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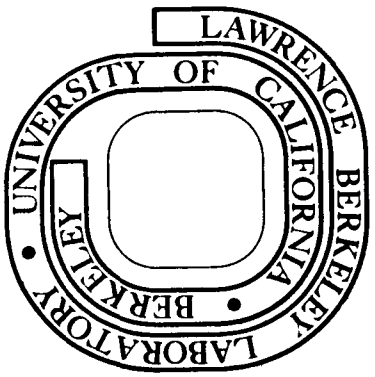

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EFFECT OF SOME RIFAMYCIN DERIVATIVES ON CHEMICALLY-INDUCED MAMMARY TUMOURS IN RATS

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SUMMARY

Five rifamycin derivatives have been compared for effectiveness in the inhibition of chemically-induced mammary tumours in rats. Daily oral administration of DMB (dimethylbenzyldesmethylrifampicin) starting two weeks before the carcinogen challenge was the most effective, both in inhibiting or delaying the onset of tumours and in slowing the growth of those that occurred. Rifampicin, dirifampin, RC-16 (rifazacyclo-16) and R-8_2 (rifazone-8_2) had lesser inhibitory effect.

* This work was supported in part by the U.S. Atomic Energy Commission and in part by the Natioanl Cancer Institute, Grant No. NCI-1-RO-1-CA-14828-2.

Several rifamycin derivatives have been shown to inhibit (1) RNA-instructed DNA polymerase (RIDP) in crude viral ext racts; (2) virus-induced transformation in tissue culture; and (3) the growth of tumours in vivo. These results have been summarized in an earlier paper [4]. M ore recently, Bissell, et al. have shown that one rifamycin derivative, R-8_2 (rifazone-8_2), not only inhibits transformation in chick fibroblasts but affects the growth of transformed cells [1].

We reported the inhibition of DMBA (dimethylbenzanthracene)-induced mammary tumours in female rats by DMB [4]. At that time intraperitoneal injection of an emulsion of the drug (10 mg/4 day) seemed most effective. This method of administration requires the intraperitoneal injection of a large amount (2 ml) of an oil-lecithin emulsion. We have tried other methods of administration to find one which would be as effective and less traumatic. Giving the drug subcutaneously was ineffective, probably due to poor absorption. Ulcerating sores frequently appeared at the injection site. A small pilot experiment indicated that giving the drug orally every day instead of every 2 days (as previously reported) and starting drug treatment one week before the carcinogen challenge, was as effective as i.p. administration and much less traumatic to the animal. Starting drug treatment two weeks after the carcinogen challenge had no effect on tumour onset or growth. Oral administration starting 1 week before the carcinogen has therefore been adopted as the method of choice for the administration of rifamycin derivatives. We report here the results of an experiment designed to determine the optimum length of time for DMB administration.

We have also compared four other rifamycin derivatives (rifampicin, dirifampin, RC-16 (rifazacyclo-16) and R-8_2) to DMB as inhibitors of

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mammary tumours. Dirifampin inhibits RIDP *in vitro* at approximately the same level as DMB [7]; RC-16 and R-8_2 are much more effective inhibitors [7,8]. Although rifampicin is not as effective an inhibitor of RIDP it has been reported to inhibit transformation in tissue culture and several tumours *in vivo* [4].

DMB was kindly supplyed by Gruppo Lepetit, Milan, Italy. Rifampicin was obtained from Schwartz-Mann. Dirifampin, RC-16 and R-8_2 were synthesized in this laboratory [7,8]. All drugs were administered orally as a 3% solution in a mixture containing 5% vegetable lecithin (Nutritional Biochemicals) in Wesson Oil. The dose of all drugs was 6 mg/day, and was started one week before the carcinogen challenge.

DMBA was kindly supplied in a stable oil-water emulsion by Upjohn Co. A total of 6 mg in three doses at 3-day intervals was injected intravenously. This schedule of carcinogen administration has been reported by Huggins to produce mammary tumours in 100% of the rats in 10 weeks [2]. In our laboratory, this schedule produces tumours in 85% of the rats in 10 weeks.

