Oncolytic Effect of Egypt Virus on a Human Epidermoid Carcinoma Grown in X-Irradiated Rats.*† (19490)

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It has been reported previously that certain neurotropic viruses possess an oncolytic property for transplantable mouse tumors, and that each of these viruses has its own tumor spectrum, *i.e.* it will destroy or inhibit certain mouse tumors, multiply without damage to the cancer in others, and fail to survive in a few(1). On the basis of these animal experiments, preliminary work has been done on the treatment of human patients with a variety of exotic viruses(2). Unfortunately, no method has existed for determining prior to use on a human patient, whether the virus employed has any oncolytic ability for the specific human tumor against which it is to be directed. The recent finding, however, that human tumors as well as normal tissues could be grown in x-irradiated heterologous hosts (3) especially in the rat, which, in contrast to mice, is immune to most of the viruses used, has made available what is hoped will be such a screening tool. Report will hereby be made of a human tumor against which Egypt 101 virus showed oncolytic ability both in x-irradiated rats and in tissue culture. Even though it has not been possible, to date, to test the virus in the original tumor donor, since he has not shown recurrence of his disease after operation, it is felt that both the method and the oncolysis are noteworthy.

Materials and methods. The tumor used, obtained immediately after surgical removal, was a metastatic epidermoid carcinoma from the jaw of a 75-year-old male, "C.K.", who had had a primary carcinoma of the lip removed 18 months previously. As soon as received, the tumor, handled with aseptic technic, was minced fine enough with scalpels so that it would pass through a No. 18 needle when suspended in a buffered Ringer's solution. 0.5 cc of this suspension was implanted subcutaneously in each flank of 16 weanling female rats (Carworth Farms) that had received 2 total body doses of x-irradiation, 150 r each, on consecutive days, 3 days prior to the implantation. Seven days after implantation, microscopic examination of the tumors of one of the rats showed "healthy", well vascularized growths, full of mitotic figures. As previous experiences had indicated that the human tumors either grew or failed uniformly in all the animals implanted, it was presumed that the "K" tumor was suitable for virus study. Accordingly 9 days after implantation 9 of the 15 remaining animals were injected directly into one tumor only with 0.1 cc of Egypt 101‡ virus as a 40% stock mouse brain emulsion, an inoculum which subsequently titered 10^{-7} intracerebrally in mice. This virus was chosen because it had been used clinically with no ill effects on patients and because prior tests had also shown that the rat whether normal or irradiated was insusceptible to it. One virus treated animal was killed daily, following inoculation, for 7 days (except that 3 were killed the 1st day). A cross section of each tumor was taken for histological examination and the remaining tumor after grinding, was made up to a 10% suspension in horse serum saline and titered for virus, intracerebrally, in mice. Control animals were killed on the 1st, 3rd, 5th (3 rats) and 7th days after the inoculation. Tumor material obtained from the 3 control rats killed 14 days after implantation was used in several ways: it was transferred successfully to a 2nd generation of x-irradiated

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‡ Obtained through the courtesy of Dr. John Paul of Yale University; it is immunologically identical with West Nile virus.

