Comparative Effects of Purified Diets and a Natural Food Stock Ration on the Tumor Incidence of Mice Exposed to Multiple Sublethal Doses of Total-Body X-irradiation\textsuperscript{1,2}

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SUMMARY

Studies were made of the effects of multiple sublethal doses of total-body X-irradiation (six weekly exposures of 200 R each) on the subsequent appearance of tumors in female mice fed either highly purified diets or a natural food stock ration. Six months after the first X-ray exposure, mice fed the purified diets showed a markedly higher incidence of a number of tumors (lymphosarcoma of the mandibular lymph nodes, lymphosarcoma of the thymus, uterine adenocarcinoma, Harderian gland adenocarcinoma, and others) over that of similarly X-irradiated mice fed the stock ration. Nonirradiated mice of similar age fed identical diets were virtually free of such tumors. The protective factor or factors in the natural food stock ration is apparently distinct from any of the known nutrients.

INTRODUCTION

Considerable data are available indicating that certain natural food stock rations contain a factor or factors apparently distinct from those present in purified diets containing the known nutrients that protect against tumor formation in experimental animals. Such findings were obtained both in respect to tumors induced by administration of carcinogenic agents and in spontaneously appearing tumors. A number of investigators have reported that the use of purified diets resulted in a shorter induction time and a higher tumor incidence in rats administered 2-acetylaminofluorene (2, 11) or 4-acetylaminobiphenyl (13) than was the case in animals fed stock rations. Ershoff (6) observed that the incidence of pituitary tumors in rats following the oral administration of estrogens was significantly greater in animals fed a highly purified diet than in those fed a stock ration. Silverstone et al. (12) found that the incidence of spontaneous hepatoma was significantly lower in mice of the DBA and C3H strains when fed a diet consisting of natural foodstuffs than when maintained on a semipurified ration. Whereas these comparative differences between stock rations and purified diets relative to tumor incidence have been evaluated in terms of carcinogenic compounds and spontaneously appearing tumors, to our knowledge this effect has never been assessed in regards to exposure to sublethal doses of total-body X-irradiation. The present investigation was undertaken to determine the comparative effects of a natural food stock ration and purified diets on tumor formation following exposure to multiple sublethal doses of total-body X-irradiation in the mouse.

MATERIALS AND METHODS

Four hundred and eighty female mice of the Swiss Webster strain (Simonsen Laboratories, Inc., Gilroy, Calif.) ranging between 12 and 16 gm in body weight were divided into 7 groups whose average weight per mouse was 14.3 gm. Groups I and VII consisted of 90 mice each; the other groups, 60 mice each. Groups I, II, III, IV, V, and VI were fed purified diets (Diets I to VI respectively) whose compositions are indicated in Table 1. Group VII was fed a natural food stock ration (Purina Laboratory Chow in meal form, manufactured by Ralston Purina Company, St. Louis, Mo.). The mice were placed in metal cages with raised screen bottoms (5 animals per cage) and were provided the test diets and water ad libitum. The mice were fed daily and any food not consumed 24 hours after feeding was discarded. The mice were weighed weekly during the course of the experiment. After 28 days of feeding, the average body weight of mice in the various groups was as follows: Group I, 26.4 gm; Group II, 25.9 gm; Group III, 27.0 gm; Group IV, 28.4 gm; Group V, 27.8 gm; Group VI, 27.8 gm; and Group VII, 26.0 gm. At this time 30 mice were selected at random from each of the groups to serve as nonirradiated controls. The remaining mice in each group were administered a 200 R dose of total-body X-irradiation which was repeated once weekly until a total dose of 1200 R (6 exposures) had been administered. The animals to be irradiated were placed in a wooden box divided into 30 equal compartments, 1.25 inches wide, 3 inches long, and 1.5 inches deep. The partitions and top were made of 1/8-inch cellulose acetate sheeting. The tops and bottoms of each compartment were perforated with holes to provide ventilation. The container was rotated slowly on an electrically driven turntable to ensure equivalent exposures. The following radiation factors were employed: GE Model Maximizer 250; 250 kv; 0.5 mm Cu and 1

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mm A1 filters plus a Cu parabolic filter; HVL, 2.15 mm Cu; target distance to top of box, 82 cm; and dose rate, 18.4 R per minute (measured in air, at top of box).

