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RAPID BODY WEIGHT GAIN INCREASES THE RISK OF ULTRAVIOLET RADIATION-INDUCED SKIN CARCINOGENESIS IN SKH-1 HAIRLESS MICE

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Abstract

Although it is well known that caloric restriction reduces the risk of chronic diseases including cancer, the role of weight gain in the development of ultraviolet light-induced tumors has not, to our knowledge, been investigated. In view of the increase in obesity worldwide, we asked the question whether there is any relationship between body weight gain and skin tumor development. We subjected three groups, each comprising 30 SKH-1 hairless female mice, to UV radiation (30 mJ/cm^2 twice weekly for 17 weeks) and observed tumor formation over the ensuing 8–13 weeks: Group 1 received pelleted diet; Group 2 received pellets during the irradiation period and was then switched to powder; and, Group 3 received powder exclusively. At the end of the experiment, the mean body weight of Group 1 was 32.1 ± 0.5 g, whereas that of Groups 2 and 3 was 39.0 ± 1.5 g and 39.5 ± 1.4 g, respectively. Tumor incidence reached 90% at 8 weeks after completion of irradiation for the animals in Group 3 and at 13 weeks for the animals in Group 2. Similarly, at 8 weeks after irradiation when all animals of Group 3 were euthanized, tumor multiplicity was 0.8, 1.2, and 3.2 for Groups 1, 2, and 3, respectively. Thus, in comparison with the mice consuming pellets, the powder-fed mice gained weight more rapidly, and developed tumors much faster. Considering the escalating numbers of individuals worldwide who are overweight or obese, our findings provide further impetus for advocating healthier diets and maintenance of constant body weight in adults.

Keywords

ultraviolet radiation; skin cancer; obesity; body weight gain; mice

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1. Introduction

Quantitative determinations of the effects of interventions on tumor development in animal models are frequently confounded by profound changes in food consumption. Even modest caloric restriction is one of the most constant and reproducible methods for suppressing not only tumor development, but also arresting a variety of chronic degenerative diseases. Such restriction also slows the aging process in several phylogenetically diverse species [1,2].

As early as the 1940s, Albert Tannenbaum clearly demonstrated that caloric restriction decreased the incidence of a variety of both spontaneous and chemically induced mouse tumors including those of skin [reviewed in 3] and that there was a direct relationship between the tumor incidence and daily caloric intake [4,5]. Work in the laboratories of Diane Birt and others over the past two decades has led to the identification of a variety of suspected molecular targets involved in inhibition of promotion of skin carcinogenesis by dietary energy production. These include the reduced expression of protein kinase C isoforms in mouse skin, blocking tumor promoter activation of Raf-1 and ERK1 and AP-1 expression, and the strong role of glucocorticoid hormone in mediating these effects in SENCAR mice [6,7].

Conversely, it has long been recognized that in humans obesity and being overweight is associated with chronic diseases, in particular, cardiovascular disease and diabetes, and more recently it has become clear that these conditions increase the risk for several types of cancer, including those of the breast, endometrium, colon, pancreas, liver, stomach, bladder, and kidney [8]. It is now estimated that 14% of all cancer deaths in men and 20% in women are associated with being overweight or obese [9]. Consequently it is surprising that, to our knowledge, only limited information is available on obesity (high body weight) as a potential risk factor for ultraviolet (UV)-induced skin cancer, even though nonmelenoma skin tumors are the most common type of cancer in humans and their main etiological factor, UV radiation, is the most ubiquitous environmental carcinogen. Moreover, skin cancer incidence is increasing steadily, and this increase is expected to continue as a result of depletion of stratospheric ozone, increased exposure to solar radiation, and longer life expectancy [10], emphasizing the urgent and compelling need for detailed knowledge of the potential risk factors.