Female Sprague-Dawley rats (Holtzman Co., Madison, Wisconsin) were injected with carcinogen at the age of 60 to 65 days. They were maintained at 22 to 24°C with food and water *ad lib*. Animals were weighed and palpated for tumours twice weekly. Tumours were graded as smaller or larger than 1 cm in diameter. Animals were killed when tumours became ulcerated.

The optimum administration schedule for DMB in respect to the inhibition of mammary tumours, with the least trauma to the animal, appears to be 6 mg/day orally beginning one week before the carcinogen challenge and continuing for a total of 10 weeks (Fig. 1). Not only is

the onset of tumours delayed, but the growth of those that do occur is retarded. There is not a great deal of difference in the effectiveness of the inhibition of tumour initiation between 7 and 10 weeks total DMB administration. Fifty percent of the control animals had tumours at 6 to 7 weeks, and those animals receiving DMB for only 4 weeks had reached the 50% point by 8 to 9 weeks. In comparison, those receiving DMB for 7 or 10 weeks did not reach the 50% point until after 11 weeks.

The continuation of DMB treatment for 10 weeks did markedly depress the growth of those tumours which did occur, even in comparison with those which received the drug for 7 weeks. It is possible that in the screening of other drugs, 7 weeks' administration would be sufficient. We have preferred to use the 10 weeks' schedule as our standard.

None of the other compounds tested was as effective as DMB (Fig. 2). For comparison's sake, the data for all animals given DMB only during the past several years (controls) and for those given DMBA orally as described above have been pooled, giving an 'N' for controls of 142 animals, and for DMB-treated an 'N' of 95 animals. Rifampicin, dirifampin and RC-16 did not appreciably delay the onset of tumours. R-8_2 showed some inhibition, but was not as effective as DMB. Rifampicin or R-8_2 did not inhibit the growth of tumours; dirifampin or RC-16 slowed the growth somewhat, but again were not as effective as DMB.

We have continued to observe an increased occurrence of enlarged spleens and livers in animals receiving both rifamycin derivatives and DMBA as compared to DMBA alone [4]. Histological examination of tissues from such animals has confirmed our tentative diagnosis of malignant lymphomas. In animals receiving both drug and carcinogen the occurrence is 33/210 (16%), while in animals receiving carcinogen only the occurrence

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is 11/137 (8%). This synergistic effect between rifamycin derivatives and a carcinogen has also been observed in the accelerated death of mice with radiation-induced thymic lymphomas which also received DMB as compared with those receiving irradiation only [3]. A possible explanation for this effect is the immunosupressant activity of rifamycin derivatives [5,6].

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LEGENDS FOR FIGURES

Fig. 1. The effect of varying the length of DMB treatment on the inhibition of mammary tumours induced by DMBA in female rats. A. = effect on occurrence of tumours; B. = effect on growth of tumours. DMB_4 = animals receiving DMB for 4 weeks; DMB_7 = animals receiving DMB for 7 weeks; DMB_{10} = animals receiving DMB for 10 weeks; C = controls (animals receiving carcinogen only). Data points at weekly intervals.

Fig. 2 The effect of several rifamycin derivatives on the occurrence and growth of mammary tumours induced by DMBA in female rats. A. = effect on the occurrence of tumours; B. = effect on the growth of tumours. Rif. = rifampicin, Dirif. - dirifampin, RC-16, $R\text{-}8_2$ and DMB = drugs as listed above, C = control. Data points at weekly intervals.

