The Dependence of Tumor Formation on the Composition of the Calorie-Restricted Diet as Well as on the Degree of Restriction

Albert Tannenbaum, M.D.

(*From the Department of Cancer Research, Michael Reese Hospital, Chicago 16, Illinois*)

(Received for publication June 20, 1945)

The inhibitory effect of caloric restriction on the formation of tumors in the mouse has been shown (11) to be dependent on the actual degree of caloric restriction. Previously it had been demonstrated (8) that restriction of caloric intake to about 60 per cent of the *ad libitum* intake causes an inhibition in tumor formation regardless of whether only the carbohydrate component is restricted (so-called caloric restriction *per se*) or an aliquot of the *ad libitum* ration is fed (restriction of all components). The question arose whether these two types of restricted diets affect tumor formation to the same extent. Also, since it is known that a fat-enriched diet enhances the formation of spontaneous mammary and induced skin tumors (1, 9) it was of interest to determine the effect of high-fat but restricted diets on tumor formation.

The present experiments, in which spontaneous mammary and induced skin tumors of the mouse were utilized, were performed in order to gather more data regarding the effect on tumor formation of the *degree* of caloric restriction, and at the same time to ascertain whether or not there is a differential effect due to the *composition* of the diet (at various caloric levels). Three types of restricted diets were used: (a) diets in which caloric restriction was achieved by decreasing the carbohydrate component only; (b) diets in which caloric restriction was achieved by reducing all components of the control ration (not necessarily in proportion to the caloric restriction); and (c) diets equivalent to the latter except that the fat content was increased by equicaloric substitution of fat for carbohydrate.

METHODS

The general methods, conditions, and criteria have been described (11). The special characteristics will be given under the separate investigations.

The constituents of the diets used in these experiments were Purina fox chow meal, skimmed milk powder, cornstarch,[1] and hydrogenated cottonseed oil.[2] The fox chow meal and skimmed milk powder contained the protein, vitamins, and minerals, and (as far as growth is concerned) adequate essential fatty acids. Consequently, variation or equivalence of these two materials in the diets is referred to as variation or equivalence of "essential components." The cornstarch and hydrogenated cottonseed oil utilized are practically free of vitamins, ash, and proteins; they were added to vary the caloric level and fat content of the diets. The compositions and caloric values of the diets were calculated from data supplied by the manufacturers of the foodstuffs.

It must be recognized that when caloric restriction *per se* is achieved by reducing the amount of cornstarch, there is concomitantly an increase in the percentage (not in the amount) of the essential components of the diet. Moreover, when cornstarch is replaced by an equicaloric amount of fat, the other constituents remaining constant, there is a decrease in the weight of the diet and a consequent increase in the proportion of the essential components. These latter points can be seen in the tables in which the diets are described.

EXPERIMENTAL

Experiment 1.—The 3 groups of this experiment were composed of either 50 or 54 dba male mice, all born between April 14 and May 23, 1941. They were placed on the respective experimental diets when they were 5 to 11 weeks old. Two weeks later they were given the first application of a 0.3 per cent solution of 3,4-benzpyrene in benzene. The solution was applied in the interscapular area by means of a dropping pipette.

The factors and conditions of the experiment were alike for all three groups, except for the differences in diet (Table IA) and the number of semi-weekly applications of the carcinogen. The mice of the restricted groups, x5 and x25, received 25 applications (in 12 weeks), whereas those of the *ad libitum* group, x12 were given only 17 (in 8 weeks). There were special reasons for this procedure: the x12 group was also being used as a control (for other experi-

[1] Anheuser-Busch cornstarch 712-87.

[2] Kremit (partially hydrogenated cottonseed oil) generously furnished by Armour & Co.

mental groups carried on at the same time) in which only a moderate tumor response was desired. Seventeen applications of the carcinogen solution would have produced only a few tumors in the restricted groups; therefore, these two groups were given 25 applications in order that the tumor response would be in a range where differences between the restricted groups, if any, would be more readily recognized.

The composition of the diet, mean caloric intake, and mean weight of the mice are given in Table Ia.

TABLE Ia: DIET, MEAN CALORIC INTAKE, AND MEAN WEIGHT OF MICE OF EXPERIMENT 1

Group	Diet constituents (gm. per mouse per day)				Mean daily caloric intake	Percentage composition of diets				Mean weight of mice in gm.								
										Weeks after 1st application of carcinogen								
	Fox chow meal	Skimmed milk powder	Corn-starch	Total		Protein	Fat	Carbo-hydrate	Ash	−2	0	8	12	20	30	40	50	General † level
x12	1.4	0.9	1.9	4.2	13-14 *	16	2	69	4	24	28	34	37	38	39	38		38
x5	1.4	0.9		2.3	8.1	29	3	52	7	25	23	22	21	22	22	21	21	21
x25	0.8	0.5	1.0	2.3	8.2	16	2	68	4	24	22	24	22	22	22	21	22	22

* Calculated from food consumption, which varied during course of experiment.
† Approximate mean weight during course of experiment following last application of carcinogen.