Six months after the first X-ray exposure, 12 mice were selected at random from each of the X-irradiated and nonirradiated groups. These were sacrificed and their heads were removed, placed in 10% neutral formalin for fixation, demineralized, and embedded in paraffin in the routine manner. Sagittal sections of the heads passing through the Harderian glands were cut at 7 μ in thickness, and slides were prepared and stained with hematoxylin and eosin. The above animals as well as the remaining animals in each group were examined for the presence of grossly visible tumors in the various tissues and the incidence of such tumors was recorded. Grossly visible tumors were removed, placed in 10% neutral formalin for fixation, embedded in paraffin, sectioned at 7 μ in thickness, and stained as the above.

RESULTS

Body Weight and Survival

The average body weight of nonirradiated and X-irradiated mice at the time of the first X-ray exposure and at the termination of the experiment (which occurred 6 months after the first X-ray exposure) is indicated in Table 2. All groups gained weight during the course of the experiment with the average weight increment in both the X-irradiated and nonirradiated groups being less for mice fed the stock ration (Group VII) than for those fed the unsupplemented purified diets (Groups I, II, and III). On most diets the weight increment of X-irradiated mice was less than that of nonirradiated mice in the same dietary group. In all dietary groups the percentage of animals surviving until the termination of the experiment was significantly less in the case of X-irradiated mice than for nonirradiated mice in the same dietary group. Data on percent survival in the various groups are summarized in Table 2.
Incidence of Grossly Visible Tumors

A highly significant difference in the incidence of grossly visible tumors was observed between mice in the X-irradiated and nonirradiated series. In the nonirradiated series, with the exception of mammary tumors in 1 animal each on Diets III and VI and tumors of the thymus gland in 1 animal each on Diets I, II, and IV, no grossly visible tumors were observed. In contrast, over 50% of the mice in the X-irradiated series fed purified Diets I, II, and III had grossly visible tumors. The principal tumor observed in X-irradiated mice fed Diet I (which contained dextrose as the source of dietary carbohydrate and cottonseed oil as the source of dietary fat) was a tumor of the mandibular lymph node which was observed grossly in 53.7% of the surviving animals in this group. Tumors of the thymus gland and uterus were observed grossly in 26.8% and 17.1%, respectively, of the animals in this group. Grossly visible tumors of the lung, liver, spleen, kidney, mammary gland, pancreas, and other tissues were also noted in some (i.e., less than 10%) of the animals in this group. Supplemening Diet I with 10% desiccated whole liver (Diet IV) or 10% yeast (Diet V) had little, if any, effect on the tumor incidence of X-irradiated mice. A supplement of 15% alfalfa meal (Diet VI), however, resulted in a highly significant increase in the incidence of grossly visible uterine tumors and an increased incidence of grossly visible mandibular lymph node and thymic tumors. Replacing the cottonseed oil in Diet I with a comparable amount of lard (Diet II) resulted in a substantial increase in the incidence of grossly visible mandibular lymph node, thymic, and uterine tumors in X-irradiated mice. Replacing the dextrose in Diet I with a comparable amount of corn starch (Diet III) resulted in an increased incidence of grossly visible thymic and uterine tumors in X-irradiated mice. In contrast to the high incidence of grossly visible mandibular lymph node, thymic and uterine tumors in X-irradiated mice fed the purified diets (Groups I–VI), a highly significant reduction in the incidence of such tumors occurred in X-irradiated mice fed the stock ration (Group VII). Findings are summarized in Table 3.

