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Studies on Carcinostatic Substances. XXXII.[*1] Acquired Resistance of Yoshida Sarcoma by Treatment with Derivatives of Nitrogen Mustard.

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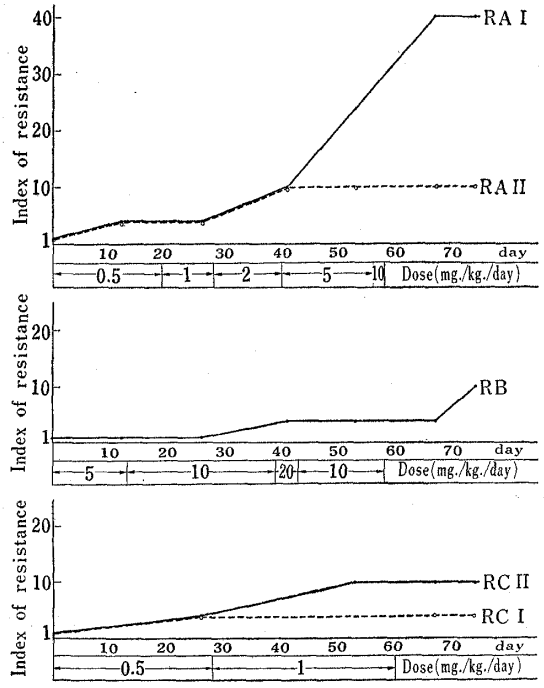
It was previously reported by Hirono, *et al.*[1] that Yoshida sarcoma acquired a resistance against N-methyl-bis(2-chloroethyl)amine N-oxide (HN_2-O) when the tumor rat was treated repeatedly with the same compound for a long period.

This paper deals with the experiment of inducing resistance against the same tumor by intraperitoneal administration of three kinds of derivatives, viz. HN_2-O, 2-bis(2-chloroethyl)aminoethyl *p*-(2-hydroxy-1-naphthylazo)benzoate (No. 294), and 2-chlorotriethylamine (No. 90). The first compound was chosen as the most popular derivative, the second is an example of the ones which retain their effective concentration for a long time in the ascites of rat when injected intraperitoneally, and the last one is a monofunctional derivative of nitrogen mustard which has been proved to have almost no anti-tumor activity.

Index of resistance was demonstrated by the ratio of minimum effective concentration (MEC) *in vitro* of N-methyl-bis(2-chloroethyl)amine (HN_2) on the resistant tumor to that of the original one. For this purpose, the experimental method of morphological determination of MEC similar to that of the screening procedure for anti-tumor substances with tissue culture technique was employed.[2]

The development of resistance of the strains during treatment is graphically demonstrated in Figs. 1~3. The resistance of the tumors was also observed in animal experiment and the minimum effective doses (MED) of HN_2-O on the rat bearing each of the



Development of Resistance during Treatment

Fig. 1. CH_3-$N(CH_2CH_2Cl)_2$ (HN_2-O)

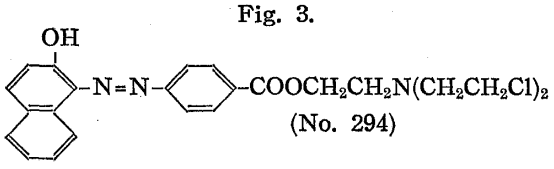
↓

O

Fig. 2.

$(C_2H_5)_2NCH_2CH_2Cl$ (No. 90)

Fig. 3.



(No. 294)

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1) I. Hirono, *et al.* : Gann, **45**, 469(1954).

2) M. Ishidate, *et al.* : This Bulletin, **7**, 873(1959).

Table I. Minimum Effective Dose of HN_2-O on Resistant and Original Strains of Yoshida Sarcoma

Tumor strain	Original	RA-I	RA-II	RB	RC-I	RC-II
Min. effective dose (mg./kg., i.p.)	1	>40	30	10	20	20

resistant strains and the original Yoshida sarcoma are summarized in Table I.

It is worth noting that (1) the tumor acquired resistance against HN_2 by treatment with No. 90 which was inactive as an anti-tumor agent and (2) the two strains which were treated with the same agent and by the same procedure were found to differ from each other in their grade of resistance. In the case of treatment with HN_2-O, it was necessary to inject the agent every day, but, in the case of No. 294, the tumor acquired resistance by a single injection for one generation of rat on the day of inoculation.

Out of these five strains, RA-I and RC-II were respectively transplanted into rats and henceforth they have been kept in the animals without any treatment. Change of resistance of these two strains during the following 12 months is shown in Fig. 4.

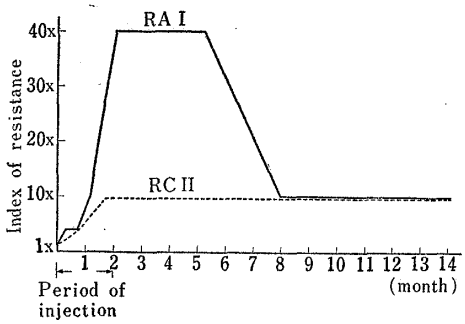

Fig. 4. Change of Resistance after Treatment

It was proved that the morphological and pathological features of the resistant tumors were not different from those of the original Yoshida sarcoma and the life span of rats inoculated with these strains were found to be quite the same as that of the rats bearing the original Yoshida sarcoma.

An investigation of cross resistance of these strains against various derivatives of nitrogen mustard and other anti-tumor agents is now under way.

Summary

Yoshida sarcoma acquired resistance against N-methyl-bis(2-chloroethyl)amine by treatment with three kinds of nitrogen mustard derivatives, one of which has no anti-tumor activity by itself.

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