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MODIFICATION OF SPONTANEOUS MAMMARY TUMORS IN MICE FED DIFFERENT SOURCES OF PROTEIN, FAT AND CARBOHYDRATE

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SUMMARY

The effects of different sources of dietary protein (milk, soy, wheat, fish and beef), fat (corn oil and butter), and carbohydrate (dextrin and sucrose) on the development of spontaneous mammary tumors in virgin female C3H/HeJ mice were investigated. Weanling mice were randomly divided (28 mice/group) and fed ad libitum one of 14 equicaloric diets containing either 11% or 33% protein and 5% or 30% fat or a standard mouse feed for approximately 2 years. Beginning at 6 months of age, tumor incidence, non-specific deaths, individual weights and amount of food consumed were monitored Variations in tumor incidence were most pronounced when the mice fed different sources of protein (at a high level) were compared. The mice fed the low fat diets containing either low milk protein (high carbohydrate) or high fish protein generally exhibited the lowest tumor incidence and highest percent survival. High weight gain was correlated with early tumor appearance, but not with tumor incidence later in the experiment. The mice fed a low fat diet containing low milk protein were tumor-free significantly longer than mice fed the diets containing fish or beef. The only groups with 100% tumor incidence by 120 weeks of age were those fed diets containing sucrose (table sugar) or a high fat level.

INTRODUCTION

Epidemiological and animal studies have implicated high dietary fat consumption with increased mammary carcinogenesis [2,6,7,9,13,17]. This association has been made independently of genetic factors, chemical contaminants in the diet, or other environmental factors. In addition, some

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studies have shown that the effect of a high fat diet is separate from that of obesity, that it acts on the promotion (rather than initiation) phase of tumor development, and that polyunsaturated fats may play a special role [5,15,22,23]. Recently, dietary sugar also has been positively correlated with human breast cancer mortality [12] and with chemically-induced mammary tumors in rats [14]. Fewer experiments in animals have been reported on the effects of meat intake versus that of another source of protein [21].

In each of 2 studies reported previously, we have used BALB/c mice fed the same 14 experimental diets containing protein from one of 5 different sources (milk, soy, wheat, fish and beef). In one study [10] mice were injected with herpes simplex virus Type 2-transformed cells (H238) whereas in the other [18] they were injected with 1,2-dimethylhydrazine(DMH)-transformed colon (no. 51) cells. Results indicated that tumor development could be highly affected by the source of protein in the diet. In addition, a protective effect against tumor development was especially evident in mice fed diets containing protein from a milk source. The present study was undertaken in order to test whether similar tumor-modifying effects could be observed in a spontaneous tumor system.

MATERIALS AND METHODS

Animals

Female C3H/HeJ mice, obtained as weanlings, were randomly divided into 15 groups (28—29 mice/group) and housed 7 per cage. Each group was given either one of 14 test diets or Old Guilford 96W/A, a commercial mouse feed, and allowed to feed ad libitum from specially designed feeders. The amount of food eaten per cage of mice was recorded twice weekly. The animals consumed an average of 10.77 kcals/mouse/day. During the first 32 weeks after arrival each mouse was weighed individually at several intervals in order to determine the average weight gained in each diet group. The mice were kept until they were 120 weeks of age.

Composition of the diets

A detailed decription of the diets has been previously reported [10] and will not be presented here. The 14 experimental diets were made equicaloric at 3.68 kcals/g by using the typical analysis of each ingredient provided by the manufacturer. Corn oil was the source of added fat in all of the diets (except Diet 4 in which butter was used). Dextrin was used to supply added carbohydrate (except in Diet 5 in which sucrose was substituted for dextrin) and to add necessary calories to those provided by the protein and fat sources. Varying amounts (2.7—40.3% by wt) of the non-nutritive filler Celufil (U.S. Biochemical Corporation, Cleveland, OH) were also needed in making the diets equicaloric. The second of 3 previous reports dealing with 2 transplantable tumor systems in which only Diets 1

and 8 were used provides more detailed information on the methods used in designing the various diets [19]. The diets were made up as needed in 6 kg batches and stored in a freezer until just prior to feeding. Early in the course of this study an error was made in making up Diet 6 (low soy protein—low fat) and the mice in this group rapidly expired. Data for this group, therefore, are presented only for the first part of the experiment.