Microscopic Appearance of Grossly Visible Tumors

Mandibular Lymph Node Tumors. The involved lymph nodes were grossly enlarged and pale gray in color. Microscopic examination indicated that the tumors were lymphosarcomas of the mandibular lymph nodes. The normal architecture of the lymph nodes was often completely obliterated by neoplastic lymphocytes (Fig. 1). The latter were prominently pleomorphic and were characterized by an irregularly spherical and acenentrally located nucleus enclosed in a narrow pale-staining cytoplasm. The nuclei stained strongly basophilic. Atypical mitotic figures were abundant and plasma cell infiltration was common (Fig. 2). No significant differences were observed in the microscopic appearance of mandibular lymph node tumors in the various dietary groups.

Thymic Tumors. The affected thymus glands were gray in color and were conspicuously enlarged, often filling the entire anterior and ventral portions of the thoracic cavity which frequently contained a serofibrinous exudate. The tumors were commonly adherent to the surrounding thoracic tissues. Microscopic examination indicated that the tumors were lymphosarcomas of the thymus gland and were marked by neoplasia of the lymphoid tissue which was composed of neoplastic lymphoblasts with numerous atypical as well as normal mitotic figures (Fig. 3). The neoplastic cells obscured the normal pattern of the thymus and frequently infiltrated the surrounding thoracic tissues (muscles, pleura, pericardium, etc. (Fig. 4). They were often accompanied by marked neoplastic stromal proliferation (Fig. 5). No significant differences were observed in the microscopic appearance of the thymic tumors in the various dietary groups.

Uterine Tumors. The uterine tumors were marked by uniform enlargement and a white color of the body and horns of the uteri. Microscopic examination indicated that the tumors were predominantly adenocarcinomas of the uteri and consisted of adenomatous polyps (Fig. 6) and adenocarcinomas and cystoadenocarcinomas (Fig. 7). Adenocarcinoma was the predominant tumor whereas the other two types were observed in less than 10% of the cases. The carcinomas arose from the endometrial mucosa or glands. The carcinomatous changes were frequently accompanied by squamous metaplasia of the endometrial mucosa, acanthosis, and accompanying diffuse pyogenetic changes of the cervix and uterine body. No significant differences were observed in the microscopic appearance of the uterine tumors in the various dietary groups.

Lung Tumors. The affected lungs showed focal, pinkish-gray areas of consolidation. Microscopic examination indicated that the tumors were adenomas and were marked by bronchiogenic

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Comparative effects of purified diets and a natural food stock ration on the tumor incidence of female mice exposed to multiple sub-lethal doses of total-body X-irradiation.

*Data are based on grossly visible tumors in the surviving animals of each group 6 months after the first X-ray exposure. The type of tumor was established by microscopic examination of the grossly visible tumor tissue.
adenomatous changes which consisted of papillary neoplasia of the bronchial epithelium (Fig. 8). These lesions were often accompanied by metaplastic transformation of the alveolar lining cells into columnar or cuboidal cells. Undifferentiated cell carcinoma was observed in 2 animals in Group II. With the exception of the latter, no significant differences were observed in the microscopic appearance of the lung tumors in the various dietary groups.

Miscellaneous Tumors. A variety of other tumors was observed in X-irradiated mice. These tumors were observed grossly in approximately 5% to 10% of the surviving mice in Groups I to VI but were not observed in any of the surviving mice fed the stock ration (Group VII). Among the tumors observed were tubular adenoma of the ovaries (Fig. 9), Brunner's gland carcinoma of the duodenum (Fig. 10), adenocarcinoma of the thyroid (Fig. 11), meningioma (Fig. 12), disseminated myelocytic sarcoma (Fig. 13), hepatoma, disseminated lymphosarcoma, reticulum cell carcinoma, adenocarcinoma of the mammary gland, and lipoma of the mesentery. Ductal hyperplasia of the submaxillary parotid and sublingual glands (Fig. 14) as well as of the pancreas (Fig. 13) was also observed in approximately 5% to 10% of the surviving X-irradiated mice in Groups I to VI but was not observed in surviving mice in Group VII.

