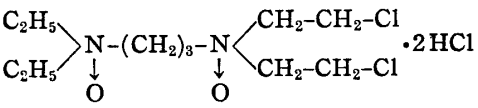


Fig. 2.



No. 484 98.6 mg./kg.

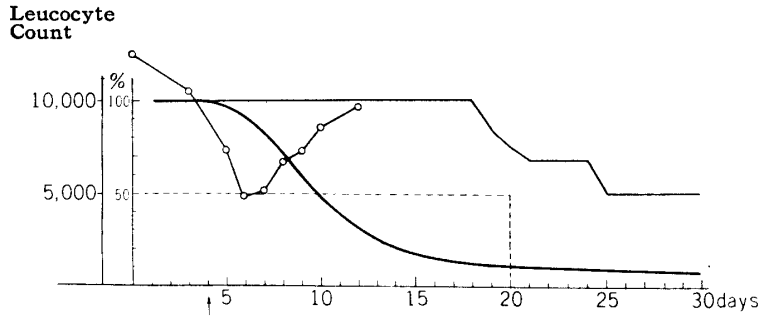
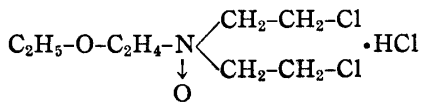


Fig. 3.



No. 204 11.5 mg./kg.

2) E. Matsui : This Bulletin, **7**, 867(1959).

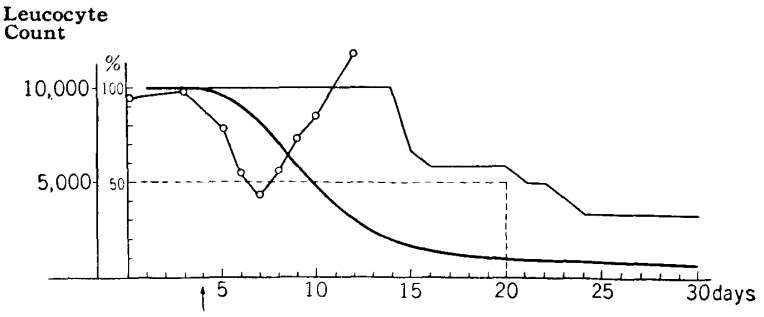
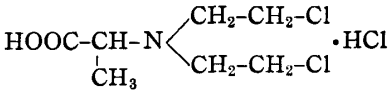


Fig. 4.



No. 243 4.0 mg./kg.

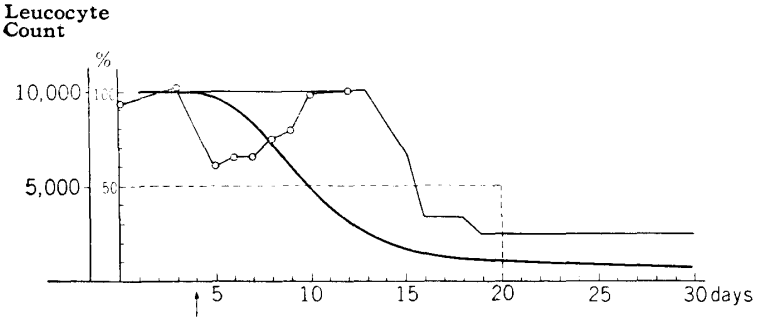
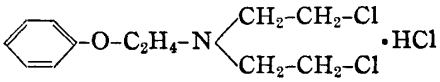


Fig. 5.



No. 471 6.0 mg./kg.

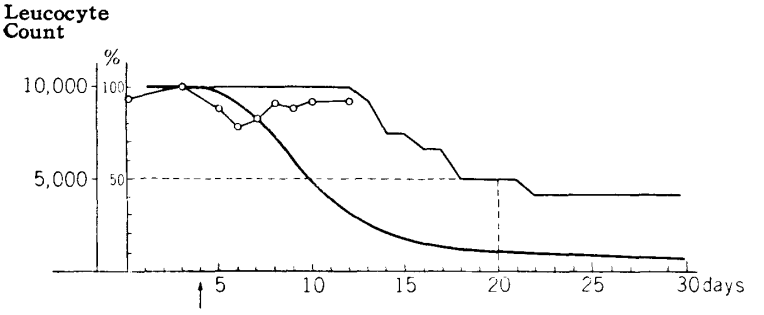
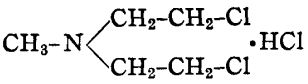


Fig. 6.