Tumor detection and percent tumor incidence

At weekly intervals beginning at 30 weeks of age each mouse was picked up by the tail and visually inspected for the presence of any unusual lumps. Whole body palpation was also done weekly with special attention given to the chest, neck, shoulders and hips. Any moveable mass or lump under the skin was tentatively classified as a tumor until confirmed by observation of continued growth through succeeding examinations. This procedure was done by the same individual throughout the entire experiment.

The date of appearance of a tumor was recorded and the percentage of tumor incidence for each diet group was calculated as follows:

$$\% \text{ tumor incidence} = \frac{\begin{array}{l}(\text{no. of living mice with tumor} + \\ \text{no. of dead mice which had tumor})\end{array}}{\begin{array}{l}(\text{total no. of living mice} + \\ \text{no. of dead mice which had tumor})\end{array}} \times 100$$

Non-specific deaths were taken into consideration and are not included in the above calculation. Necropsies to determine the exact cause of death were not routinely performed.

Percent survival

The number of living mice in each cage was checked twice weekly and non-specific deaths were recorded. The percentage of surviving mice in each diet group was calculated thus:

$$\% \text{ survival} = \frac{\text{total no. of living mice}}{\begin{array}{r}(\text{total no. of mice} - \text{no. of dead mice} \\ \text{which had tumor})\end{array}} \times 100$$

Statistics

The Test for Equality of Two Proportions, the Wilcoxin Signed Rank Test, Pearson's Correlation Method, and the Student's 2-tailed *t*-Test were used in the statistical analyses of the data.

RESULTS

Mouse weights and weight gains

At arrival the mean weights for the 15 diet groups were 14.9 ± 0.3 g to

TABLE 1

TIME OF FIRST DETECTABLE TUMOR IN EACH DIET GROUP OF MICE ARRANGED IN ORDER OF APPEARANCE OF FIRST TUMOR[a]

Diet no.	Age at first tumor appearance (in weeks)	Protein		Fat		Average kcals/ mouse/day[d]	Average weight gains (g)[e]
		Source[b]	% by wt	Source[c]	% by wt		
14	32[f]	Beef	33	Beef (10.7%)[h]	10.7[g]	9.7	11.2
10	48[f]	Fish	11	Corn oil	5	9.7	11.1
15[h]	50[f]	Several[h]	22.5	Several[h]	7.5	13.3	9.8
13	54[f]	Beef	11	Beef (3.6%) Corn oil (26.4%)	30	9.7	11.3
4	59[f]	Milk	11	Butter	30	9.9	9.8
11	61[f]	Fish	33	Corn oil	5	10.1	9.4
12	61[f]	Beef	11	Beef (3.55%) Corn oil (1.45%)	5	12.8	9.4
2	63	Milk	11	Corn oil	30	9.3	7.8
8	68	Wheat	11	Corn oil	30	9.6	8.9
7	71	Soy	33	Corn oil	5	12.3	10.8
3	72	Milk[i]	33	Corn oil	5	8.4	8.3
6	72	Soy	11	Corn oil	5	12.7	10.5
1	81	Milk	11	Corn oil	5	9.7	8.8
5[j]	82	Milk	11	Corn oil	5	11.5	9.0
9	84	Wheat	33	Corn oil	5	12.1	9.7

[a]The typical analysis of each ingredient was obtained from the various manufacturers and used to determine the total amounts of protein, fat and carbohydrate in each of the experimental diets (Diets 1—14), Vitamin Diet Fortification Mixture (United States Biochemical Corporation (USBC), Cleveland, OH), and Salt Mixture Hegsted (USBC) were added at 2% and 4%, respectively, to each of the experimental diets.

[b]Beef: pre-cooked, freeze-dried diced; Mountain House, Oregon Freeze Dried Foods, Inc., Albany, OR. Fish: fishmeal; USBC. Milk: powdered, skim; Foremost-McKesson, Inc., Willows, CA. Wheat: Do-pep (wheat gluten), Standard Flour Company, Los Angeles, CA, supplemented with 0.5% lysine (USBC) and 0.2% L-threonine (USBC). Soy: Textured Vegetable Protein, Archer, Daniels Milling Company, Decatur, IL.

[c]Beef: (see footnote b). Corn oil: "Mazola", Best Foods, Englewood Cliffs, NJ. Butter: Knudsen Dairy Products, Los Angeles, CA.