Neoplastic Changes in the Harderian Gland

A particularly high incidence of Harderian gland adenocarcinoma was observed in X-irradiated mice fed the purified diets in contrast to that of X-irradiated mice fed the stock ration. The incidence of such tumors in the varying groups was as follows: Group I, 75%; Group II, 91.7%; Group III, 58.3%; Group IV, 66.7%; Group V, 58.3%; Group VI, 66.7%; and Group VII (stock ration), 8.3%. Neoplastic changes in the Harderian gland were not observed in nonirradiated mice on any of the diets employed. The tumors were present primarily along the posteroventral and dorsal portions of the gland and were predominantly solid adenocarcinomas (Fig. 15). A few cystic and papillary carcinomas were also observed. Neoplasia was frequently accompanied by cystic dilation and accumulation of brown ceroid-like material in the normal portion of the gland. No significant differences were observed in the microscopic appearance of the Harderian gland tumors in the various dietary groups.

DISCUSSION

An extensive literature is available indicating that X-irradiation is a general carcinogen and that it induces (or helps to induce) tumors in almost all tissues of mammals irrespective of species (1, 8, 10). The carcinogenic effects of X-irradiation are dependent on a number of factors including dose rate, frequency of exposure, area exposed, age of animal at time of exposure, sex and strain of animal, and others. Present findings indicate that the carcinogenic effects of exposure to X-irradiation are also dependent on the diet employed. Mice fed purified diets and exposed to multiple sublethal doses of total-body X-irradiation consistently showed a higher incidence of a number of tumors (lymphosarcoma of the mandibular lymph nodes, lymphosarcoma of the thymus, uterine adenocarcinoma, Harderian gland adenocarcinoma, and others) than similarly X-irradiated mice fed a stock ration. The latter findings are consistent with observations indicating that the toxic effects of administration of massive doses of various drugs, chemicals, hormones, food additives, and other substances (3—5, 14, 15) or exposure to certain stressor agents including carcinogens (2, 6, 11, 13) and X-irradiation (7) were frequently found to be particularly pronounced in animals fed highly purified diets and were minimal or even absent in those fed a natural food stock ration. Present findings indicate that differences also occurred in respect to the tumor incidence of X-irradiated mice on the purified diets. Thus, X-irradiated mice in Group II which were fed a diet containing 10% lard as the source of dietary fat showed a substantially higher incidence of lymphosarcoma of the mandibular lymph nodes, lymphosarcoma of the thymus, Harderian gland adenocarcinoma, uterine adenocarcinoma, and adenoma of the lung than did X-irradiated mice in Group I which were fed an identical diet except that cottonseed oil replaced lard as the source of dietary fat. X-irradiated mice in Group III which were fed a diet containing cornstarch as the source of dietary carbohydrate showed a substantial increase in the number of thymic and uterine tumors over that of X-irradiated mice in Group I which were fed an identical diet except that dextrose rather than cornstarch was employed as the source of dietary carbohydrate. Supplementing Diet I with 10% liver (Diet IV) or 10% yeast (Diet V) resulted in a tumor incidence that differed little if at all from that of X-irradiated mice on Diet I. A supplement of 15% alfalfa meal (Diet VI) added to Diet I, however, substantially increased the incidence of lymphosarcoma of the mandibular lymph nodes, lymphosarcoma of the thymus, and adenocarcinoma of the uterus over that of X-irradiated mice on Diet I. The latter findings suggest that alfalfa meal may have contained one or more carcinogenic or cocarcinogenic agents not present in comparable amounts in the stock ration which contributed to the results obtained.

