the thyroid epithelial cells. Whether this hypothesis can be established with certainty remains for continuing studies to disclose.

The occurrence of spontaneous sialadenitis has been described in various species(10,11). The hamster is no exception(12). Salivary gland disease in this species is characterized by prominent intranuclear inclusions. These have not been observed in the salivary glands of any animals studied in this laboratory. Furthermore, no inflammatory changes have been observed in a large number of animals which had not received iodide. The possibility must, of course, be considered that iodide may activate a latent viral agent. If so, and such seems most unlikely, this agent is unaccompanied by inclusions and must be activated with great rapidity, *i.e.*, within 4 hours.

The sexual dimorphism of the submaxillary glands of the rat and mouse is well recognized(10). All of the observations reported herein have been in males. Whether such a change occurs in females remains to be studied. So, too, the effects of prolonged administration of iodide as well as the possible production of chronic sialadenitis are worthy of further investigation.

Summary. Administration of large amounts of potassium iodide to the hamster leads to an inflammatory reaction in the submandibular glands, but not in the parotid or sublingual structures. The primary site of damage is the distal duct system. Such selective damage by this agent may be related to the greater concentrating ability of iodine by the submandibular glands in contrast to the parotids and sublinguals.

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Establishment of Resistance to Gardner Lymphosarcoma (6C 3HED) and L#2 Lymphoma in C3H and A/He Mice.* (26872)

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The production of resistance to growth of Ehrlich carcinoma in C57 BL mice(1,2) and in Swiss mice(3) by intraperitoneal injections of X-irradiated Ehrlich carcinoma cells has been reported. Furthermore, Revesz(4,5) has found that if X-irradiated Ehrlich cells are mixed with non-irradiated cells in appropriate quantities there is inhibition of cancer cell growth and immunity is produced in the injected mice. However, if too few X-irradiated cells are present in the mixture there may even be an enhanced growth without immunity being produced.

We are reporting here the production of complete resistance in C3H mice to Gardner lymphosarcoma (6C 3HED) and L#2 lymphoma, and in A/He mice to Gardner tumor.

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Also partial resistance was produced in A/He mice to L#2 lymphoma. These immunized mice showed little or no protection when injected with a second completely viable non-irradiated tumor.

Materials and methods. The 113 C3H mice and 107 A/He mice used in these experiments were obtained from the Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine, and from the Cancer Research Genetics Laboratory, Univ. of California, Berkeley. Both males and females, 7-11 weeks of age at start of experiments, were used. The mice were housed in groups of 5 in hanging metal cages, 5″ × 5″ × 7″, with wire bottoms, and were given water and Ralston Purina Laboratory Chow *ad libitum*.

The ascitic forms of the Gardner lymphosarcoma, Cancer Research Genetics Laboratory, and the L#2 lymphoma, obtained from Dr. Theodore S. Hauschka, Roswell Park Memorial Inst., Buffalo, N. Y., were employed. The Gardner tumor was harvested after 7-9 days' growth in C3H mice, in which tumor was maintained by weekly intraperitoneal injections of 0.2 ml of undiluted tumor. The L#2 tumor was obtained after 7-10 days' growth in A/He mice in which tumor was maintained by weekly intraperitoneal injections of 0.2 ml of tumor diluted 1:100 with 55 mM phosphate Locke's solution. Ascites tumors were withdrawn from the peritoneal cavity under sterile conditions, and employed untreated or diluted with phosphate Locke's solution. In the irradiation process 1-6 ml of undiluted tumor were placed in special sterile 50 ml balloon flasks which were rotated under the X-ray unit at a rate of 25-30 rpm at a temperature of 28-30°C. Tumor was X-irradiated with 2000 r, at a rate of 53 r per minute and a focal distance of 45 cm. Dose rate was calibrated in air for the 250 kv X-ray head having 0.21 mm of Cu, inherent. Additional filters were 1 mm of aluminum and 0.5 mm of Cu, parabolic. The half value layer was 2.1 mm of Cu.