[d]The caloric intake values represent the averages obtained during the entire course of the experiment.

[e]The numbers represent the average weight gains obtained from the time the mice arrived (at 5 weeks of age) until 17 weeks later. The weight gains were the most rapid during this time.

[f]Significantly younger ($P \leq 0.05$, Wilcoxon Signed Rank Test) than mice fed Diets 1, 5 and 9.

[g]In order to maintain a 33% beef protein level, a 5% fat level could not be achieved.

[h]Old Guilford 96W/A (Emory Morse Company, Guilford, CT) is a standard commercially available mouse feed containing known amounts of protein, fat and carbohydrate provided by wheat, milk, soy and corn oil ingredients.

[i]One-half of the milk protein in Diet 3 was provided by Vita-free casein (USBC).

[j]In Diet 5 sucrose (C & H Sugar, San Francisco, CA) instead of dextrin (USBC) (as in Diet 1) was used as the source of carbohydrate.

16.1 ± 0.3 g. Early weight gain was rapid and by 12 weeks after arrival the mean values were 22.9 ± 0.5 g (Diets 2, 3 and 8) to 26.3 ± 0.4 g (Diet 14). Maximum weight was usually attained by 32 weeks after arrival at which time the weights were 25.4 ± 0.3 g (Diet 2) to 31.4 ± 0.9 g (Diet 10). Each mouse was weighed once more at 85 weeks and values of 27.0 ± 0.5 g (Diet 2) to 31.4 ± 1.2 g (Diet 13) were obtained. As seen in Table 1, the animals fed Diet 13 (low beef protein—high fat) and Diet 14 (high beef protein—low beef fat) were heavier than those fed the milk-containing diets (Diets 1—5). Figure 1 shows the mean weight gain/group from the time of arrival until 32 weeks of age with the diets grouped according to the levels of protein and fat. High weight gain was found to be linearly correlated with early tumor appearance (correlation coefficient = −0.55; decision point from the Linear Correlation Coefficient Table was ± 0.514). No correlation was found, however, when weight gain was examined in relation to tumor incidence at 90, 100, 110 and 120 weeks of age.

Tumor incidence

The differences in the age of the mice at the time of the first detectable tumor in each diet group are presented along with a brief description of the diets in Table 1. Table 2 gives the distribution of deaths with time in mice with and without a grossly detectable tumor.

The development of tumors for the 5 high protein—low fat diet groups is shown in Fig. 2A,B. Statistical analysis was done when the mice were 90,

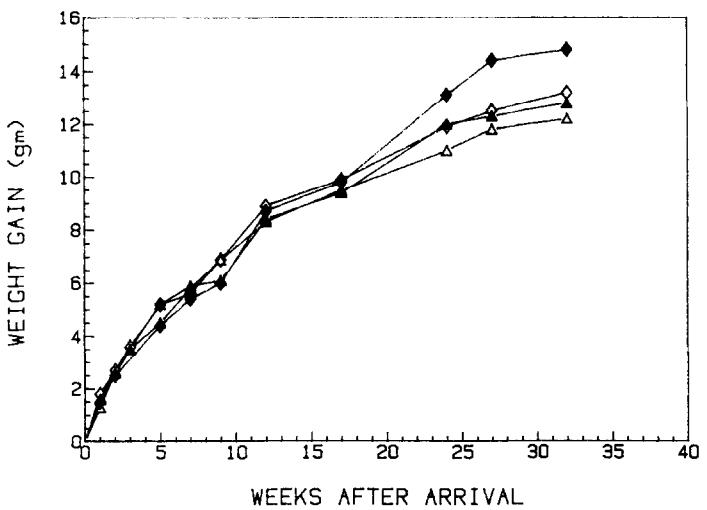


Fig. 1. Average weight gains of the mice with time. The diets were grouped according to the levels of protein and fat: (△) low protein—low fat; (▲) high protein—low fat; (◇) low protein—high fat. The average weight gains of the mice fed Diet 15 (Old Guilford 96W/A, the control diet) are also shown (◆). By 27—32 weeks after arrival the control group had gained significantly more ($P \leq 0.05$) weight than the mice fed the experimental diets.