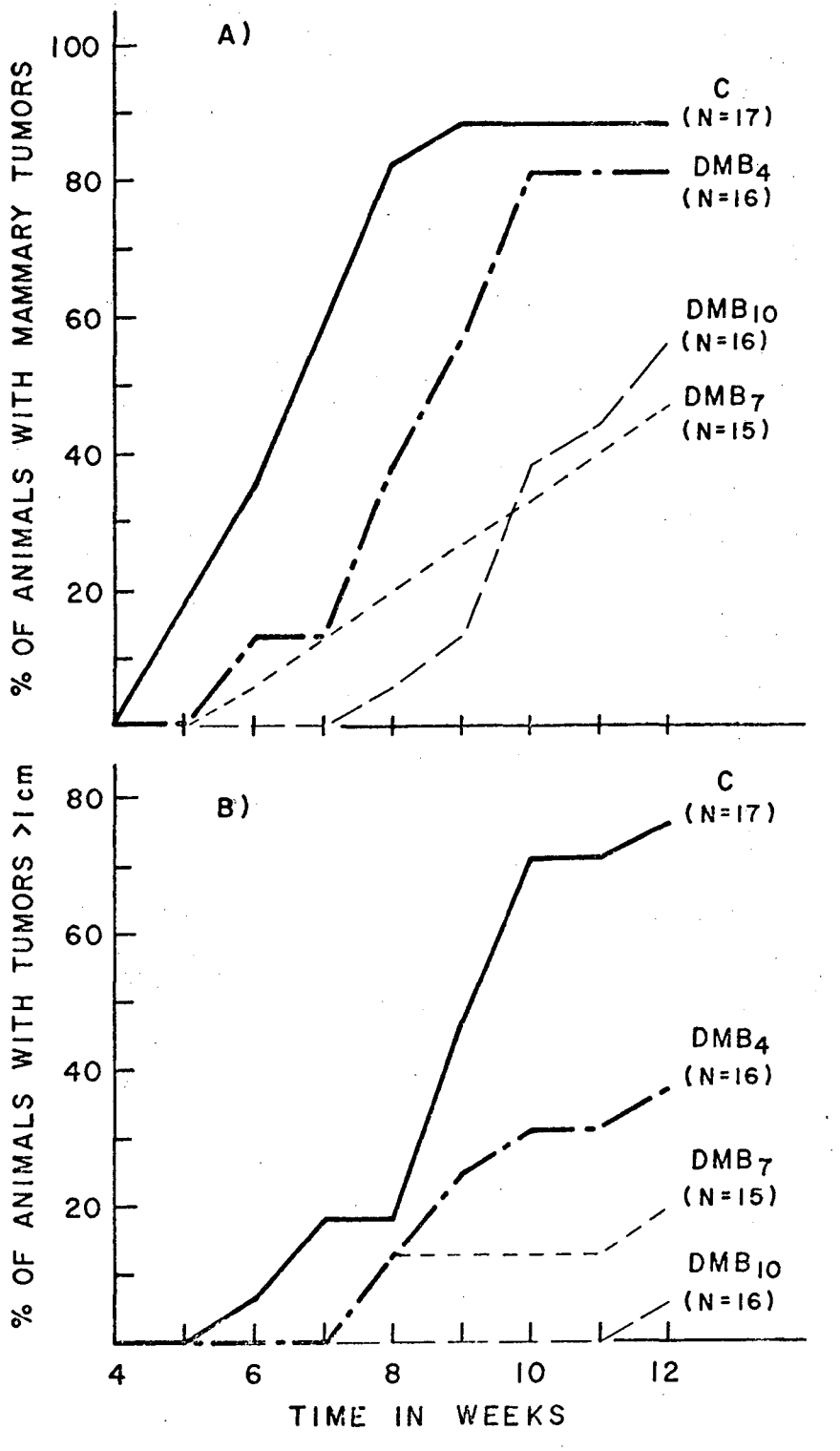
A)
% OF ANIMALS WITH MAMMARY TUMORS
100
80
60
40
20
C
(N=17)
DMB4
(N=16)
DMB10
(N=16)
DMB7
(N=15)
B)
% OF ANIMALS WITH TUMORS >1 cm
80
60
40
20
C
(N=17)
DMB4
(N=16)
DMB7
(N=15)
DMB10
(N=16)
4
6
8
10
12
TIME IN WEEKS
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Fig. 1