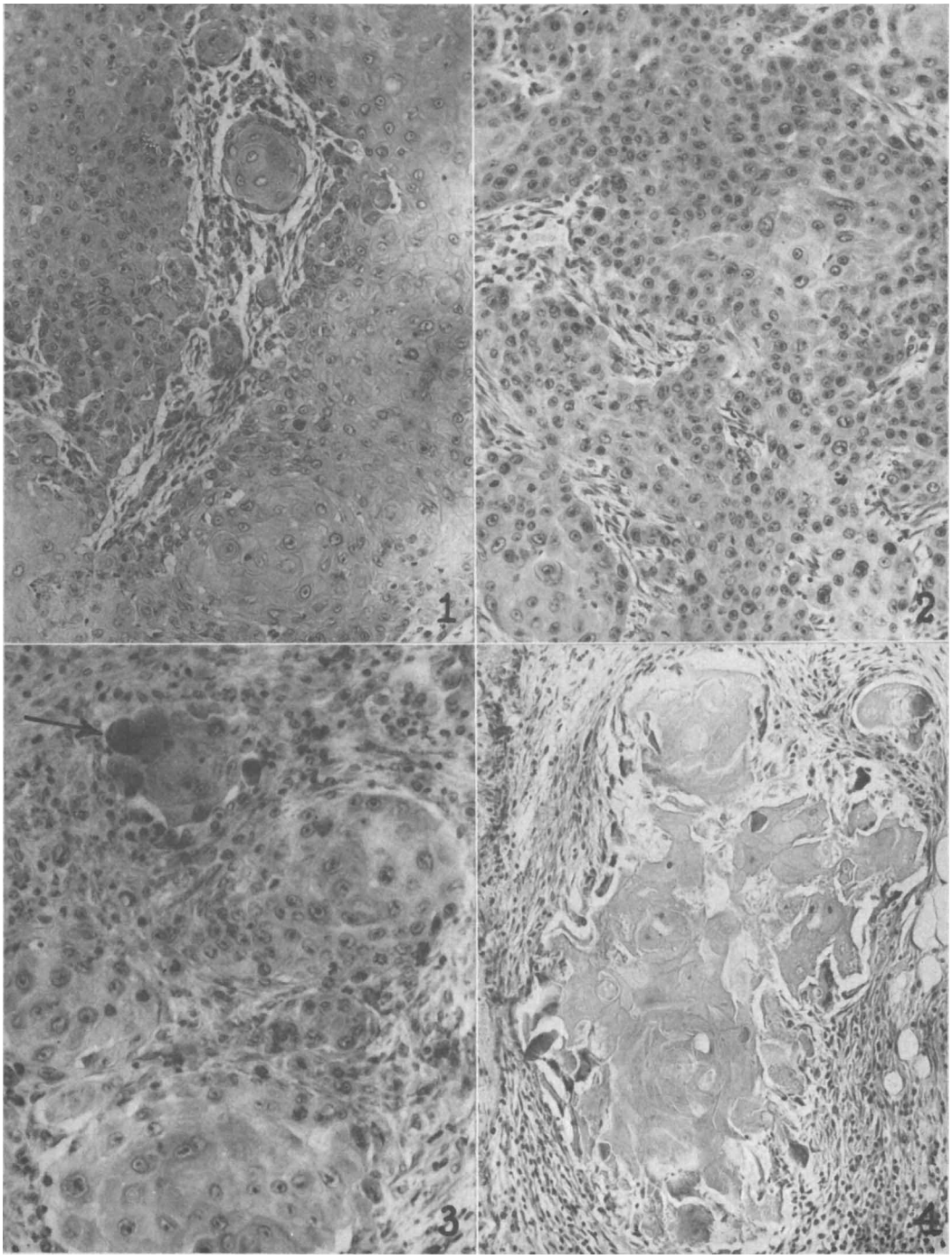

FIG. 1. Metastatic epidermoid carcinoma from neck of patient "K"; original tumor. × 120.

FIG. 2. Same "K" tumor after 11 days in an x-irradiated rat; virus injected 2 days previously. × 120.

FIG. 3. "K" tumor after 13 days in an x-irradiated rat; virus injected 4 days previously. Note degenerating cells (arrow). × 200.

FIG. 4. "K" tumor after 15 days in an x-irradiated rat; virus injected 6 days previously. × 120.

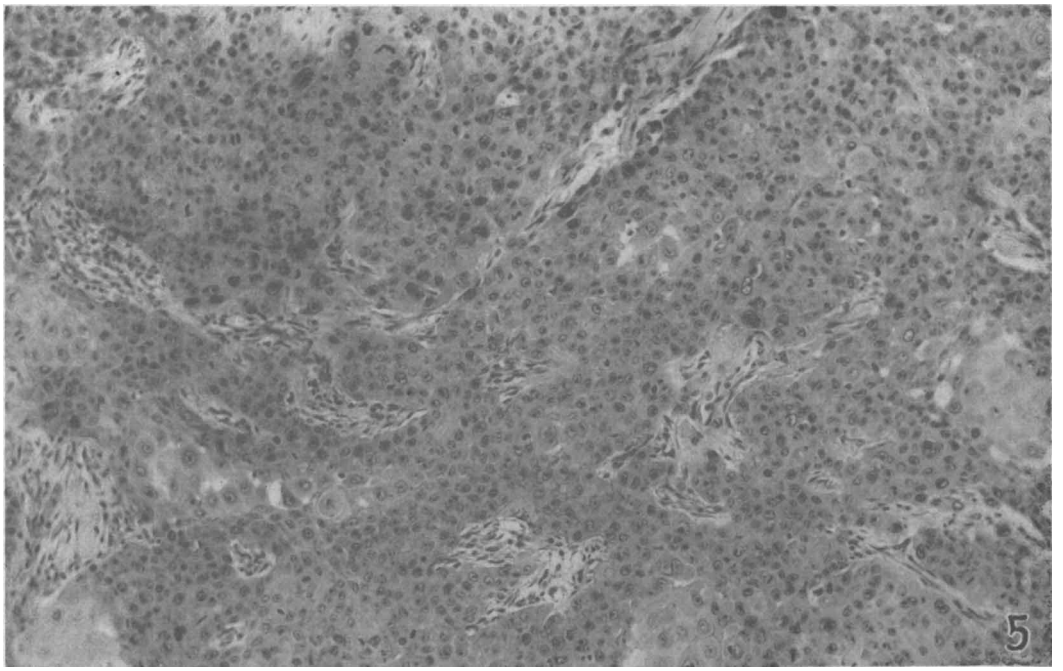

FIG. 5. Control "K" tumor grown 14 days in x-irradiated rat. × 80.