TABLE 2

DISTRIBUTION OF C3H/HeJ MICE WHICH DIED AT DIFFERENT AGES

Diet no.[c]		Age of mice in weeks[b]																		Total
		35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	
1	A	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	2	2	0	7
	B	0	0	0	0	0	0	0	2	0	0	1	0	2	1	0	0	5	4	15
2	A	0	0	0	0	0	0	1	0	2	0	0	1	0	0	1	0	2	1	8
	B	0	0	0	0	0	0	0	0	1	0	0	0	7	1	2	1	3	0	15
3	A	0	0	0	0	0	0	0	0	1	0	0	3	0	1	0	0	1	2	8
	B	0	0	0	0	0	0	0	0	0	2	0	0	5	1	2	2	4	2	18
4	A	0	0	0	0	0	0	1	2	1	0	1	1	0	1	1	0	1	1	10
	B	0	1	0	0	0	0	1	1	0	1	0	0	4	2	4	3	1	0	18
5	A	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	2	7
	B	0	0	1	0	0	0	0	2	0	0	0	1	2	4	4	0	1	2	18
7	A	0	0	0	0	0	0	0	0	1	0	1	2	0	1	5	6	0	0	16
	B	0	0	0	0	0	0	0	0	0	1	0	0	0	3	1	0	0	0	5
8	A	0	0	0	0	0	0	0	0	2	1	1	1	0	0	1	2	3	1	12

9	A	0	0	0	0	0	0	0	0	0	0	1	0	1	3	0	4	0	2	11
	B	0	0	1	0	0	0	0	1	0	0	2	0	1	3	4	0	0	0	12
10	A	0	0	0	0	0	0	1	0	0	0	3	1	1	0	0	1	5	0	12
	B	0	1	0	0	0	0	0	0	0	0	2	1	2	1	1	2	1	0	11
11	A	0	0	0	0	0	0	2	0	1	0	1	0	0	0	0	3	2	0	9
	B	0	0	0	0	0	0	0	0	0	0	0	1	2	2	1	0	2	1	9
12	A	0	0	0	0	0	0	1	0	1	0	0	2	0	0	0	1	1	3	9
	B	0	4	0	0	1	0	0	0	0	0	1	0	3	1	0	3	1	0	14
13	A	0	0	0	0	0	0	0	0	2	0	1	3	1	1	1	3	3	1	16
	B	0	0	0	0	0	0	0	2	1	0	1	0	3	0	0	3	2	0	12
14	A	1	0	0	0	0	0	1	1	1	0	2	1	1	1	0	0	2	0	11
	B	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	2	6
15	A	0	0	0	0	1	0	0	0	1	0	0	0	1	1	3	1	3	1	12
	B	0	0	0	0	2	0	0	1	0	0	1	0	2	3	0	0	0	0	9

A Mice with mammary tumors which died or were taken when moribund.
B Non-specific deaths.
[a] Deaths due to non-specific causes occurred in some of the diet groups before 30—35 weeks of age. The experiment was terminated at 120 weeks.
[b] The number of mouse deaths appearing in the A and B categories represent the deaths occurring within the previous 5 week interval.
[c] Results for the mice fed Diet 6 are not presented in this table because during the course of the experiment an error was made in making up the diet and the mice in this group expired rapidly thereafter.

100, 110 and 120 weeks of age. Significant differences are given in the legend. Three diets are depicted in each part of the figure in order to illustrate more clearly the diversity in tumor appearance with source of protein. Although the tumors in the mice fed the high soy and high wheat diets (Diets 7 and 9, respectively) appeared later than those in mice fed the corresponding fish and beef diets (Diets 11 and 14, respectively), their incidences increased at a much more rapid rate after appearance. Tumors in the mice fed the high milk protein diet (Diet 3) appeared relatively late and yet increased in incidence in succeeding weeks at a rate similar to that for Diet 14.

Figure 3A—H shows the percent tumor incidence obtained for pairs of diets differing in level of fat (A,G), level of protein (B,F,H), level of protein and fat (E), source of fat (C), or source of carbohydrate (D). The P values obtained are shown in the legend of Fig. 3A—H.

