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Influence of Total Body X-irradiation on Tumor Induction by Parotid Tumor Agent in Adult Mice. (26127)

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The parotid tumor agent, (PTA, polyoma virus), has certain limitations of action in several parameters, such as species, age, mouse strain, and tissue susceptibilities and type of response by different cells or by cells of the same morphogenetic lineage infected at different levels in their development. Differences in cell sensitivity and specificity of response to PTA have been discussed(1).

In a previous study(2), sensitivity in certain strains of mice to PTA was demonstrable through the first 14 days *post partum* without appreciable lengthening of the latent period. Beyond this age the response has not been adequately studied. Preliminary data from our laboratory indicated that sensitivity was increased in 1 to 2-month-old mice given whole-body X-radiation prior to injection of virus. The present report concerns the response of adult mice of several age groups to PTA, influence of whole-body X-irradiation on this response and a histologic comparison of the tumors and lesions in mice inoculated as adult or as newborns.

Methods and materials. Mice. C3Hf/$_{BiLw}$ mice from our pedigreed colony were used. This strain was obtained directly from Dr. J. J. Bittner in 1955 and has been inbred continuously since that time, brother x sister. Frequency and types of neoplasms in a sample of mice of this subline have been reported (2). Parotid tumors within the breeding colony or following acute whole-body-X-irradiation have been observed only very rarely (3), despite a high frequency of serologic convertors(4). Age of these mice at radiation and injection of virus is given in the results.

Virus. PTA has been passaged in the continuous tissue culture line derived from a lymphocytic leukemia, designated P-388D_1. The origin of virus and cell strain have been described(2,5). Source of virus was the tenth serial passage obtained by collecting tissue culture fluid at time of maximum CPE. This material had been stored at −70°C for 8 months prior to use here. The HA titre of the 10-fold diluted material, used for intravenous inoculations in adult mice was 1:40. Intravenous injection of 0.5 ml of virus was accomplished in Groups 1 and 2 (Tables I and II) through the lateral tail vein. This represented an inoculum of $10^{4.9}$ adult mouse ID_{50}/mouse as determined by the MAP test(6). Although HA titre and mouse ID_{50} are relatively low, this preparation has proved to be highly oncogenic and primarily thymotrophic, inducing thymic epithelial tumors in nearly all mice inoculated at birth (7).

The adult C3Hf/$_{BiLw}$ mice in the third group, (Table IV) received either tissue culture explants of salivary gland tissue or tissue culture fluid plus cell scrapings from these cultures. Explants of salivary gland tissue were exposed to tenth passage P-388 D_1- passaged PTA prior to explanting as gelatin-sponge-matrix cultures as previously described(8). Transfers of explants or tissue culture fluids were made on the 48th day. (See(8) for morphologic changes observed *in vitro* in these intact fragments of salivary

TABLE I. Induction of Tumors in C3Hf/$_{BiLw}$ Adult Mice with Parotid Tumor Agent, PTA.

Treatment	No. of mice: Initial	No. of mice: Alive after 1 mo	No. litters (pos./total)	No. parotid tumors	Mean latent period in mo (range)	Tumor sites other than salivary
X-radiation	23	15	0/3	None	—	—
PTA	23	23	0/3	"	—	—
X-radiation + PTA	22	16	3/3	7*(43.8%)	8.7 (4½-15½)	Thymus—1 Hair follicle—1 Subcut. connective tissue—1

* Four mice had unilateral parotid tumors.

Two mice of the first group had neoplasms of reticular tissues as follows: 1 lymphocytic leukemia (15 mo), 1 reticulum cell sarcoma (14 mo); one mouse of the second group had a lymphocytic leukemia (14 mo).

TABLE II. Induction of Tumors in Thymectomized, Adult C3Hf/$_{BiLw}$ Mice Following Intravenous Injection of Parotid Tumor Agent, PTA (Polyoma Virus).

Treatment	No. of mice: Inoc.	No. of mice: Alive after 1 mo	No. litters (pos./total)	No. parotid tumors	Mean latent period in mo (range)	Tumor sites other than salivary
X-radiation + PTA	41	35	5/5	10*(28.6%)	7.9 (3-14½)	Hair follicle—3 Thyroid—1 Bone—2 Mammary—3

* Five mice had unilateral parotid tumors (right or left).