rats in which it grew well; it was grown on the chorioallantoic membrane of the chick where it exhibited a high degree of mitotic activity; and it was placed in roller tube tissue culture, using a horse serum-placental extract-Gey's solution mixture as nutrient. Each of the 16 plantings (2 to a cover slip) in the tissue culture showed good and remarkably uniform outgrowth of tumor 7 days after planting. On this day in order to test the effect of the virus on the tumor cells *in vitro* culture, as well as in the rat, the 8 cover slips with growing tumor were placed, 2 each, in 4 tubes. In 2 of the tubes the feeding material was mixed with the same Egypt 101 stock mouse brain emulsion used in the rats, in such manner that the final titer of virus in the tubes was 10^{-3}. To the feeding mixture in the 2 control tubes, normal mouse brain emulsion was added in amount equal to that in the virus treated tubes. The final volume of fluid material present in each tube was 1 cc. For the first 3 days, 0.1 cc was removed daily from each of the 2 tubes containing virus, the material pooled and titered in mice. A similar amount was withdrawn from the control tubes. Replacement was made of any amount removed by an equal quantity of ordinary tissue culture feeding solution. After the first 3 days, *i.e.* on the 4th, 7th, 9th and 10th days, 0.2 cc was taken from each tube and separate titrations done. Ten days after the original addition of virus (17 days after planting) all the cover slips were fixed and stained for microscopic examination. By this time it was estimated that all the original fluid in the tubes had been replaced by fresh extract.

Observations and discussion. All the tumors grown in untreated control rats were found to be in excellent condition, identical morphologically to the original carcinoma (Fig. 1), well vascularized and full of mitotic figures. Those removed on the 14th day after implantation (3 rats) were especially large, solid and free from necrosis (Fig. 5). The tumors removed from treated rats (4 animals) in the first 2 days after injection with virus were also growing well (Fig. 2); while those removed on the 3rd, 5th and 6th days were entirely dead (Fig. 4) and those of the 4th day degenerating (Fig. 3). The tumors of the 7th day had a peculiar appearance in that large central areas of the tumor were dead but foci about these regions remained alive. It