In general, the tumor incidence was lowest in the low protein—low fat diet groups during the entire lifespan of the mice. The mice fed Diet 10 (low fish protein), however, had significantly higher ($P \leq 0.05$) tumor incidence by 110 weeks of age than mice on the comparable diets containing milk or beef. Although tumorigenesis was usually enhanced with high protein (with fish protein, however, the opposite effect was evident, see Diets 10 and 11), high fat made even a greater increase in tumor incidence. Mice fed Diet 2 (the high fat diet containing milk protein) was the only group fed a high fat diet in which tumor incidence (78%) was less than 100% by 120 weeks of age. Statistical significance ($P \leq 0.05$), however, was obtained only at 110 weeks between Diets 2 and 8 and between Diets 2 and 13. The lowest cumulative tumor incidence was found in the mice fed the low fat diets containing either milk (Diet 3, 66.7%) or fish (Diet 11, 55%) as the protein source.

Mouse survival

The survival curves of the mice in most of the diet groups are shown in Fig. 4. In general, the mice fed low levels of protein and fat had fewer deaths due to non-specific causes and thus a higher percent survival than those fed either the high protein or, especially, the high fat diets. The mice fed milk or fish protein together with a low level of fat tended to have the highest survival rates (Diets 1 and 11).

Comparison of different sources of carbohydrate and fat

Mice fed the diet containing sucrose (Diet 5) had higher tumor incidence than the mice fed the comparable diet containing dextrin (Diet 1) beginning at 90 weeks of age, although statistical significance ($P = 0.05$) was obtained only at 120 weeks (Fig. 3D). Survival, however, was significantly lower ($P \leq 0.05$) by 110 weeks for the sucrose-fed mice. The group of mice fed butter (Diet 4) as the source of fat had a higher tumor incidence (Fig. 3C) and lower survival rate than the group fed the comparable diet containing