Six mice had neoplasms of reticular tissues, as follows: 1 granulocytic leukemia (14 mo), 2 lymphocytic leukemia (13, 14 mo), 3 reticulum cell sarcomas (14, 15 and 15 mo). These were not associated with parotid tumors.

gland tissue exposed to PTA). Explants were transferred under the renal capsule or into the subcutaneous connective tissues or to both areas and tissue culture fluids plus scrapings of cells were introduced into the subcutaneous connective tissues of the right axilla. Each animal received fluid and cells of one culture, both in test and control series. Comparable materials from control cultures were introduced into 10 adult mice. All mice had received 300 r whole-body X-irradiation 2 days before introduction of materials.

Radiation. Two X-ray tubes placed opposite each other were operated at 200 kvp and 15 ma.; the filtration achieved was with 0.25 mm Cu and 0.55 mm Al. Radiation distance was 54 cm from each focal spot to the center of the mouse. Dose rate was 120 r/min. Mice in Group 1, (Table I) were given 300 r whole-body X-irradiation at 2 months of age, Group 2 (Table II) at 6 weeks of age; this group was thymectomized at 1 month of age, and Group 3 mice (Table IV) were nearly equally divided into 1 month and 2 month-old mice at time of receiving 300 r, whole-body X-irradiation. Virus and materials containing virus were introduced 2 to 3 days after irradiation.

Autopsies. Necropsies as described earlier (2) were performed on most of the animals in this study. Tissues were fixed in either Zenker-formol or Tellyesniczky's fluid, and stained with hematoxylin and eosin.

Results. The results in C3Hf/$_{BiLw}$ mice, 2 months old at time of treatment, are given in Table I. Whole-body X-irradiation or virus alone were ineffective in inducing parotid gland tumors. These mice have now lived for 17 months. Whole body X-irradiation (300 r), followed by intravenous injection of 0.5 ml of virus resulted in typical pleomorphic parotid gland tumors in 2 of 7 males and 5 of 9 females at a considerably lengthened latent period. Four of these appeared as unilateral parotid gland tumors only, at 4½, 7, 10 and 15½ months, respectively. The relative resistance of adult mice is reflected further in the rare occurrence of other primary sites of tumors and/of lesions.

Ten of 35 C3Hf/$_{BiLw}$ mice thymectomized

TABLE III. Tumors and Lesions in Twenty Non-irradiated (C3Hf × AKR)$_{F_2}$ Mice Inoculated as Newborn with Tenth Tissue-Culture Passage of PTA.*

Site	Type of reaction	No. mice showing reaction	Comments
Parotid glands	Tumor	19	All tumors microscopic size and in most instances multifocal.
Sublingual glands	"	17	
Submandibular glands	"	6	
Lacrimal glands	"	11	Only 1 tumor grossly visible at necropsy.
Thymus	"	20	All tumors grossly evident and associated with signs of respiratory insufficiency. Tumor also present in 2 ectopic thymuses.
Thyroid gland	"	13	
Tracheal submucosal glands	"	9	
Glands of oro-pharynx and nasal mucosa	"	6	All tumors microscopic in size.
Harderian gland	"	1	
Mandible	Tumor (ameloblastoma-like tumor)	1	
Renal cortex	Alteration of tubular epithelium	Marked 15 Moderate or mild 5	

* These mice represent the first 20 consecutive animals to come to autopsy out of a total of 118 that received a subcut. inj. of 0.05 ml of 1:100 dilution of culture fluid. This virus preparation was the same used to inoculate all other animals in this report, but dosage is only 1/100th that used intrav. in adult mice. Interval between inoculation and autopsy for these 20 mice ranged from 16 to 40 days.

TABLE IV. Tumors in Adult C3Hf/$_{BILw}$ Mice Receiving Organ Explants or Tissue Culture Fluids of PTA-Infected Salivary Gland Tissue.