No. 24 0.5 mg./kg.

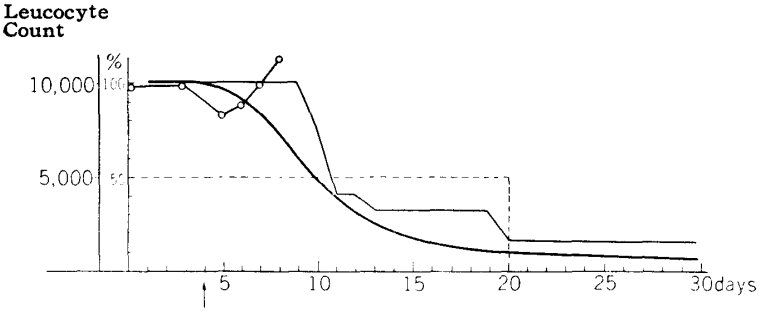
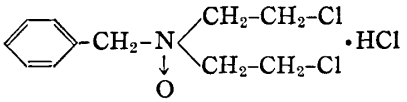


Fig. 7.



No. 183 10.4 mg./kg.

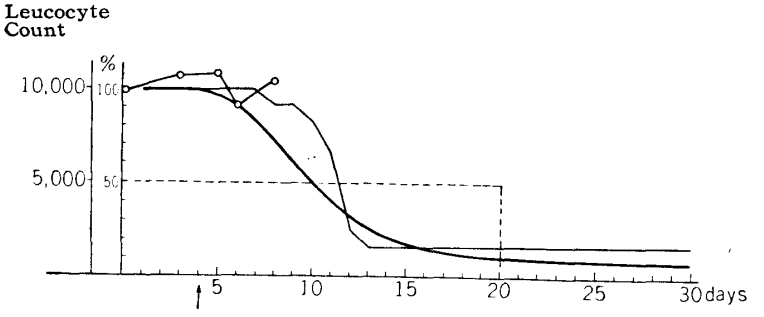
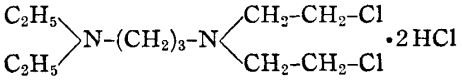


Fig. 8.



No. 483 0.63 mg./kg.

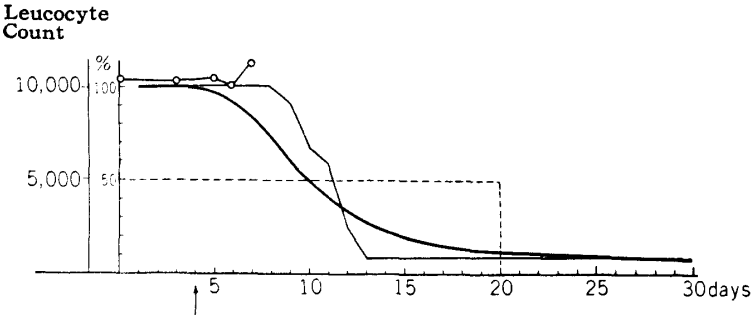
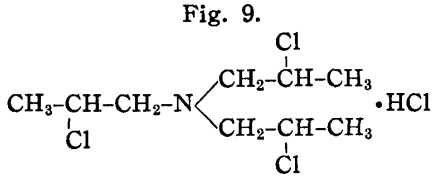


Fig. 9.



No. 513 4.8 mg./kg.

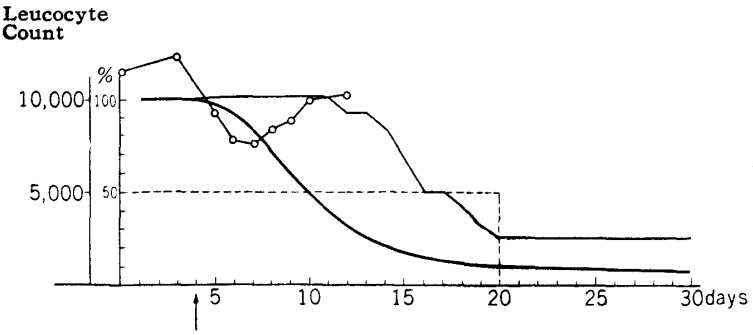
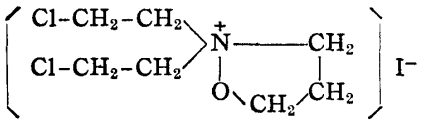


Fig. 10.



