The objective of the present investigation was to contribute toward determining the degree to which susceptibility and resistance to carcinogenic action are intrinsic within target tissue, and the extent to which host factors determine tissue reaction. The target tissue investigated was lung; the carcinogen used was urethan.

PRELIMINARY EXPERIMENT

Lung tumors have been induced in several strains of mice by carcinogenic hydrocarbons (1, 2, 11, 17, 20, 21, 25), miscellaneous chemical agents (4, 5, 12), and x-rays (7, 13, 14). By far the most effective method of inducing lung tumors in mice, administration of urethan, was discovered by Nettleship and Henshaw in 1943 (22). In most strains tested for their response to urethan, 100 per cent of the injected mice developed primary pulmonary adenomas, some as early as 3 months after initiation of treatment (6, 9, 10, 23, 24). In all animals the lung was the specific target organ for the carcinogenic action, the incidence of other tumors not being significantly increased.

This investigation has been aided by a grant from the National Cancer Institute.

† Submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Minnesota.

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Received for publication May 4, 1951.
Bagg albino (19), and CBA strains,1 the NH not having been tested for its response.

Three strains were refractory to the induction of lung tumors with urethan. The DBA was relatively resistant (16 per cent incidence), as it is to methylcholanthrene (7 per cent incidence). The spontaneous incidence was also very low. The FA and FB strains (genetically related to one another and to the F strain of mice) might be considered a special group, since their response to urethan appears to be inflammatory rather than neoplastic (Fig. 9). Their spontaneous incidence was also very low; response to methylcholanthrene has not been determined.

MATERIALS AND METHODS

Preliminary experiments revealed that the DBA was a good choice for a strain resistant and the Bagg albino for a strain susceptible to the carcinogenic action of urethan.

Bagg albino mice (100 per cent incidence of urethan-induced lung tumors) were mated to DBA mice (16 per cent incidence) to obtain F1 hybrid offspring. Approximately one-half of the experimental animals were from Bagg albino male×DBA female matings and the others from the reciprocal cross. Hybrids injected with urethan responded as did the Bagg albino parent—that is, 100 per cent developed lung tumors (Table 1).

When the hybrids reached 70 days of age, pulmonary tissue was grafted into their ears. Lung tissue from 1-day-old Bagg albino litters was grafted into the left ear of the hybrid, and similar tissue from DBA litters was grafted into the right ears of the same animals. Subcutaneous pockets were made into the host’s ear (by employing iridectomy scissors and fine forceps), into which small pieces of lung tissue were forced. The most successful grafts were made with a piece of tissue about 2 mm. in diameter. With larger pieces necrosis of the ear resulted. Within 1 week the incision was healed, and within 2 weeks the grafts were well vascularized.

Fifty-nine mice were operated upon. One week after the grafting was completed, 27 mice taken at random were placed on experiment, while the remainder were kept as controls. Experimental animals were injected with a solution of 10 per cent urethan (1 mg/gm body weight) once a week for 6 weeks. All mice were killed 8—13 months later. All ear grafts were fixed in Zenker’s solution and sectioned serially, unless the presence of a tumor in the graft was obvious, in which case sections were made only to confirm the gross diagnosis.

1 A. Kirschbaum, unpublished data.

RESULTS

Bagg albino tissue residing in injected hosts.—It was generally noted that the Bagg albino lung tissue grew more rapidly and attained a larger size than did DBA lung tissue residing in the same host. Of 27 Bagg albino transplants, 8 regressed within 1 month and were excluded from the data. Two hybrids died 5 months after the experimental procedure (grafting and injection) had been completed and were not available for autopsy.

Nine of the seventeen ear grafts were obviously tumorous (Table 2). In some cases nodules 1—2 mm. in diameter were visible under the skin of the ear. In one instance the tumor had attained a size of 5 mm. (Fig. 1). In some grafts the presence of a tumor was not apparent until the ear had been removed and the graft dissected out. In three additional instances adenomas were demonstrated by examination of serial sections of the grafts. Thus, urethan induced pulmonary adenomas in 76 per cent of the susceptible Bagg albino lung tissue grafts present within a susceptible host.

Microscopically, the adenomas were well developed and identical histologically with those found in the lungs in situ of animals injected with urethan (compare Figs. 2 and 3, 4 and 5). Some areas contained phagocytes with hemosiderin in association with accumulations of lymphocytes (Fig. 8). In some cases the tissue resembled atelectatic lung.

DBA tissue residing in injected hosts.—The right ear of the F1 hybrids contained the DBA lung tissue. Of 27 DBA grafts, 8 regressed and

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1 A. Kirschbaum, unpublished data.
were excluded from the data. The 8 failures of both the Bagg albino and DBA tissues were not, however, found in the same hybrid host. At the termination of the experiment the DBA grafts studied were in general smaller than the Bagg albino. All grafts less than 1 mm. in diameter were excluded from microscopic study. None was observed grossly to contain tumors (Table 3). Microscopically, however, it was noted that one graft had a small well formed adenoma (Fig. 7). The grafted DBA tissue resembled the Bagg albino tissue histologically in all respects, except for the lower incidence of induced lung tumors. An incidence of only 6 per cent (one out of seventeen cases) was obtained in DBA lung tissue residing in a host susceptible to the lung tumor-inciting action of urethan.