Material	No. of mice	No. of parotid tumors	Latent period in mo (range)	Tumor sites other than salivary in parotid-tumor bearing mice	Primary tumor sites in mice not bearing parotid tumors
Salivary gland explants	10	7*	4 (3-6)	Thymus, hair follicle, bone, mammary, thyroid, subcut. conn. tissue, other salivary glands	None
Tissue culture cells	20	3†	13.3 (10-18)	Subcut. conn. tissue	Thymic epithelial tumors—2 Lipo sarcoma—1 Bone—1

* Bilateral parotid tumors involving also other salivary glands.
† Unilateral parotid tumors only.

at 1 month of age and given 300 r whole-body X-irradiation and PTA at 6 weeks of age presented tumors of the parotid gland (Table II). The frequency and latent period were strikingly similar to those observed in intact adult mice, despite the fact that this preparation of virus is thymotrophic when introduced into mice less than 48 hours of age, inducing thymic epithelial tumors in nearly all recipients with involvement of the salivary gland tissues and other primary sites seen upon microscopic examination.

In this second group of adults, pleomorphic parotid tumors were nearly equally distributed among the sexes. Five tumors were unilateral only with no other primary sites of tumor. Tumors of primary sites in these mice with bilateral parotid tumors were indeed infrequent (Table II) in comparison with the response of neonatal mice.

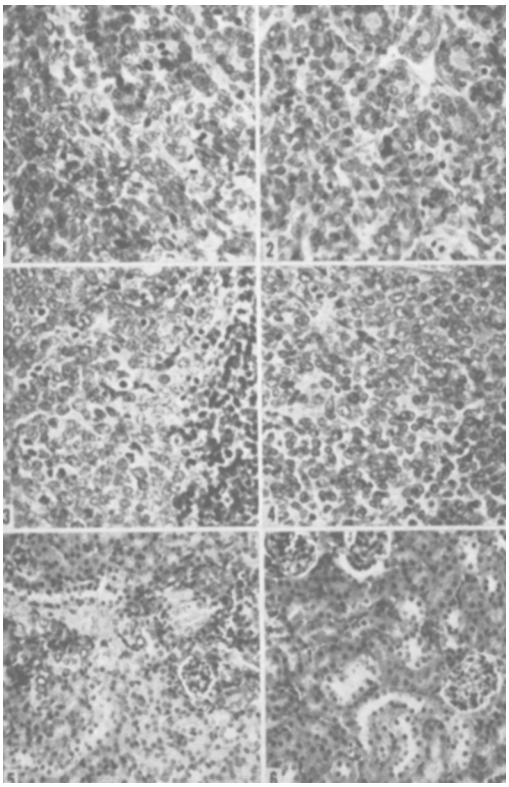

FIG. 1. Section of parotid tumor from a female ($C3Hf/_{Lw} \times AKR)_{F_2}$ mouse that had received no X-ray. Mouse received PTA of 10th P388 D_1 tissue culture passage at birth and was autopsied at 5 wk of age. Note poorly-defined tubular structures (upper right) surrounded by and adjacent to round and spindled cells presenting a sarcoma-like appearance, typical of PTA-induced parotid tumor. Hematoxylin and eosin, ×215.

FIG. 2. Section of parotid tumor from a female $C3Hf/_{Bi}$ mouse, thymectomized at one mo of age and irradiated with 300 r to whole body at 6 wk of age. Mouse received PTA of 10th P388 D_1 tissue culture passage 2 days following irradiation. Autopsy was performed when mouse was 5 mo old, at which time it bore a large tumor of the left parotid gland and 2 mammary tumors. Histologically, this tumor did not differ from that illustrated in Fig. 1. Hematoxylin and eosin. ×215.

FIG. 3. Section of thymic epithelial tumor from a female ($C3Hf/_{Lw} \times AKR)_{F_2}$ mouse that had received no X-radiation and was inoculated with 10th P388 D_1 tissue culture passage of PTA at birth. Autopsy was performed at one mo of age. Epithelial tumor cells form solid mass at left. Lymphocytes occupy lower right corner of field. Hematoxylin and eosin. ×215.

FIG. 4. Section of thymic epithelial tumor from a female $C3H/_{Bi}$ mouse that had received a transplant of PTA-infected salivary gland from tissue culture, at one mo of age. This animal also received 300 r of total-body X-irradiation on day of transplantation. At autopsy 3 mo later, tumors were found in all salivary glands, exorbital lacrimal glands, thymus, thyroid, hair follicles, and bone. Thymic tumor shown here contained fewer lymphocytes than that in Fig. 3, but was otherwise identical. Hematoxylin and eosin. ×215.