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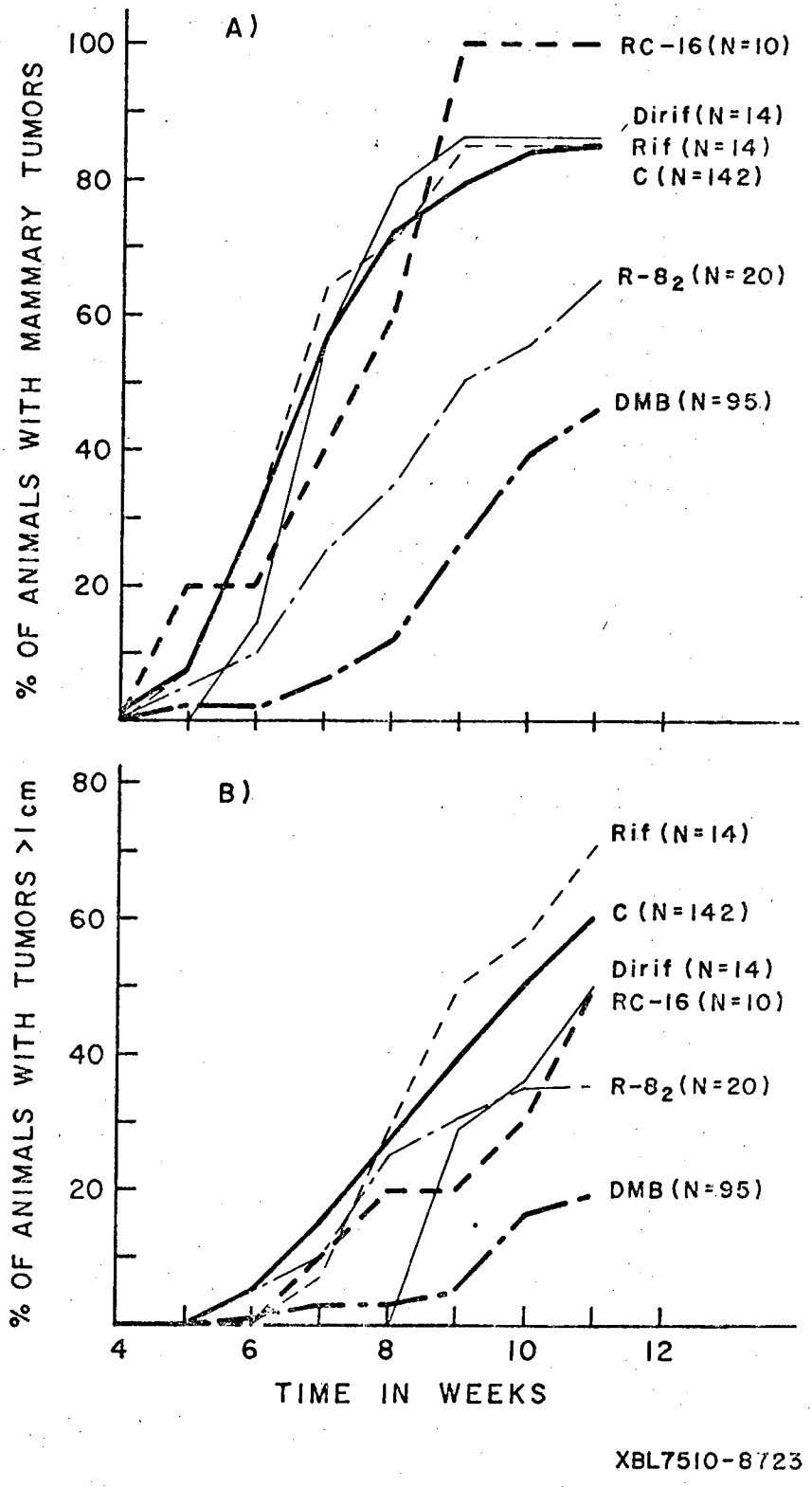

Fig. 2

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