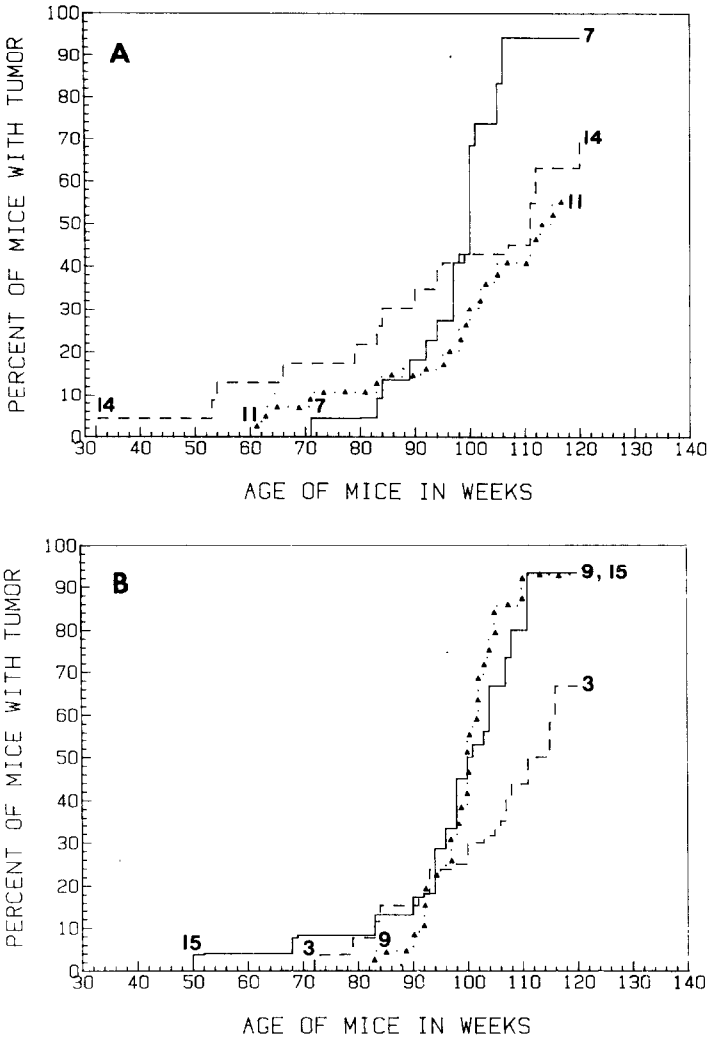
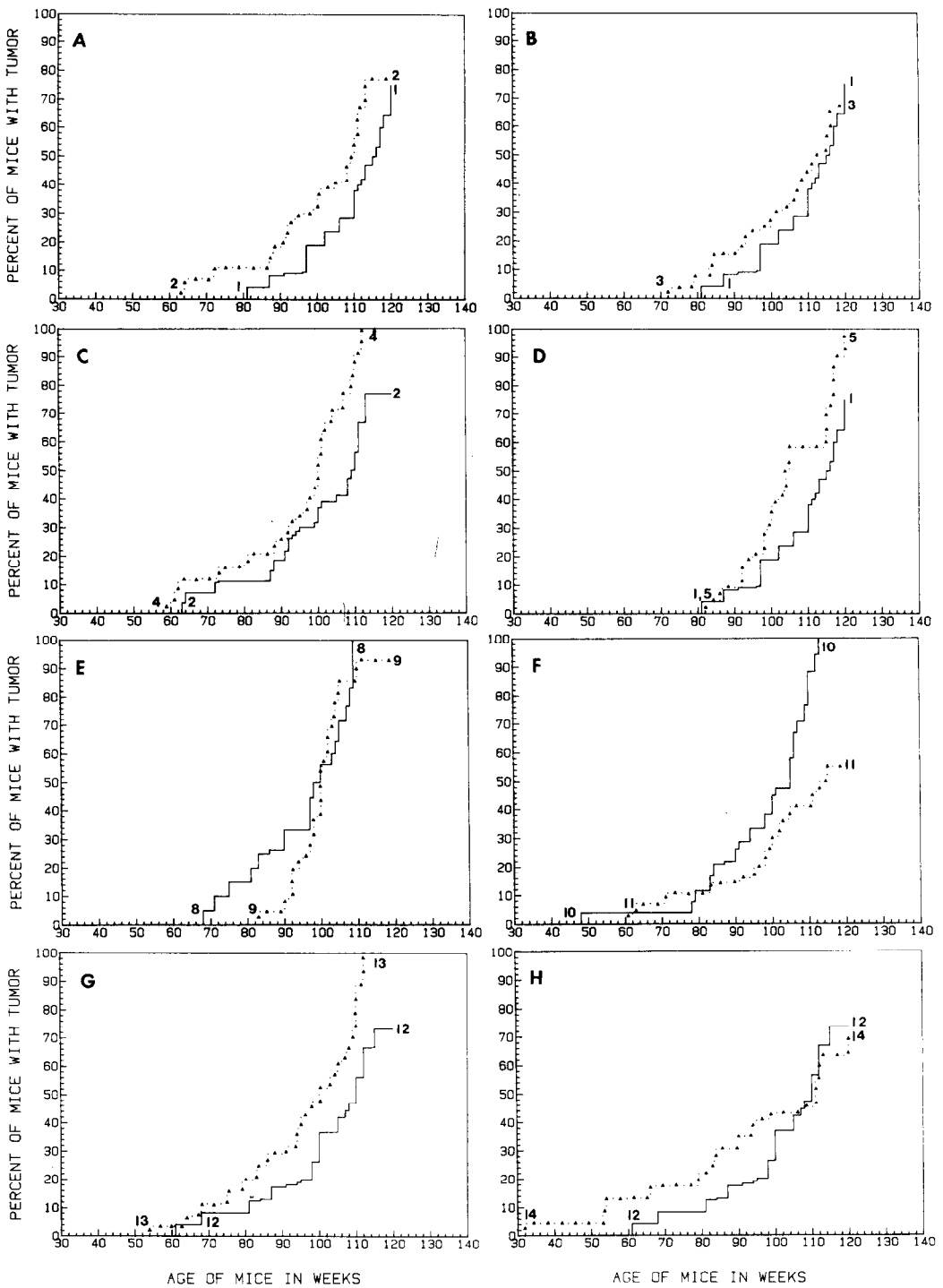


Fig. 2. Tumor incidence in mice fed diets containing high protein levels. (A) Tumor incidence in mice fed low fat diets containing high levels of protein derived from soy (Diet 7), fish (Diet 11) or beef (Diet 14) sources. (B) Tumor incidence in mice fed low fat diets containing high levels of protein derived from either milk (Diet 3) or wheat (Diet 9) sources. Tumor incidence for the group fed Diet 15 (Old Guilford 96W/A, the control diet), which contained 7.5% fat and 22.5% protein derived from various sources, but primarily from wheat, is included here. Significant differences ($P \leq 0.05$) were observed at: 100 weeks: Diets 3 vs. 7, Diets 7 vs. 11; 110 weeks: Diets 3 vs. 9, Diets 3 vs 15, Diets 7 vs. 14, Diets 11 vs. 14, Diets 11 vs. 15, Diets 14 vs. 15; 120 weeks: Diets 7 vs. 11, Diets 9 vs. 11, Diets 11 vs. 15. Significance was not obtained between additional pairs of diets at 120 weeks of age probably because of the small numbers of animals still alive at this time.