No. 524 1.7 mg./kg.

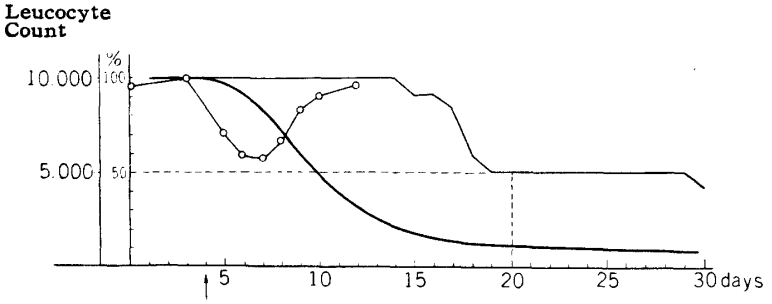
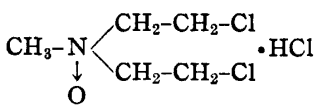


Fig. 11.



No. 27 15 mg./kg.

Discussion

Covering all results of experiments, a tendency can be approximately affirmed that the deeper the fall of leucocyte number, the longer the animals remained alive but when the results were examined more closely, there was not always a complete parallelism between anti-tumor and leucopenia-inducing effects.

For instance, a pair of compounds, Nos. 27 and 484, caused almost the same rate of leucocyte depression, but the anti-tumor effect of the former in the same dose appeared to be far better than that of the latter (Figs. 1 and 2). It was almost the same with the pair of Nos. 204 and 243, as shown in Figs. 3 and 4.

Only slight depression of leucocyte number was noticed in the compounds Nos. 24, 183, 483, and 513, and each of them had but a poor effect in prolonging the life-span of the animals (Figs. 6, 7, 8, and 9). In these cases too, there could be found a divergency to some extent among the mutual rate of their two effects in question. For example, No. 24 alone showed a comparatively noticeable effect of life-prolongation, although its leucopenia-inducing effect appeared almost the same as the rest of compounds.

No. 524 was one of the typical derivatives with latent activity prepared by one of the authors, but the attempt to elevate its tumor selectivity by this kind of masking seemed to be of no avail, as indicated in Fig. 10.

By the usual screening technique with the ascites tumors, a chemotherapeutic index ($CI = LD_{50}/MED$) of the compounds was compared with each other. As presented in Table II, it was found that the compound with a larger CI was not invariably the more effective in prolonging the life-span of the animals or inducing leucopenia. For example, Nos. 243 and 471 having such a large CI, as seen in Table II, did not have life-prolongation comparable to No. 27 (Figs. 1, 4, and 5).

It was also interesting that, even at about one-half equivalent dose, needless to say at the same dose, No. 27 caused far stronger leucopenia than No. 24, as indicated in Fig. 11. This fact seemed to demonstrate that, if the leucocyte depression equal to that of No. 27 is to be expected in No. 24, a far larger quantity might be necessary for injection, which would be nearly close to its lethal dose.

Such above-stated phenomena could be understood by considering that the tendency seen in the mutual rate of the two activities of each compound is due to its own chemical property depending on its individual structure, even among the similar derivatives of nitrogen mustard.

Therefore, it may be possible to find by this experimental procedure, a better one among the numerous candidate compounds prepared or collected, as a practical cancer chemotherapeutic which shows less damage upon the hematopoietic tissues of the host than on the tumor itself.

The authors express their gratitude to Prof. T. Yoshida, and Drs. H. Satoh and H. Nakamura for their kind advices in the course of this study. This work was supported by Grant-in-Aid of Scientific Research from the Ministry of Education.

Summary

Each of 10 different derivatives of nitrogen mustard was tested on 12 Yoshida sarcoma rats for their life-prolongation and leucopenia-inducing effects. It was found that some showed a very strong effect of life-prolongation on the tumor rats, although the leucopenia-inducing effect of such compounds was quite equal to those of other compounds.

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