TABLE Ib: EFFECT OF COMPOSITION OF A CALORIE-RESTRICTED DIET ON FORMATION OF BENZPYRENE SKIN TUMORS IN DBA MALE MICE

Group	Number of mice (effectual total) *	Number of applications of carcinogen	Mice developing skin tumors			Per cent mice developing carcinomas	Number of mice tumor-free and alive at end of experiment (54th week)
			Number	Per cent	Mean time of appearance (weeks)		
x12	54	17	35	65	34 ± 2.6	46	17
x5	48	25	12	25	38 ± 6.1	13	33
x25	49	25	19	39	34 ± 7.1	27	23

* Number of mice alive at 13th week of experiment.

It is to be noted that, in comparison with x12, x5 was restricted only in carbohydrate. On the other hand, group x25 was fed a ration prepared by restricting all components (essentials as well as calories) of the x12 diet. The two restricted diets were approximately equicaloric.

The control mice grew normally, while those of the two restricted groups maintained a fairly constant weight; the mean weights of the latter groups were approximately the same. The experiment ran for 54 weeks. Table Ib contains the data on tumor incidence and average time of appearance.

At the termination of the experiment the carbohydrate-restricted group (x5) had 25 per cent skin tumors in comparison with 39 per cent for the all-component-restricted group (x25). These compare with 65 per cent for the "control" group (x12). While a direct comparison can be made only between x5 and x25, the x12 group may be considered a qualitative control, particularly since more tumors were formed here than in the restricted groups in spite of fewer applications of the carcinogen. With regard to the formation of carcinomas the percentages for x5, x25, and x12 are 13, 27, and 46, respectively. The curves of tumor formation are given in Fig. 1.

Although caloric restriction inhibits tumor formation, the method by which the restriction is achieved apparently modifies the degree of the effect. It appears that the mice receiving the diet restricted in carbohydrate only developed fewer tumors, and at a later mean time of appearance, than did the mice receiving the diet restricted in all components.

Experiment 2.—Four groups of 50 virgin dba female

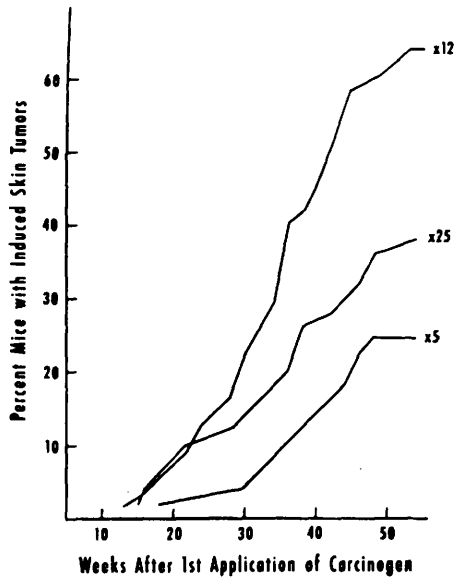

FIG. 1.—Cumulative curves of formation of induced skin tumors, illustrating effect of composition of diet on inhibition caused by caloric restriction. x12, *ad libitum* control; x5, restricted in carbohydrate only; x25, restricted in all components.

mice, all born between April 14 and 23, 1941, were utilized in this experiment. The groups were constructed by litter-mate distribution. After weaning, the mice were fed Purina fox chow checkers until they were 9 to 10 weeks of age, when the experimental diets were instituted; these were respectively, *ad libi-*

TABLE IIA: DIET, MEAN CALORIC INTAKE, AND MEAN WEIGHT OF MICE OF EXPERIMENT 2

Group	Diet constituents (gm. per mouse per day)					Mean daily caloric intake	Percentage composition of diets				Mean weight of mice in gm.						
	Fox meal chow	Skimmed milk powder	Corn-starch	Kremit	Total		Protein	Fat	Carbo-hydrate	Ash	Age in weeks 10	30	50	70	90	110	General † level
T2	1.2	0.8	1.4		3.4	11-12 *	17	2	67	4	22	32	34	35	32	31	33
T5	1.2	0.8	0.7		2.7	9.6	21	2	62	5	21	22	25	24	23	24	24
T25	0.7	0.5	1.5		2.7	9.7	13	2	73	3	22	21	24	24	22	23	24
T15	0.7	0.5	0.5	0.32	2.0	8.9	17	18	53	4	21	22	25	25	22	24	24

* Calculated from food consumption, which varied during course of experiment.
† Approximate mean weight between 50 and 110 weeks of age.