No data are available as to the factor or factors in the stock ration responsible for its protective effect. It has been suggested (3, 4) that certain natural foodstuffs contain unidentified nutritional factors apparently distinct from any of the known nutrients that are essential for optimal adaptation or resistance to various stressor agents. These factors may be dispensable under "normal" conditions, or their requirements may be met by amounts ordinarily present in the diet or through the synthetic activity of the intestinal flora or the animal's own tissues. After exposure to certain stressor agents, however, requirements may be increased to the extent that deficiencies occur, manifested by tissue pathosis or injury. These are preventable, at least in part, by administration of

5There is evidence to indicate that the deleterious effects of ionizing radiation in animals are due, at least in part, to excessive formation of peroxides and related substances such as epoxides, hydroperoxides, and others. The possibility that a natural food stock ration may contain a higher concentration than the purified diets of certain antioxidants that prevent or minimize the formation of peroxides and related substances, providing in effect a carcinostatic action, is deserving of further study.
increased amounts of the deficient factor or factors. It is possible that the protective effect of the stock ration in X-irradiated mice, under conditions of the present experiment, resulted from the presence of such a factor or factors. Further studies are directed toward the concentration, isolation, and identification of such factors as well as their modus operandi and their effectiveness in preventing tumor formation or prolonging the period of tumor induction in animals exposed to other carcinogenic agents.

REFERENCES


Fig. 1. Mandibular lymph node of an X-irradiated mouse fed Diet I. The normal architecture of the node is completely obliterated by the lymphosarcoma cells which are also present in the subcapsular space. H & E, x 45.

Fig. 2. Higher magnification of Fig. 1. Note pleomorphic lymphosarcoma cells and numerous mitotic figures. H & E, x 500.

Fig. 3. Thymus of an X-irradiated mouse fed Diet II. The invasive lymphosarcoma cells are infiltrating into the tissues surrounding the capsule (A) and into the skeletal muscle (B). H & E, x 125.

Fig. 4. Higher magnification of Fig. 3. The tumor cells are typical of lymphosarcoma and show numerous mitotic figures. H & E, x 500.

Fig. 5. Thymus of an X-irradiated mouse fed Diet III showing stromal proliferation. H & E, x 250.

Fig. 6. Uterus of an X-irradiated mouse fed Diet III. Note pedunculated adenomatous polyp arising from the endometrial mucosa. H & E, x 150.

Fig. 7. Uterus of an X-irradiated mouse fed Diet I. An endometrial adenocarcinoma is invading the myometrium and exhibits a solid (A) as well as a cystic (B) appearance. H & E, x 125.

Fig. 8. Lung of an X-irradiated mouse fed Diet II exhibiting a well-differentiated papillary bronchiogenic neoplasia. H & E, x 125.

Fig. 9. Ovary of an X-irradiated mouse fed Diet I. Note tumor structure typical of tubular adenoma. H & E, x 125.

Fig. 10. Duodenum of an X-irradiated mouse fed Diet VI. Note the cystadenocarcinoma arising from the Brunner's gland. H & E, x 250.

Fig. 11. Thyroid of an X-irradiated mouse fed Diet III. Note the nodular adenocarcinoma which is composed of well-differentiated secretory acini mixed with transitional and undifferentiated hyperchromatic cancer cells. H & E, x 250.

Fig. 12. Head of an X-irradiated mouse fed Diet I. Note an early meningothelial meningioma arising from the dorsal arachnoidal tissue. H & E, x 125.

Fig. 13. Pancreas of an X-irradiated mouse fed Diet V. Note proliferation of the interlobular duct (arrows) and invasive myelocytic sarcoma (A). H & E, x 125.

Fig. 14. Submaxillary salivary gland of an X-irradiated mouse fed Diet VI. Note the hyperchromatic cells lining a hyperplastic intralobular duct (A) and a normal-appearing duct (B). H & E, x 150.

Fig. 15. Harderian gland of an X-irradiated mouse fed Diet I. Advanced adenocarcinoma involving the posterior portion of the gland. Note ceroid-like material (arrow) in the normal portion of the gland. H & E, x 125.
Dietary Effects on Tumor Incidence of Irradiated Mice
Comparative Effects of Purified Diets and a Natural Food Stock Ration on the Tumor Incidence of Mice Exposed to Multiple Sublethal Doses of Total-Body X-irradiation
