II. Treatment of Flexner-Jobling Carcinoma in Sprague-Dawley Rats with 2,4,6-Triethylenimino-s-Triazine (TEM)*† (19654)

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We have previously reported(1) that the treatment of the King A rat bearing transplanted sarcoma 231 with suitable doses of 2,4,6-triethylenimino-s-triazine and related products resulted in complete regression of the tumor in the majority of the animals. These rats were then resistant to further transplants of the tumor.

It seemed important to know how different types of rat tumor would respond to treatment with the ethylenimines. From a preliminary investigation of tumor growth in mice it appeared that the compounds might have a greater retarding action on the growth of carcinomas than sarcomas(2). To see if this might be true of rat tumor, we have chosen to investigate first the response of the well known Flexner-Jobling carcinoma to therapy with 2,4,6-triethylenimino-s-triazine.

Materials and methods. Young Sprague-Dawley rats were used as carriers of the Flexner-Jobling carcinoma. The animals were selected for the particular experiment so as to secure maximum uniformity. The methods used were essentially the same as previously described(1). An isotonic saline solution containing the required dose of the drug in 0.1 ml was prepared each day and this dose injected intraperitoneally either twice or 4 times daily during the course of therapy. In all cases the treated rats were pair-fed with the controls. The rats were treated only after the carcinoma had established itself as a growing neoplasm. Treatment was started from 8 to 14 days after the implantation and the tumors averaged from 180 to 1000 mm^2.

Results. Eight young male rats, averaging 140 g, were implanted with the carcinoma. Four of the group served as controls and the other 4 were treated, beginning on the 8th day after the implantation, with 0.057 mg/kg of TEM 4 times daily. This treatment was continued for 4 days. Reversal of tumor growth took place in all 4 rats on the third day after treatment began and the regression continued to completion without further medication. The only toxic symptom observed was loss of appetite during the therapy. A few days after the drug was stopped the rats regained their normal appetite and went on to gain weight and develop normally. The tumors in the controls grew rapidly and ultimately resulted in the death of the animals. The tumors ulcerated in the untreated animals, as is usual for this tumor, but did not in the treated rats. The cured rats proved to be resistant to further transplants of the carcinoma.

Four young male rats of about 85 g gross weight, bearing 9-day tumors, were treated with 0.053 mg/kg of TEM twice daily, and this therapy continued for 18 days. The tumors regressed completely in 3 and partially in one. In this latter case, regression of the tumor proceeded for 7 days and then stopped; growth began again and continued at an accelerated rate in spite of the treatment.

Twelve young rats, averaging 170 g, were selected 11 days after implantation when the tumor size was about 700 mm^2; 8 were treated 4 times daily with 0.053 mg/kg of TEM and 4 maintained as untreated controls. This treatment continued for 4 days, during which time the rats received about the same amount of drug as had caused complete regression of the tumors in all the animals in the group bearing the 8-day tumor. From the previous results, no further treatment with the drug should have been necessary. However, after the period of therapy the tumor in but one animal showed a reversal of growth. Two days after the animal had been off the drug, regression of the tumor started in another rat of the group. The rate of growth of the tu-

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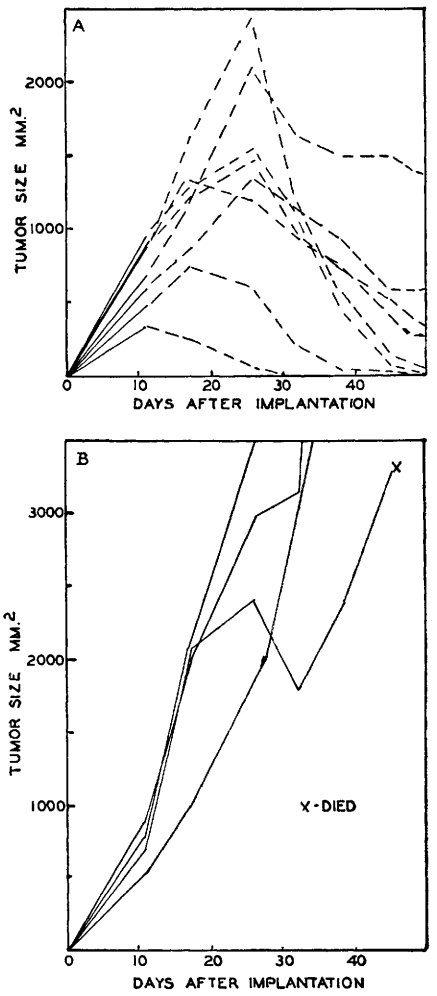

FIG. 1 A. Growth curves of the carcinoma in young rats treated 4 days with .053 mg/kg of TEM 4 times daily; 3 days no treatment and 34 days treatment with .053 mg/kg of the drug twice daily. The solid portions of the curves indicate the periods of no treatment.