We have been devising strategies for protection against UV radiation-induced skin cancer using a high risk mouse model originally developed by Lu et al. [11] and Conney et al. [12]. In this model, SKH-1 hairless mice are chronically exposed to low doses of UVB light (30 mJ/cm^2/session twice a week for ~20 weeks) comparable to human outdoor occupational or sunbathing exposures to sunlight - estimated to be in the range of 50–100 mJ/cm^2/day [13]. Although at the end of irradiation the animals are tumor-free, they are at high risk for developing skin cancer. Indeed, during the subsequent 10–15 weeks, nearly all of them develop skin tumors without further exposure to UV radiation. With this model we have shown that daily topical treatment with broccoli sprout extracts as a source of the chemoprotective agent sulforaphane reduced the incidence, multiplicity, and volume of skin tumors by about 50% [14]. In subsequent studies we noted that the rate of appearance of tumors was related to the body weight of the animals. Therefore, we hypothesized that rapid body weight gain may increase the risk for UV radiation-induced skin carcinogenesis. To this end, we examined the relationship between body weight gain and tumor appearance, using a diet that was identical in composition, and differed only in physical presentation (pellets versus powder). The daily food consumption of mice fed powdered diet was ~18% higher than that of mice consuming pellets and not only did the mice gain weight more rapidly with the powder, but there was a striking and contemporaneous correlation between the rate of body weight gain and acceleration of tumor development.

2. Methods and Materials

2.1 Animals

Female SKH-1 hairless mice, 5- to 6-week-old, were purchased from Charles River, Wilmington, MA. The animals were maintained in a 12-h light/12-h dark cycle with 35% humidity, and given free access to water and AIN-76A purified diet without antioxidants in a powder or pellet form (Harlan Teklad, Madison WI) throughout the duration of the experiment. Three groups of 30 animals were examined: Group 1 was fed pellets for 30 weeks; Group 2 was fed pellets for 17 weeks and then was switched to powder for 13 weeks, at which time point the experiment was terminated. Group 3 received only powdered diet. The experiment was in compliance with the National Institutes of Health Guidelines, approved by the Johns Hopkins University Animal Care and Use Committee.

2.2. Ultraviolet irradiation and evaluation of skin tumors

UV irradiation was provided by UV lamps (FS72T12-UVB-HO, National Biological Corporation, Twinsburg, OH) emitting UVB (280–320 nm, 65% of total energy) and UVA (320–375 nm, 35% of total energy). The radiant dose was quantified with a UVB Daavlin Flex Control Integrating Dosimeter and further calibrated with an IL-1400 Radiometer (International Light, Newburyport, MA). After acclimatization for one week, the animals were irradiated twice a week for 17 weeks, with 30 mJ/cm^2/session. Tumors (defined as lesions > 1 mm in diameter) and body weight were recorded weekly. Tumor volumes were determined by measuring the height, length, and width of each mass that was larger than 1 mm in diameter. The average of the three measurements was used as the diameter and the volume was calculated ($v = 4\pi r^3/3$). Total tumor burden represents the sum of the volumes of all tumors.

2.3. Statistical analysis

All values reported herein with confidence intervals represent ± 1 SD from the mean unless otherwise noted. Animal weights were compared using ANOVA, followed by Bartlett's test for homogeneity of variances and Bonferroni post-hoc recalculation of pairwise significance. Tumor incidence was compared using Kaplan-Meier and Cox proportional hazard estimates followed by log rank comparisons to test for differences in incidence. Weekly tumor burden and multiplicity were compared using a pairwise ANOVA model. All comparative statistics were calculated using STATA 10.0 (Stata Corporation, College Station, TX).

3. Results and Discussion

3.1. SKH-1 hairless mice with access to powdered diet ad libitum gain weight much more rapidly than their counterparts that are fed pellets

Initially, the animals were randomized into three groups of 30: two groups (Group 1 and 2) received pelleted diet and one group (Group 3) received powdered diet (non-compressed, non-pelleted form of the same feed). The diet compositions were identical and consisted of (% by weight): protein (17.7), carbohydrate (64.5), and fat (5.2), delivering 3.8 Kcal/g. The actual components of the diets were (g/kg): casein (200.0), DL-methionine (3.0), sucrose (500.0), corn starch (150.0), corn oil (50.0), cellulose (50.0), vitamin mix (10.0), and choline bitartrate (2.0).