A
B
C
D
E
F
G
H
PERCENT OF MICE WITH TUMOR
AGE OF MICE IN WEEKS

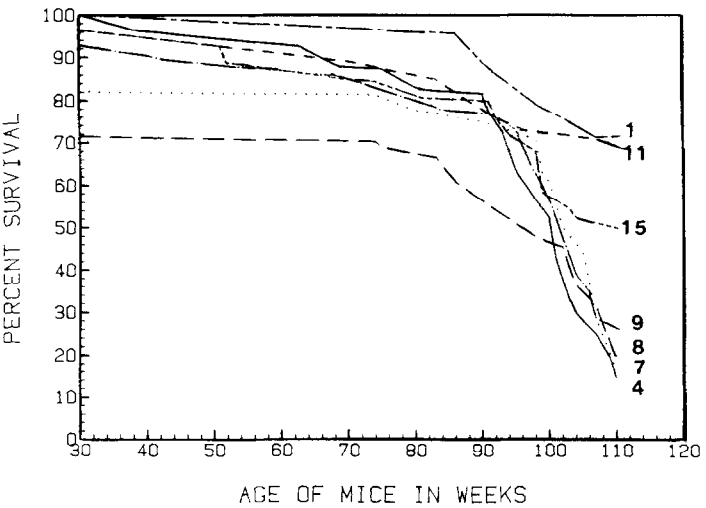


Fig. 4. Survival of the mice with time. The percentages have been adjusted for deaths due to tumor. Survival curves for mice fed Diets 2, 3, 5, 10, 12, 13 and 14 resembled the curve for animals on Diet 15 (Old Guilford 96W/A, the control diet) and are not shown here. At 90 weeks of age the % survival of mice fed Diet 11 was significantly greater ($P \leq 0.05$) than that for Diets 7, 9, 14 and 15. By 110 weeks the Diet 1 group had significantly greater percent survival than the Diet 5 group.

corn oil in place of butter (Diet 2), although the differences seen were not statistically significant. In addition, while 25% of those on the high corn oil-containing diet were still alive by 120 weeks of age, all of the mice on the high butter-containing diet were dead.

DISCUSSION

Enhancement of mammary tumors in animals has been consistently observed with increased dietary fat [3,5,7,23]. Recently Carroll and Hopkins [4] demonstrated that in a high fat diet a small amount of polyunsaturated fat is required in order for tumor enhancement to occur. In the present study, mice fed a high level of butter had significantly enhanced tumor incidence compared to those fed a low corn oil diet (Diet 1, 38.1%;

Fig. 3. Tumor incidence in mice with time. (A) Diet 1, low milk protein—low fat; Diet 2, low milk protein—high fat. (B) Diet 1, low milk protein—low fat; Diet 3, high milk protein—low fat. (C) Diet 2, low milk protein—high fat (corn oil); Diet 4, low milk protein—high fat (butter). (D) Diet 1, low protein—low fat—high dextrin; Diet 5, low milk protein—low fat—high sucrose (table sugar); $P \leq 0.05$ at 120 weeks. (E) Diet 8, low wheat protein—high fat; Diet 9, high wheat protein—low fat. (F) Diet 10, low fish protein—low fat; Diet 11, high fish protein—low fat; $P \leq 0.05$ at 110 and 120 weeks. (G) Diet 12, low beef protein—low fat; Diet 13, low beef protein—high fat; $P \leq 0.05$ at 110 weeks. (H) Diet 12, low beef protein—low fat; Diet 14, high beef protein—low fat.