FIG. 5. Section of kidney from same mouse as in Fig. 3. Small inflammatory infiltrates are present about portions of renal tubules that contain enlarged epithelial cells with "ballooned" nuclei and intranuclear inclusions. Such changes were very numerous in kidneys of mice inoculated with PTA as newborns. Hematoxylin and eosin. ×215.

FIG. 6. Section of kidney from same mouse as in Fig. 4. No significant changes are present. Lesions of type shown in Fig. 5 were consistently absent from kidneys of mice inoculated with PTA as adults. Hematoxylin and eosin. ×215.

The histologic appearance of primary tumors encountered in Groups 1 and 2, irradiated adult $C3Hf/_{BiLw}$ mice, did not differ significantly from that described earlier for many strains of mice receiving virus as newborns. A direct comparison with tumors induced in non-irradiated mice that received the same virus preparation, but in smaller dose and by subcutaneous route at birth, has limited value because the salivary gland tumors in the irradiated mice were much larger than those in the non-irradiated mice inoculated as newborns. Mice of the latter group reached the stage of terminal illness from thymic tumor effects at a time when their salivary tumors were still only microscopic in size. Nevertheless, the basic tumor pattern, with a marked tendency toward loss of epithelial character and gain of spindle-cell features, was observed in both irradiated and the non-irradiated groups (Fig. 1 and 2). Likewise, tumors of thymus (Fig. 3 and 4), hair follicle, and thyroid found in irradiated mice differed histologically in no way from PTA-induced tumors in these same tissues in non-irradiated mice. Microscopically, no evidence was seen to indicate that X-irradiation had any qualitative effect on the pathogenesis of those tumors that developed.

Quantitatively, however, no tumors were observed in submaxillary, sublingual, and accessory glands of the oropharynx, or in submucosal glands of the nasal passages and trachea in the X-irradiated adult animals. Furthermore, the renal cortical epithelial lesions so characteristic of PTA infection in newborn mice were absent in these adult animals, even when parotid tumors were present (Fig. 5 and 6). The most sensitive histologic indi-

cator for this particular strain of the agent would thus appear to be parotid tumor development.

Table III presents results of tumor response of mice inoculated when less than 24 hours of age with the same virus preparation used in the adult study.

Two mice of the thymectomized group were positive for HI antibodies (HI>200) at time of virus inoculation and have remained tumor-free. All others in this group and in Group 1 (Table I) were HI negative. Sera of the mice in Group 3 were not tested for HI antibodies.

Preliminary results have been reported on transfer of PTA-infected explants of salivary gland tissue to irradiated mice(8). There are several interesting aspects of the completed results (Table IV) of these mice which have now reached 17 months of age. Response of adult irradiated mice receiving 48 day explants of salivary gland tissue from cultures exposed to PTA was similar to that of mice receiving PTA at birth. Bilateral parotid gland tumors appeared early at 1 to 2 months after transfer of cultures, and there were observed primary tumors of other salivary glands, of the lacrimal glands, and of thymus, hair follicles, bone, thyroid gland, mammary glands and subcutaneous connective tissues. In contrast, only 3 of 20 mice receiving cells adherent to the glass of these test cultures, developed parotid tumors. These were unilateral, there was only one other primary site involved in tumor formation, and the latent periods were long. In addition 2 thymic epithelial tumors were observed at 14 and 15 months, respectively, in mice receiving fluid and cells of the test cultures. In one of these mice there was no gross evidence of parotid tumors; histologic sections were not available. In the other, the histologic sections presented no evidence of parotid tumors. A liposarcoma and a bone tumor appearing in mice receiving cells and fluid from cultured explants are probably unrelated to PTA, as they have been seen occasionally in control C3Hf/$_{BiLw}$ mice, and were histologically distinct from PTA-induced tumors of similar tissue origin.