The average food consumption was 2.88 ± 0.19 g/day/mouse for the animals receiving pellets and 3.48 ± 0.14 g/day/mouse for those receiving powder. At the start of the study, the body weights were essentially the same: 22.5 ± 0.4 g, 22.8 ± 0.7 g, and 21.1 ± 1.0 g for Groups 1, 2, and 3, respectively. Figure 1 shows that the average body weight gain of the two groups (Groups 1 and 2) that were offered pelleted diet was essentially identical (29.0 ± 0.8 g and 29.9 ± 0.7 g) from the beginning of the study until the end of the irradiation period (17 weeks). In

contrast, the body weight of the powder-fed mice (Group 3) increased much faster, such that the differences in weight gain were significant ($P<0.05$) by the end of the first week after initiation of the experiment. By the 17th week of feeding, the weights were 29.0 ± 0.8 g, 29.9 ± 0.7 g, and 38.7 ± 1.5 g for Groups 1, 2, and 3, respectively. In order to confirm the trend of much more rapid weight gain for the mice receiving powder within the same experimental group, the animals in Group 2 (fed pellets through week 17) were switched to powder. As early as 2 weeks after the switch, the weight of these mice (31.5 ± 0.6 g) was already significantly higher ($P<0.05$) than the weight of the mice that remained on pellets (29.0 ± 0.8 g). Furthermore, at the end of the experiment, the body weight of the animals that were switched to powder was essentially identical to the body weight of the animals that were fed powder throughout the experiment (39.0 ± 1.5 g and 39.5 ± 1.4 g for Groups 2 and 3, respectively), in sharp contrast to the weight of the mice that remained on pellets (Group 1) which weighed 32.1 ± 0.5 g. The weekly weight gain differences became significantly different only at 18 weeks, and although not always significant at $P<0.05$, the group switched to powdered diet at week 17 (Group 2) consistently gained more weight than did the pellet-fed Group 1. Notably, as shown in Figure 1, the incremental weight gain of the mice that were switched to powder (Group 2) paralleled the weight gain curve, at similar body weight, for the animals that received powder throughout the experiment (Group 3). Overall, there were significant differences in weekly body weight ($P<0.001$) as determined by ANOVA, followed by post-hoc Bonferroni recalculation of pairwise significance.

3.2. SKH-1 hairless mice that have ad libitum access to powdered diet develop UV radiation-induced skin tumors much more rapidly than those receiving pellets

The earliest lesions larger than 1 mm were observed 1 week after the end of the irradiation schedule in all groups (Figure 2A). Even at this early time point however, the tumor incidence for Group 3 (30%) was significantly higher than the tumor incidence for Groups 1 and 2 (10 and 3.3%, respectively), indicating that the animals that were gaining weight rapidly were almost immediately at a higher risk for tumor development than were their “lean” counterparts. Tumor incidence was compared using Kaplan-Meier and Cox proportional hazards transformations of the data (not shown), followed by log-rank comparisons to test for differences between groups. Significance of the χ^2 test of these overall log-rank comparisons between Groups 1 and 2 was $P=0.0012$, and $P<0.0001$ for the comparison between Groups 1 and 3 and between Groups 2 and 3. Thus, 3 weeks after being switched to the powdered diet, at which time point their body weight had increased significantly (Figure 1), the 16.7% tumor incidence for Group 2 (initially the lowest) already exceeded the 10% tumor incidence for Group 1 (Figure 2A). Indeed, at this time point the tumor incidence for Group 3 was already 53%. Overall, the time point at which at least 50% of the animals had developed tumors correlated with body weight gain and was week 12, week 9, and week 3 for Groups 1, 2, and 3, respectively. Tumor incidence for Group 3 reached 90% 8 weeks after ending irradiation and all animals within this group were euthanized. In contrast, 5 more weeks were required for tumor incidence to reach 90% for Group 2 and even at that time point, when the experiment was terminated, tumor incidence was still below 70% for Group 1. When Groups 2 and 3 were compared, there was a striking correlation between tumor incidence and body weight (Figure 2C). Very importantly, the almost immediate increase in weight gain when switched to powdered diet correlated with more rapid tumor development. This observation suggests that tumors were already initiated and promoted, and that rapid body weight gain stimulated principally tumor growth and progression.