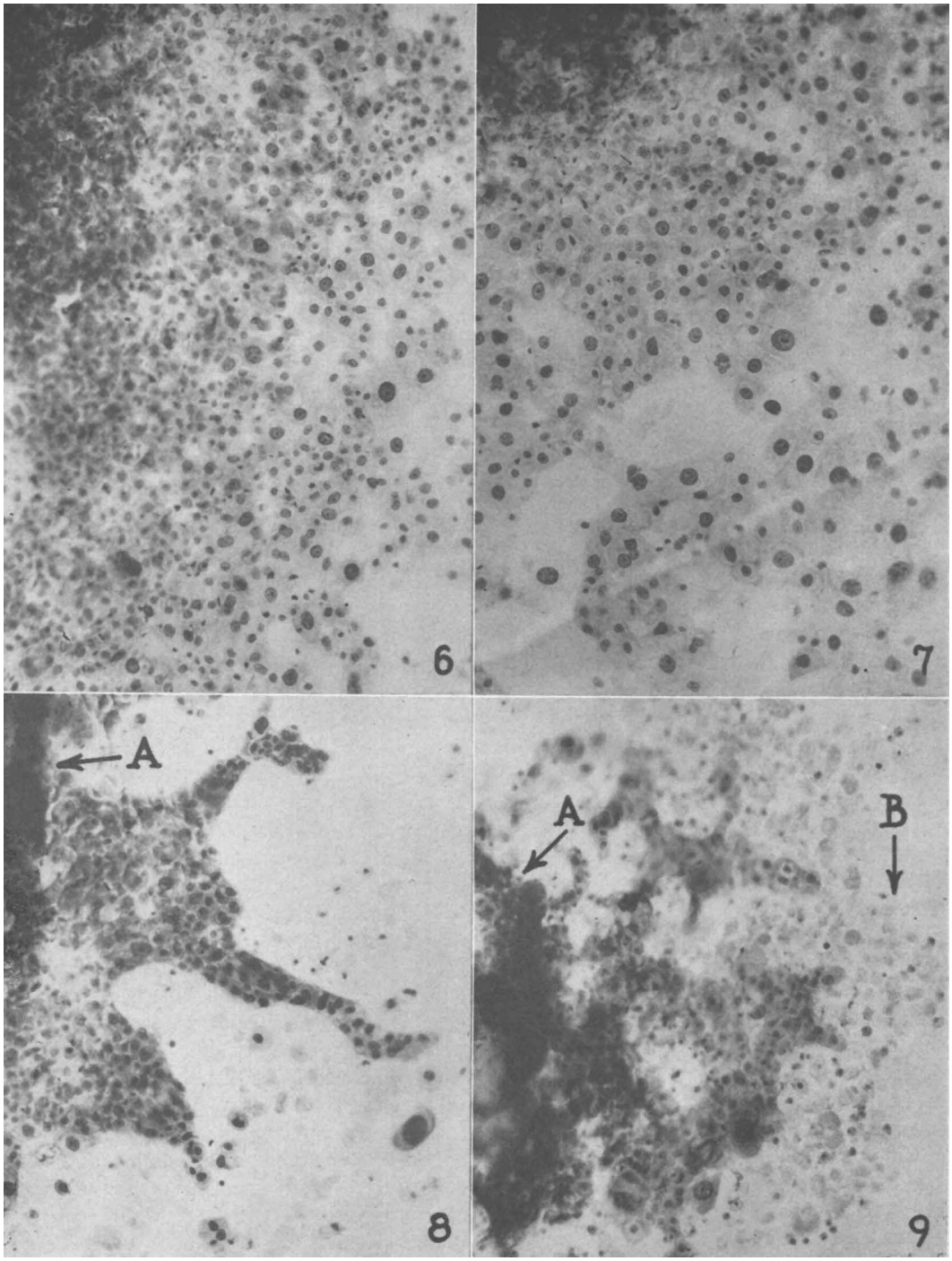

FIG. 6 and 7. Two control tissue cultures of "K" tumor, first grown 14 days in an x-irradiated rat (Fig. 5) and then 17 days in tissue culture. × 80.

FIG. 8 and 9. Two virus treated tissue cultures of "K" tumor, same age (17 days) and from same rat as controls (Fig. 5 and 6); Egypt 101 virus added 10 days previously after the tissue cultures had grown 7 days normally. Tumor cells have retracted toward original planting (arrows "A") or lysed (arrow "B"). × 80.

TABLE I. Titer of Egypt 101 Virus in Tissue Cultures of "K" Tumor.*

Day after virus added to cultures	Virus titers† Tube 1	Tubes 1 & 2 (pooled)	Tube 3
1		$10^{-5.25}$	
2		$10^{-5.50}$	
3		$10^{-6.25}$	
4	$10^{-6.25}$		$10^{-5.25}$
7	$10^{-4.25}$		$10^{-5.50}$
9	$10^{-3.75}$		10^{-4}
10	10^{-5}		10^{-5}

* Original titer of virus in tissue culture $= 10^{-2}$. Virus added to cultures, 7 days after planting.

† Titers determined by intracerebral inoculation of test fluid into Swiss albino mice. See text.

should be noted that though the virus had been injected into the tumor of one flank only, both tumors of any one animal were comparable. (The injected tumors were found to titer, in mice, approximately $10^{-3.5}$ except that the 5th and 6th days were 10^{-1} and the 7th $10^{-2.5}$. The uninjected tumors had no titer for the first 2 days, 10^{-1} on the 3rd and 6th days, and 10^{-2} on the 4th and 7th days.)

Microscopic examination of the tissue cultures of "K" tumor showed an even more striking contrast between the control and virus treated tumors. Each of the 8 explants in the control tubes was still in luxuriant condition (Fig. 6 and 7) 17 days after planting, while every one of the 8 treated explants showed complete lysis of the tumor cells or retraction toward the original site of outgrowth (Fig. 8 and 9). (Since the human carcinoma possessed very little rat stroma at the time of planting and had been almost "pure" tumor (Fig. 5) very few normal rat cells had grown out. It is noteworthy that the few fibroblasts and macrophages present appeared undamaged even in the virus treated tubes.) The virus titrations of the tissue culture fluid (Table I) showed that considerable multiplication of virus occurred. By the 10th day all the original volume of virus containing feeding fluid had been replaced by ordinary nutrient, yet the titers remained very high. It would have been interesting to see how long these titers would have persisted after complete lysis of the tumor cells. It is possible that the normal rat cells present accounted for some of the titer, yet they were so few in number that the amount must have been very small indeed.