All tumor injections were made intraperitoneally under sterile conditions. The Gardner tumor, containing about 240,000 cells per mm^3, was injected in amounts ranging from 0.2 ml diluted 1:100 with 55 mM phosphate Locke's solution to 0.2 ml undiluted. The L#2 tumor, containing about 150,000 cells per mm^3, was injected in amounts varying from 0.1 ml diluted 1:100 with phosphate Locke's solution to 0.5 ml undiluted. The quantities of each tumor used for the various experiments are shown in Table I.

Primarily this study involved 4 phases, attempts to establish resistance to Gardner lymphosarcoma in C3H and A/He mice, and to L#2 lymphoma in C3H and A/He mice. In each phase were 2 groups of mice, controls and test animals. Test animals initially received 8 injections (2 per week) of X-irradiated tumor. Four to 7 days after the last injection of X-irradiated tumor the mice received a challenging injection of non-irradiated viable tumor. A second challenging injection of viable tumor followed 20-90 days later (Table I). Control animals were untreated mice that received a single injection of non-irradiated tumor. Controls were utilized for the initial injection of X-irradiated tumor and for each of 2 challenges with non-irradiated tumor.

An additional phase of the study, not shown in the table, was to determine whether mice that were made immune to one of the tumors showed resistance to the second tumor. Consequently, after establishing immunity in C3H mice to Gardner lymphosarcoma and giving them 2 challenges with viable Gardner cells, they were then challenged 38 days later with 0.5 ml non-irradiated L#2 lymphoma cells. Similarly, C3H mice that were immunized and twice challenged with L#2 lymphoma cells, were subsequently challenged 30 and 80 days later with 0.2 ml (1:100 diluted) non-irradiated Gardner lymphosarcoma cells. Also, A/He mice that were immunized and twice challenged with Gardner lymphosarcoma cells, were subsequently challenged 40 days later with 0.1 ml (1:100 diluted) non-irradiated L#2 cells.

Results. The data in the table show that 8 injections of X-irradiated Gardner lymphosarcoma offered complete protection against growth of viable non-irradiated Gardner tumor in both C3H and A/He mice for both challenges. All of the C3H control animals

TABLE I. Production of Resistance to Gardner Lymphosarcoma (6C 3HED) and L#2 Lymphoma in C3H and A/He Mice.

Treatment*	Survivals‖	Survival time (days)	Amt and dilution of tumor
A. Gardner lymphosarcoma in C3H mice			
Controls	0/39	10–13 (12)§	.2 ml 1:100
Test animals—X-irradiated tumor	36/36	—	*Idem*
1st challenge (5)†	36/36	—	"
2nd challenge (22)‡	36/36	—	"
B. Gardner lymphosarcoma in A/He mice			
Controls	4/23	9–20 (12)§	.2 ml undil.
Test animals—X-irradiated tumor	23/23	—	*Idem*
1st challenge (4)†	23/23	—	"
2nd challenge (33)‡	23/23	—	"
C. L#2 lymphoma in C3H mice			
Controls	3/23	7–11 (8)§	.5 ml undil.
Test animals—X-irradiated tumor	15/15	—	.4 ml "
1st challenge (5)†	15/15	—	.5 ml "
2nd challenge (30)‡	13/15	(5)§	.5 ml "
D. L#2 lymphoma in A/He mice			
Controls	0/30	9–14 (11)§	.1 ml 1:100
Test animals—X-irradiated tumor	24/31	18–30 (25)§	" 1:100–1:10
1st challenge (7)†	13/24	2–34 (21)§	" 1:100
2nd challenge (90)‡	6/13	13–33 (26)§	" 1:100

* Controls received 1 inj. of non-irrad. tumor. Test mice received 8 inj. of 2000 r X-irrad. tumor followed by 2 inj. of viable non-irrad. tumor (challenges).

† Days since 8th inj. of X-irrad. tumor.

‡ Days since 1st challenge.

§ No. in parentheses is avg.