Similarly, tumor multiplicity (Figure 2B) and burden also correlated with body weight gain. As early as 2 weeks after completion of irradiation differences in multiplicity were already significant ($P<0.05$) between Groups 2 and 3. By 3 weeks after irradiation, tumor multiplicity was significantly different ($P<0.05$) between Groups 1 and 3. Eight weeks after irradiation,

tumor multiplicity was 0.8, 1.2, and 3.2 for Groups 1, 2, and 3, respectively. At the end of the experiment, 13 weeks after irradiation, tumor multiplicity was still consistently lower for the animals that were maintained on pellets (2.4 tumors per mouse) compared to those that were switched from pellets to powder (4.2 tumors per mouse). At 8 weeks post-irradiation, when all animals from Group 3 were euthanized, the average tumor burden (the sum of the volumes of all tumors per mouse) was similar for Groups 1 (2.5 cm^3) and 2 (2.4 cm^3) and significantly lower ($P<0.05$) compared to Group 3 (15.3 cm^3).

The exact mechanism(s) by which rapid weight gain increases the rate of tumor development in this model are not clear and require further study. Our findings, however, are supported by the recent recognition that adipocytes are important components of the tumor microenvironment by producing various cytokines and other biologically active factors that promote inflammation and angiogenesis [15,16]. Interestingly, Katiyar and Meeran [17] have reported that obesity exacerbates a number of markers of oxidative stress in the skin of mice that were chronically exposed to UV radiation. Compared to wild-type animals, genetically obese leptin-deficient mice had higher levels of photo-oxidative damage of macromolecules (e.g., lipids and proteins), more severe depletion of antioxidant defenses (e.g., glutathione, catalase), greater activation of MAPK and NF-κB signaling pathways in their skin, and higher levels of circulating pro-inflammatory cytokines, suggesting that obesity could be a risk factor for UV radiation-induced skin carcinogenesis. Conney and his colleagues [18] showed that voluntary exercise or partial lipectomy inhibited UV radiation-induced skin carcinogenesis in the same high-risk animal model. Evaluation of apoptotic indices revealed enhanced apoptosis in the epidermis of the animals that exercised and those that underwent partial lipectomy, and it was suggested that fat cells may secrete substances that inhibit apoptosis in cells with mutations and possibly in developing tumors. In a non-UV induced mouse papilloma model, evidence points towards inhibition of ERK1, and -2 signaling and AP-1 regulation in relation to body weight [6], thus providing additional testable hypotheses for a UV radiation-induced skin carcinogenesis model such as the one utilized here.

The reasons for the higher consumption of powdered diet in comparison with pelleted diet are not clear, and we can only suppose that its intake required less effort (e.g., less climbing and chewing), or that perhaps its physical presentation was more appealing to the animals. It has been observed previously that apparent food consumption in rodents increases and that food utilization decreases with the softness of the diet [19]. It is interesting to note that although the animals fed pellets were not calorically restricted, compared to the animals fed powder, they could probably be considered as having a “limited caloric intake”. This is important in view of the fact that most experiments with rodents involve animals that receive pelleted diets and interpretations of the experimental results should take in consideration the dietary intake as a critical factor. In conclusion, our findings imply that: (i) the development of skin tumors is substantially delayed in the lighter mice compared with their heavier counterparts; (ii) this delay is shortened as the body weight gain increases; and (iii) substantial protection against tumor development could perhaps be achieved even in high-risk adults simply by ensuring constant maintenance of their body weight. In view of the escalating numbers of individuals who are overweight and obese worldwide our findings are adding to the increasing justification for controlling these conditions.

Acknowledgments

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Abbreviations

ANOVA, analysis of variance; AP-1, activator protein-1; UV radiation, ultraviolet radiation.