**Ear-grafted animals not treated with urethan.**

Thirty-two animals were set aside with ear grafts but were given no treatment with urethan. Five animals were excluded because of premature death. Of the remaining animals, 25 retained viable Bagg albino lung tissue grafts during the entire experimental period of observation; 16 DBA grafts remained viable. Animals were killed 11—13 months after the grafts were transplanted; serial sections of the grafts were made. One Bagg albino graft had a tumor which might have been induced by transplantation per se but which probably represents a spontaneous occurrence (1 per cent incidence of spontaneous lung tumors in Bagg albinos before 400 days of age). The grafted tissue was well maintained and identifiable histologically as lung (Figs. 6 and 8).

**DISCUSSION**

The ear as a site for transplantation of lung tissue proved very favorable. The majority of transplants became established readily, grew, and were histologically pulmonary in character (Figs. 6 and 8). The hybrid host was highly susceptible to the lung tumor-inciting action of urethan and supported the growth of tissue from the two parent stocks. Urethan diffuses throughout the body, assuring adequate exposure of the grafted tissue to carcinogenic action. The 76 per cent incidence of lung tumors obtained in the Bagg albino lung tissue indicates a high susceptibility of the grafts to the carcinogenic action of urethan, especially when one considers the relatively small amount of tissue acted upon. It seems logical to suppose that, were it possible to have grafted the entire lung into the ear, a 100 per cent incidence would have been observed. DBA tissue grafted into the ears of the same hosts reacted to urethan as does tissue of this genetic type in its natural environment—that is, within a genetically resistant DBA host.

It can be concluded that the intrinsic capacity of pulmonary tissue to respond to the carcinogenic action of urethan was manifest in tissue grafts. DBA pulmonary tissue maintained its resistance, although residing in a susceptible host during exposure to the carcinogenic chemical. Pulmonary tumors were induced in susceptible Bagg albino grafts under identical conditions.

**SUMMARY**

Bagg albino (susceptible to the induction of pulmonary tumors by urethan) and DBA (resistant) mice were mated to obtain F1 hybrids (susceptible). Lung tissue from 1-day-old mice of each of the parent strains was transplanted to the left and right ears, respectively, of the hybrids. Hosts were then injected with urethan. Although the two types of lung tissue grafts were in genetically identical hosts, twelve out of seventeen grafted Bagg albino lungs became tumorous, whereas only one out of seventeen DBA grafts exhibited a tumor. It has been determined, therefore, that susceptibility and resistance to the carcinogenic action of urethan on the lung are intrinsic properties of the target pulmonary tissue and that the host does not determine this reaction.

**REFERENCES**


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FIG. 1.—Urethan-induced pulmonary adenoma in Bagg albino ear graft (left ear). Tumor, 5 mm. in diameter, exhibited histologic structure illustrated in Figure 3. X 14.

FIG. 2.—Histologic structure of pulmonary adenoma induced by urethan and appearing in situ. Composed of closely packed, alveolar-like, cuboidal epithelial whorls with relatively little fibrous stroma. X 350.

FIG. 3.—Section of pulmonary adenoma induced in Bagg albino ear graft. Structure identical with that of urethan-induced adenoma developing in situ. X 350.

FIG. 4.—Urethan-induced pulmonary adenoma which developed in lung of Bagg albino × DBA F1 hybrid. X 45.

FIG. 5.—Urethan-induced pulmonary adenoma developing in Bagg albino pulmonary tissue graft. X 45.

FIG. 6.—Bronchiolar epithelium within pulmonary tissue graft 18 months after grafting. Section indicates normal appearance of such epithelium. X 650.

FIG. 7.—In upper portion of this section is a circumscribed pulmonary adenoma which developed in a pulmonary graft of DBA tissue residing in a susceptible F1 hybrid. This is the only instance in seventeen where a tumor developed in resistant tissue within a susceptible host. Histologic appearance of grafted pulmonary tissue is well shown in lower portion of section. Alveoli contain hemosiderin. X 45.

FIG. 8.—Section of pulmonary tissue graft 18 months after grafting. Pulmonary alveoli normal, blood-filled capillaries present in interalveolar septa, hemosiderin in alveoli, phagocytes filled with hemosiderin present. X 350.

FIG. 9.—Inflammatory nodules in lung of FA mouse treated with urethan. Nodules of this type are not neoplastic and are to be distinguished from nodules whose histologic structure is shown in Figures 2 and 3. Leukocytic infiltration and squamous-like cells present. X 350.


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Intrinsic Tissue Response to Induction of Pulmonary Tumors

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Cancer Res 1951;11:644-647.