‖ Ratios express No. of mice surviving, over total No. of mice in the group treated.

died and all except 4 of the A/He controls died. Similarly, 8 immunizing injections into C3H mice with X-irradiated L#2 lymphoma gave complete protection for the first challenge, while all except 3 controls died. All except 2 of these mice survived a second challenge.

In experiments with L#2 lymphoma and A/He mice 7 animals died as a result of injecting 2000 r X-irradiated tumor. A plausible explanation for these deaths is that there may have been inadequate X-irradiation thus allowing completely viable cells to remain. Average survival time of these 7 mice was 25 days compared to 11 days for the controls. Of the 24 remaining mice 13 survived the first challenge of non-irradiated L#2 tumor with an average survival time of 21 days, and 6 of these 13 survived a second challenge of L#2 tumor with an average survival time of 26 days. Survival time of the A/He test mice was significantly longer than that of the controls ($P<0.01$).

In preliminary attempts to determine whether mice that were immunized to one of the tumors were also immune to the second tumor, cross challenge experiments were performed. With C3H mice immunized to Gardner lymphosarcoma a challenge with L#2 tumor produced death in all except 3 of the 30 mice with no prolongation of survival time. C3H mice immunized with L#2 lymphoma and challenged 2 times with Gardner tumor produced death in all except 4 of 13 mice with an insignificant prolongation of survival time ($P>0.1$). A/He mice immunized with Gardner lymphosarcoma and challenged with L#2 tumor produced death in 8 of 10 animals but with a prolonged survival time ($P = .02$).

Discussion. In addition to employing X-irradiated cancer cells for production of immunity in mice a number of other technics and materials have been employed with varying degrees of success(1). However, more nearly complete protection has been afforded by use of X-irradiated cells. Different amounts and rates of X-irradiation were not

studied in these experiments so that optimum irradiating conditions for the cancer cells may not have been achieved. A 2000 r dose, found to be most effective in our earlier investigation(1,2) with Ehrlich ascites carcinoma cells, was employed. It would appear that metabolizing and perhaps partially viable cancer cells are required for production of resistance since our earlier experiments showed that 2000 r to 4000 r treated Ehrlich ascites cells injected into C57 BL mice gave nearly an optimum production of resistance, while 8000 r was ineffective. Also Donaldson and North(6) have shown that a certain amount of nitrogen mustard is highly effective, although larger quantities reduce the effect.

To date we have not determined the mechanism involved in production of immunity by repeated injections of X-irradiated cells; however, in C57 BL mice immunized with X-irradiated Ehrlich carcinoma (1,2) blood serum, spleen, lung and liver tissues have high titres of complement-fixing antibodies. Such studies have not been made on tissues from animals immunized against Gardner lymphosarcoma and L#2 lymphoma. It is possible that X-irradiation alters the histocompatibility genes and this in turn produces different antigens, which results in an alteration of the array of antibodies in the animals. Another possibility is that X-irradiation modifies the cells in such a fashion as to alter and slow their growth and make them more antigenic.

It is not surprising perhaps that cross challenging of immunized mice gives different results with the various tumor-mouse combinations but it is most interesting that there is only slight protection. In this regard unpublished data in our laboratory show that C57 BL mice repeatedly injected with X-irradiated normal liver and spleen have no protection against growth of Ehrlich carcinoma cells.

Summary. It has been possible to produce complete protection in C3H mice to Gardner lymphosarcoma and to L#2 lymphoma, and in A/He mice to Gardner lymphosarcoma. Partial immunity was produced in A/He mice to L#2 lymphoma. This was accomplished by semiweekly intraperitoneal injections of 8 doses of 2000 r X-irradiated tumor. C3H mice immunized with Gardner and cross challenged with L#2 tumor and C3H mice immunized with L#2 tumor and challenged with Gardner tumor showed no significant amount of protection. However, A/He mice immunized with Gardner and challenged with L#2 tumor were partially protected. Plausible mechanisms of immunity production are discussed.

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