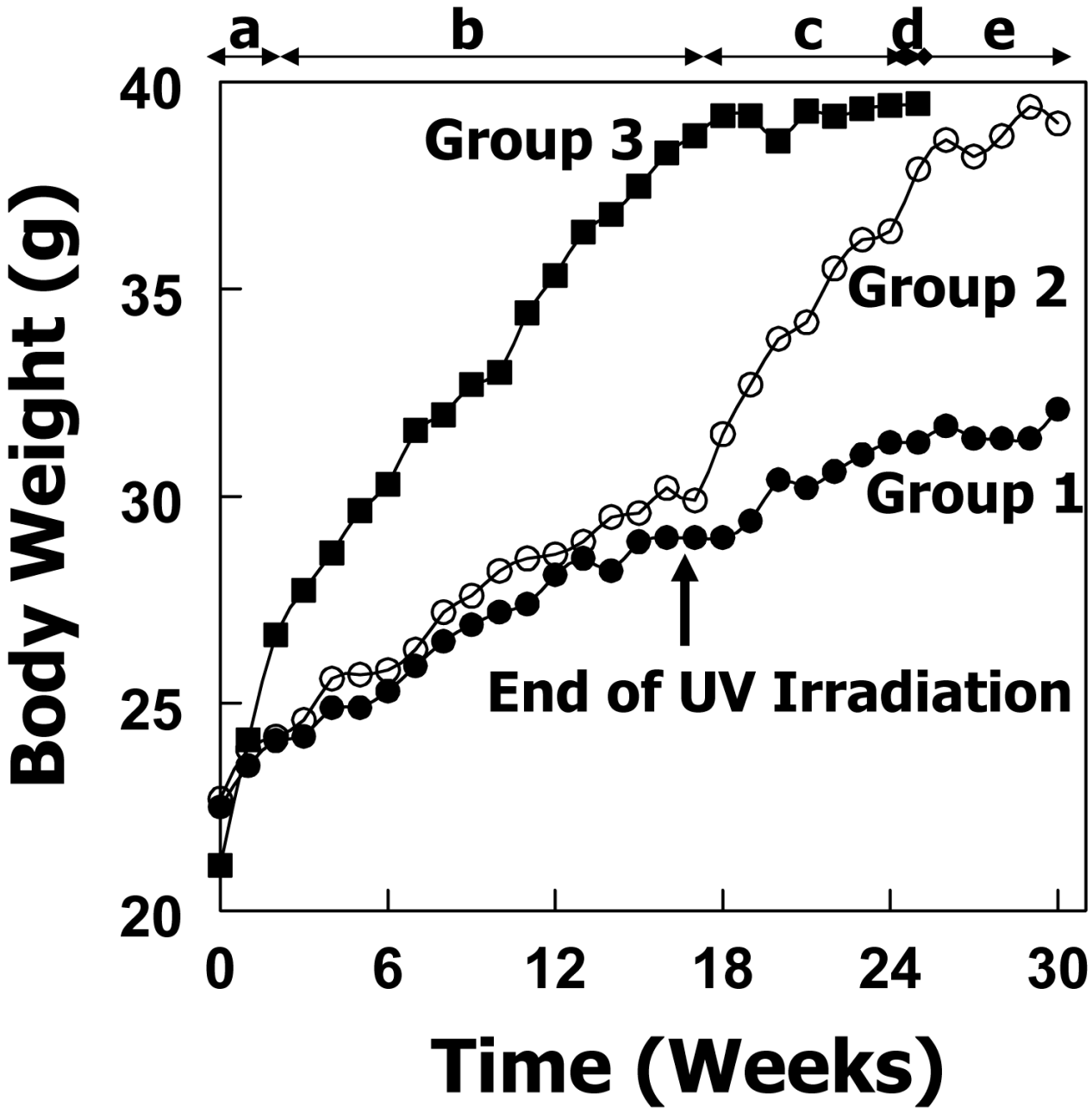

Figure 1. SKH-1 hairless mice fed powdered diet gain weight much more rapidly than their counterparts that are fed pelleted diet