It thus seems apparent that Egypt 101 possessed oncolytic activity against the epidermoid carcinoma of "C.K." whether grown *in vivo* in a complex host such as a rat, or *in vitro* in tissue culture. The final test, of course, would be to treat "C.K." with Egypt virus if he has a recurrence of his tumor.

It is hoped that this method of testing the oncolytic activity of a virus against any particular tumor will be of considerable practical value and give a more rational approach to therapy with oncolytic viruses. The work with viruses on transplantable mouse tumors has indicated a very specific tumor spectrum for the oncolytic activity of each virus used. Unpublished data of ours indicates that this finding will hold true for the human tumors as well since it has been found that in 2 cases (a recurrent mixed tumor of the parotid and a metastatic squamous carcinoma), Egypt 101 reproduced in the tumors grown in rats but caused no apparent damage. These tumors were transferred after 14 days into 2nd generations of x-irradiated rats and still had titrable virus (10^{-3}) when the 2nd groups of animals were killed (28 days after surgical removal).

Since Moore(4) has indicated that the oncolytic activity of a virus can be increased by passage in mouse tumors it is also possible that a similar procedure and result can be carried out by continued virus passage in human tumors borne by the x-irradiated rats. Work is now under way to test this.

Summary. 1. Egypt 101 virus was found to possess oncolytic activity against a human epidermoid carcinoma grown either in x-irradiated rats or in tissue culture. 2. It is suggested that this rat-tissue culture method of testing the oncolytic activity of a virus for any particular tumor may be employed in estimating the possible value of virus therapy for each individual patient.

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Failure of Rutin and Related Flavonoids to Influence Mortality Following Acute Whole Body X-Irradiation.* (19491)

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The flavonoids were the first compounds reported to reduce the mortality in animals following whole body x-irradiation. The rationale for the use of the flavonoids in the attempt to prevent or treat radiation injury has been based upon the claim that these compounds reduce the hemorrhage resulting from increased capillary fragility(1). Rekers and Field(2) observed a reduced hemorrhagic tendency and obtained improved survival rates among irradiated dogs pretreated with rutin or other flavonoids. Clark, Uncapher, and Jordon(3) obtained increased survival of guinea pigs treated with calcium flavonate. Sokoloff, Redd, and Dutcher(4) found that CVP, a citrus "vitamin P" compound, significantly reduced the mortality of rats exposed to 800 r of x-radiation. Opposed to these findings are the negative results of other workers. Kaplan(5) and Cronkite *et al.*(6) used rutin in mice, and Kohn and coworkers (7), and Patt and his associates(8) used rutin in rats without observing any effect on radiation mortality. Similarly CVP had no beneficial effect in mice(9), and rutin and hesperidin failed to influence the mortality or hemorrhagic changes in irradiated young chicks(10).

In view of the conflicting reports regarding the value of the flavonoids in reducing the mortality of animals exposed to acute whole body x-radiation, the present investigation was undertaken in order to explore further the possible usefulness of these compounds.

Materials and methods. The flavonoids used include: rutin in pure form, rutin in de-ionized form, rutin solubilized with piperazine hexahydrate, quercitrin, quercetin, hesperidin, dihydroquercetin, naringen, calcium flavonate, hesperidin methyl chalcone, xanthorhamnin, hemoeriodictyol, morin (de-ionized), citrus vit. P (CVP), and lemon juice infusion (LPI). CVP is reported(9) to contain 4% of a quercitrin-like substance, 15% eriodictin, 80% hesperidin-glucose, and 0.38% calcium phosphorus ash. Lemon peel infusion contains eriodictyol glycoside, hesperidin, perhaps other flavonone glycosides, i-inositol, some fractions of the vit. B complex, and some natural soluble carbohydrates. A total of 1132 CF_1 female mice and 232 female Sprague-Dawley rats were used. The mice were 7 to 9 weeks in age and weighed 18 to 24 g prior to irradiation. The rats were 10 to 18 weeks old and weighed 116 to 246 g at the time they were started on the flavonoid treatment. In addition, 70 female guinea pigs with a weight range of 205 to 400 g were included in the studies. The mice and rats were fed Wayne Dog Blox. The guinea pigs were given Purina Rabbit Chow Meal supplemented daily by fresh cabbage and timothy hay. Animal weights, deaths, and food and drinking water consumed, if containing a flavonoid, were recorded daily. In all tests the administration of the compounds was con-

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