Diet 4, 90.9% at 110 weeks of age), whereas in the group fed a high corn oil (unsaturated fat) diet significant enhancement was not seen (Diet 1, 38.1%; Diet 2, 56.3% at 110 weeks of age). In previous mouse studies we have found strikingly different results when comparing the effects of corn oil and butter. Butter, rather than corn oil, markedly restricted tumors in one system [10] while no significant differences were seen in the other [18]. Weight gain was found to be an insignificant factor in these other 2 tumor systems. In the present study, a high fat level significantly increased tumor incidence (at 110 weeks of age) only in mice fed beef-containing diets, although all of the groups fed high fat had higher incidence compared to the equivalent groups fed low fat. These findings illustrate the complexity of the relationship between dietary fat and tumorigenesis and emphasize the need for further investigation.

High weight gain, shown previously to enhance mammary tumor development, is associated with early tumor appearance in our study (see Table 1). The source of protein, however, is a modulating factor. The mice fed soy protein gained almost as much weight as those fed beef, yet the first appearance of tumor in the soy-fed group occurred considerably later. The type of fat may also play a role in early tumor appearance. Tumor appeared early in the mice fed the beef diets, all of which contained some beef fat due to the high percentage of fat in the beef protein source (the fat in Diet 14 was all beef fat), whereas none of the other diets contained beef fat. No correlation was seen between the percent tumor incidence at 90, 100, 110 and 120 weeks of age and the average weight gain per group. The 5 groups in which tumor incidence reached 100% (Diets 4, 5, 8, 10 and 13) gained from 8.9 g to 11.3 g, whereas the 5 groups with the lowest tumor incidence at 120 weeks (Diets 1, 3, 11, 12 and 14) gained from 8.8 g to 11.2 g. These data suggest that in this system the sources of dietary protein and fat have a profound effect on mammary tumor development in the older mice, more so than weight gain. It is interesting to note that the mice fed the low fat diets containing milk (Diet 1) or fish (Diet 11) had higher percent survival values than the group fed the commercially available mouse diet (Diet 15) and were also among those that had the lowest percent tumor incidence throughout much of the experiment.

In the present study, 32% (40/124) of the mice fed Diets 1—5 (milk protein) which died from 30—120 weeks of age had mammary tumors, whereas 56% (108/194) on Diets 7—15 (other protein sources) had mammary tumors during this same time (Table 2). Factors affecting the tumor incidence include: whether the mice are conventional or virus free (C3Hf), and whether the mice are bred or kept as virgin females. Presence of the mammary tumor virus in the milk of their mothers and breeding tend to increase the fraction of tumors. The mice used in the present study were conventional female mice (C3H/HeJ — not virus free) and were kept as virgins. The 56% mammary tumor incidence found as an average for groups 7—15 is typical of the expected results for presently supplied conventional virgin female C3H/HeJ mice. The 32% incidence found in the milk-protein

fed mice, is much lower than expected and is apparently diet related.

It should be noted that some of the tumor incidence curves begin early and have shallow slopes (Diets 12 and 14) whereas others begin later but have much steeper slopes (Diets 7 and 9). The increase in incidence of the former curves may be due to exogenous mouse mammary tumor virus, whereas those of the latter may represent induction of endogenous viruses which are known to be involved in mammary tumor appearance late in the lives of female mice in some species. Had more virus been transmitted through their mother's milk early in life, greater response differences with dietary protein may have been noted. It is evident that mammary tumor incidence in C3H/HeJ mice supplied by the Jackson Laboratory has declined appreciably. The same substrain used in our laboratories approximately 12—14 years ago and fed a standard commercial diet began to develop tumors about 6 months earlier than those fed Diet 15 in the present study (unpublished data). This decline is referred to in recent information from the Jackson Laboratory announcing the present availability of a new inbred strain C3H/OuJ with high mammary tumor incidence.

An association between sugar intake and tumors has recently been reported by several investigators [1,8,11,12,14,16]. In the study reported here, significant enhancement of tumor incidence was seen in the group fed sucrose, but only when the mice were 120 weeks of age (Fig. 3D). The differences in average weight gain between these 2 groups, however, was substantial. Our previous studies, using H238 or no. 51 cell-induced tumors, showed that both tumor volume and incidence were slightly (but not always significantly) greater in the sucrose-fed groups when compared to the dextrin-fed groups [10,18].

The C3H mouse mammary tumor model is the third model used with the same 15 diets containing low and high levels of 5 different sources of protein [10,18]. In all 3 systems, milk as the protein source, particularly at the low (11%) level, appears to have a restricting effect on tumor development. Experiments which may help to clarify the possible mechanism(s) involved are currently underway in our laboratories.

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