Female SKH-1 hairless mice were exposed to UVB radiation (30 mJ/cm^2/session) twice a week for 17 weeks. At the onset of the experiment, when the animals were 5- to 6-week-old, they were divided into three groups of 30 each. All were fed AIN-76A diet (n=30 animals per Group). Group 1 (●) was fed pellets for 30 weeks. Group 2 (○) received pellets for 17 weeks and then were switched to powder for 13 weeks, when the experiment was terminated. Group 3 (■) was fed powder for 24 weeks, at which time point all animals from that group were euthanized. Body weights were monitored weekly and are shown as mean values. The arrow indicates the end of the UV irradiation schedule, which was also the time when the animals

from Group 2 were switched from pellets to powder. Note that the animals from Group 2 began increasing their body weight as soon as they were changed to the powder. There were significant differences in weekly body weight ($P<0.001$) as determined by ANOVA, followed by post-hoc Bonferroni recalculation of pairwise significance: (nsd – no significant difference; average weight per mouse); significant differences are indicated by the symbols at the top of the graph: **a-** 1,2>3; **b-** 1,2,3 nsd; **c-** 3>1,2 ; **d-** 3>2>1; **e-**3,2>1; **f-**2>1.

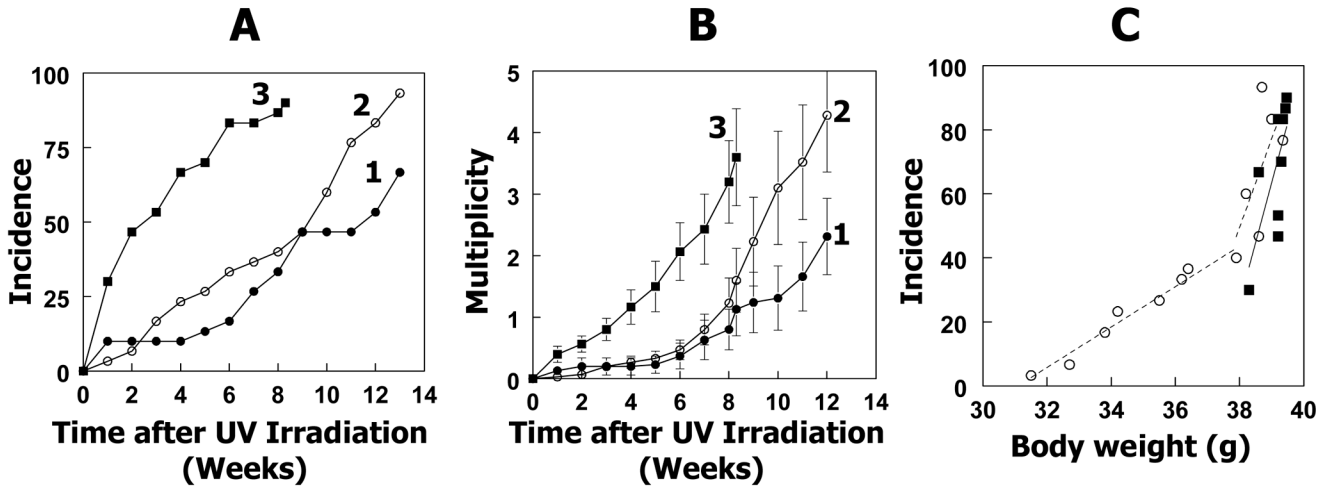

Figure 2. SKH-1 hairless mice fed powder develop UV radiation-induced skin tumors much more rapidly than their counterparts that were fed pellets

SKH-1 hairless mice (n=30 animals per Group) were irradiated with UVB (30 mJ/cm^2/session) twice a week for 17 weeks and received AIN-76A diet. During the period of irradiation, Group 1 (●) and Group 2 (○) were fed pellets, whereas Group 3 (■) was fed powder (n=30 for each Group). After completion of irradiation (time 0 on the graphs), the animals from Group 1 (●) remained on pellets, whereas those from Groups 2 (○) and 3 (■) were fed powder. The appearance of tumors was monitored on a weekly basis, and tumors were mapped, counted, their sizes were recorded. (A) Tumor incidence, i.e., percent of mice with tumors within each group; log-rank comparisons for equality between Groups were significant (P<0.0001) for Group 1 v. 3 and Group 2 v. 3, and at P=0.0012 for Group 1 v. 2; (B) Tumor multiplicity, i.e., number of tumors per mouse, expressed as mean values ± SEM, (n=30 animals per Group). (C) Tumor incidence (% mice with tumors) as a function of body weight.