In only one of the necropsied tumor-bearing mice was there gross or microscopic evidence of a tumor at the site of transfer of explants or tissue culture fluids and cells. This was a fibrosarcoma in the right axilla of a mouse also bearing a unilateral parotid tumor. This mouse had received tissue culture scrapings of cells and fluid. More recently, however, progressively growing neoplasms at site of transplant(9) have been observed following transfer of several cultures of salivary gland tissue to mice in the neonatal period after varying periods of PTA exposure *in vitro*.

One animal in 10 C3Hf/$_{BiLw}$ mice receiving control explants, developed a unilateral parotid tumor at 10 months of age. This is not surprising in view of the ease of cross-contamination and extreme stability of this virus.

Nine neoplasms (6.5%) of reticular tissues (leukemia) were found among the 139 mice under observation. This frequency is somewhat lower than that previously observed in this strain of mouse(2) but many mice are still alive. These were not found in excess in the mice receiving PTA.

Discussion. The data presented show that tissues of non-immune adult mice of the C3Hf/$_{BiLw}$ strain are capable of responding to PTA (polyoma virus) by development of neoplasms of the parotid gland. The resistance of adult mice is similar to a strain resistance observed in C57Bl/$_{Ka}$ mice to PTA (1). Relatively few primary sites are involved in tumor induction and the latent period is long. The striking thymotrophic character of the Passage X P388D$_1$ strain of PTA, as observed in mice receiving virus shortly after birth was not expressed by adult irradiated mice. Thus thymectomy would not be expected to influence the response in adults.

The role irradiation plays in influencing sensitivity of adult tissues needs to be investigated. Additional information obtained in this laboratory shows that:

1) whole body X-irradiation given 3 days to 1 week after introduction of virus is as effective in modifying sensitivity as irradiation given prior to virus, and

2) shielding of a thigh during irradiation does not influence the response.

Despite the fact that infection by polyoma virus occurs spontaneously in our breeding colonies at a frequency greater than 30% in mice over 3 months of age(4), an extremely low incidence of parotid tumors, and at lengthened latent periods has been observed in mice of the following inbred strains receiving fractionated whole-body X-radiation at total doses of 400 to 672 r: C57Bl/$_{Ka}$, 400 mice, 0 parotid tumors; RFM, 80 mice, 0 parotid tumors; C3Hf/$_{Lw}$ 92 mice, 1 parotid tumor; AKR/$_{Lw}$, 165 mice (thymectomized) 2 parotid tumors; C3Hf/$_{BiLw}$, 159 mice, 2 parotid tumors. These strains were observed throughout life.

The likelihood that relative resistance of non-immune adult mice to the tumor-inducing effects of PTA is a dose-response phenomenon is indicated by 2 observations: 1) Irradiated C3Hf/$_{BiLw}$ adults given 48-day PTA-infected submandibular gland explants have revealed a high potential response by developing primary tumors in a variety of sites at short latent periods and in a majority of animals, resembling the response of mice inoculated shortly after birth, and 2) adult submandibular explants infected with PTA *in vitro* appear to be more susceptible to the proliferation-inducing effects of virus than explants of tissue from newborn mice(1). Unfortunately no quantitative data are available at the moment to answer the questions.

It is clear that a peculiar susceptibility of the newborn mouse to PTA is not a prerequisite for induction of tumors as evidenced particularly by the response of those C3H mice, discussed above, that received virus from infected explants of salivary gland tissues.

Summary. Pleomorphic tumors of the parotid gland have been induced by PTA (polyoma virus) in non-immune adult C3Hf/$_{BiLw}$ mice given 300 r whole-body X-radiation. The latent period was long and few other primary sites were involved in tumor induction following intravenous injection of a virulent thymotrophic virus preparation. Thymic epithelial tumors, induced in 100% of mice injected in the neonatal period, did not appear in mice injected as adults. Thymectomy therefore could not be shown to have an influence on the expression of tumors in adult-injected mice. The response of X-rayed, adult C3Hf/$_{BiLw}$ mice receiving salivary gland explants exposed *in vitro* to PTA, was in contrast similar to the response of mice receiving virus within 24 hours *post partum.* Tumors arising at many primary sites in a relatively short period of time were observed. No differences were found in the pathogenesis of the tumors in the groups studied here. The typical renal cortical epithelial lesions characteristic of PTA infection in newborn mice were absent in those animals receiving virus as adults.

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