B. Growth curves of the carcinoma in the untreated controls, pair-fed with 1 A.

mors in the other 6 rats indicated little or no retardation by the treatment. One day later the therapy was resumed, but with only 2 daily injections. This was continued for 34 days, during which time 4 tumors regressed completely; one having reached a size of 2400 mm^2 before growth stopped and regression began. Regression of the tumor in a fifth animal in the group was continuing when the experiment was terminated, 52 days after implantation, while in the other 3 animals the tumor size appeared to be contained at the lowest level reached. All of the animals were in good condition. Those with tumors were sacrificed for histological studies. The animals in which the tumors regressed were later tested and found to be resistant to further transplants of the tumors. They were cured. All of the untreated controls died of the carcinoma between 34 and 49 days after the implantation. The comparative growth of the tumors in the treated and untreated rats is illustrated in Fig. 1A and Fig. 1B.

Fourteen male rats averaging about 270 g and bearing 14-day tumors of 650 mm^2 average size were selected for a comparative study with the doses of TEM which we used most effectively in the treatment of sarcoma 231. Four animals received 0.016 mg/kg; 4, 0.023 mg/kg; and 2 animals, 0.046 mg/kg twice daily, during the entire course of treatment, lasting 29 days. The remaining 4 animals were untreated and served as controls. There was also a group of 4 normal controls pair-fed with the animals bearing the tumor in order to check on the adequacy of the food intake. The treated animals in which the tumor regressed completely and the normal controls gained weight. There were 2 complete regressions in the first group, 3 in the second, and 2 in the third. In the group receiving the smallest amount of drug, the tumors in 2 of the animals continued to grow at about the same rate as before treatment until 15 days of therapy, when they stopped growing and regressed for the next 5 days; then, growth was continued for the remaining 9 days of treatment. The therapy was discontinued and soon thereafter the tumors began to grow again and the growth continued at an accelerated rate until the carcinoma killed the animals. However, these animals lived 64 days while all untreated controls were dead by the 28th day. In a group of 4 animals treated with 0.023 mg/kg twice daily, the tumor had regressed completely in 2, almost to completion in one, and only slightly in the other when the therapy was discontinued. Both of the existing tumors continued to regress without further therapy; the small one to completion and the larger one to about half its size. At this stage the growth of the

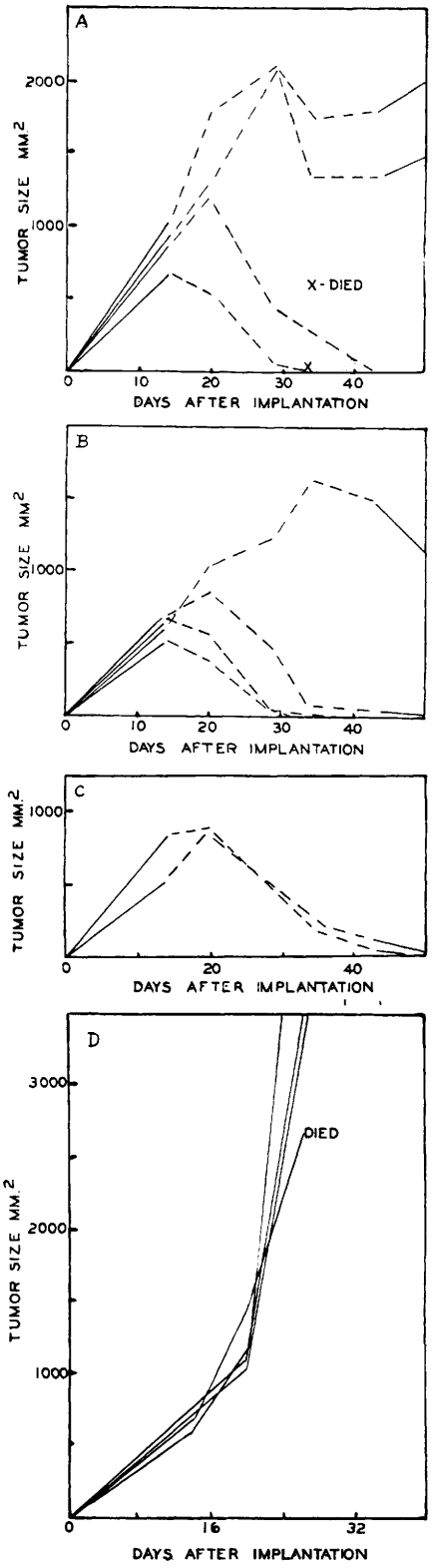

FIG. 2 A. Growth curves of the carcinoma in rats treated with .016 mg/kg TEM, twice daily. The solid portions of the curves denote the periods of no treatment.

B. Growth curves of the carcinoma in rats treated with .023 mg/kg of TEM, twice daily. The solid portions of the curves indicate the periods without treatment.

C. Growth curves of the carcinoma in rats treated with .046 mg/kg of TEM, twice daily. The solid portions of the curves represent the periods of no treatment.

D. Growth curves of the carcinoma in rats untreated and serving as controls for 2 A-2 C.

latter began again and continued at a moderate rate. The animal lived more than 64 days, showing that treatment had helped to hold in check the killing power of the tumor. The tumor in both of the animals treated with 0.046 mg/kg twice daily regressed to small size during the period of treatment and continued to complete regression after the therapy was discontinued. The comparative effects of treatment with different doses are shown in Fig. 2A-2D.

There were 26 rats treated in the several groups at the different dose levels and complete regression of the tumor occurred in 18. The tumor did not regress in any of the 13 control animals.

The 18 rats in which the tumor was completely cured were all resistant to the growth of further transplants of the tumor and in no case did the tumor which had regressed reappear.

Several of these rats were sacrificed soon after the third challenge for immunity and their organs and cavities examined. No tumors or lesions were found. About 6 months later another group was sacrificed and examined with similar results. The remaining animals were alive one year after being cured of the tumor. They were normal in weight and well-being. They were sacrificed along with the controls that had survived and a comparative examination made. No tumors or lesions were found in either group.

Discussion. The Flexner-Jobling carcinoma in the Sprague-Dawley rat responded to treatment with triethylenimino-s-triazine over a wider range of doses than sarcoma 231 in the King A rat. In the latter no regressions were obtained with 0.2 mg/kg/D either in a short or long course of treatment and the best re-

sults were obtained with about 0.04 mg/kg/D. Regressions of the carcinoma have been obtained with 0.032 to 0.230 mg/kg/D and without toxic manifestations by the hosts.

In general this carcinoma ulcerates in its later stages of growth. However, in the treated animals this occurred less often. In most of the cases where regression started early in the treatment it proceeded to completion without ulceration of the tumor.

It would appear that the Sprague-Dawley rat is less sensitive than the King A rat to TEM. The animals gained weight under treatment.

Summary. 1. The treatment of Sprague-Dawley rats bearing Flexner-Jobling carcinoma with 2,4,6-triethylenimino-s-triazine resulted in complete regression and cures of the tumor in about 70% of the animals treated. There were no spontaneous regressions among the controls. 2. This carcinoma responded to treatment with doses ranging from 0.016 to 0.046 mg/kg, injected twice daily for the entire course of treatment and the rats showed no marked symptoms of toxicity. 3. A short course of treatment with 0.230 mg/kg/D divided in 4 equal doses and given but 4 days, resulted in complete regressions of the tumors in all of the animals treated when the therapy was begun 8 days after the implantation but this method did not give equally good results in another group treated 11 days after implantation. In this case it was necessary to institute a second course of treatment with 0.052 mg/kg, twice daily for 34 days to obtain complete regression of the tumors in 50% of the animals. 4. All of the rats in which the tumor regressed completely were then resistant to further implants of the tumor. 5. Rats that were cured lived for more than a year without the tumor reappearing. In comparison with the controls they were normal. 6. No gross manifestations of toxicity were seen with the therapeutic doses of TEM used. 7. There was no evidence of carcinogenicity of TEM in the therapeutic doses used in the Sprague-Dawley rats up to one year after the end of the period of therapy.

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A Critical Examination of the Histochemical Staining Technic for Ascorbic Acid. (19655)

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The acid silver nitrate method for the identification and localization of ascorbic acid in tissues has been used extensively since its introduction by Gough and Zilva(1). Some workers(2-5) claim good specificity and precise localization, although they admit that a negative reaction does not exclude the presence of the vitamin. Others(6-8) do not consider precise localization to be possible by this technic, but offer no or inconclusive evidence for their contention.

In the study to be presented, the sensitivity, specificity, and localizability of the histochemical method for ascorbic acid have been investigated by test tube and model experiments and by the use of animal tissues.

Experimental. 1. A solution of acetic acid and silver nitrate was added to the test substances in centrifuge tubes. The precipitate was washed and the unreduced silver removed by thiosulfate or ammoniacal thiosulfate solution. A large number of substances was tested, and positive reactions (a precipitate of metallic silver) were obtained with ascorbic acid, 3,4-dihydroxyphenylalanine, hydroquinone, and a mixture of calcium and magnesium

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