TABLE IIB: FORMATION OF SPONTANEOUS MAMMARY TUMORS IN DBA VIRGIN FEMALE MICE: MODIFICATION OF INHIBITORY EFFECT OF CALORIC RESTRICTION BY COMPOSITION OF RESTRICTED DIET

Group	Diet characteristics			Number of mice (effectual total) †	Mice developing mammary tumors by end of experiment (136 weeks)			Mean age at death of non-tumor mice (weeks)
	Mean daily caloric intake	Relative * amount of "essentials"	Per cent fat		Number	Per cent	Mean age at appearance (weeks)	
T2	11.5	1.0	2	47	35	74	89 ± 3.1	74 ± 9.7
T5	9.6	1.0	2	49	23	47	99 ± 3.6	102 ± 5.2
T25	9.7	0.6	2	49	32	65	86 ± 3.3	89 ± 5.5
T15	8.9	0.6	18	47	41	87	80 ± 3.2	71 ± 11.5

* Relative amount of protein, vitamins, and minerals: T2 = 1.
† Number of mice alive in group when first tumor appeared: mice 45 weeks old.

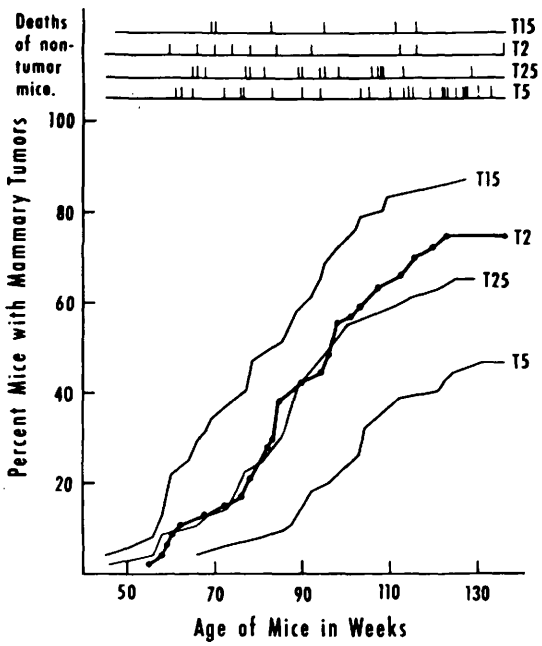

FIG. 2.—Cumulative curves of formation of spontaneous mammary tumors illustrating dependence of inhibitory effect of caloric restriction on composition of restricted diet. T2, *ad libitum* control; T5, restricted in carbohydrate only; T25, restricted in all components (low fat); T15 similar to T25 but with high fat content.

tum (T2), calorie (carbohydrate) restricted (T5), all components restricted (T25), and the latter with fat substituted for some of the carbohydrate (T15). The 3 restricted diets were approximately equicaloric. The composition of the diet, mean caloric intake, and mean weight of the mice are shown in Table IIA.

The experiment was terminated at a time when only 3 mice were alive (136 weeks of age). The experiment ran smoothly and the mice were in good condition throughout. The data with regard to mammary tumor incidence and the time of appearance of these tumors are shown in Table IIB and Fig. 2.

The following results, in comparison with tumor production in the *ad libitum* control (T2), were obtained in the 3 restricted groups: The high-fat group (T15) developed more tumors and, on the average, at a significantly earlier time; the group restricted in essential components (T25) developed fewer tumors, but at about the same time; the group restricted in carbohydrate only (T5) developed significantly fewer tumors, and at a later time. The actual percentages of mammary tumors, on an effectual total basis, in the T2, T15, T25, and T5 groups were 75, 87, 66, and 47 respectively.

This experiment clearly indicates that although caloric restriction may inhibit tumor formation, the

components of the restricted diet are of great importance, for they may be of such a nature (*i.e.*, high-fat) that under proper conditions tumor production is enhanced over and above the control level. It is also obvious that the augmenting effect of the high-fat diet is not due to a caloric effect (since the 3 restricted groups were approximately equicaloric) but to some unexplained essential action of fat.

In both Experiments 1 and 2, diets restricted in carbohydrate only (caloric restriction *per se*) inhibited tumor formation to a greater extent than did an equicaloric diet restricted in all components. In Experiment 1, performed with induced skin tumors, and with diets restricted to approximately 60 per cent of the *ad libitum* intake, the differences were not statistically significant. On the other hand, in Experiment 2, performed with spontaneous mammary tumors and diets restricted to 80 per cent of the *ad libitum* intake, the differences were statistically significant. The agreement in results between the experiments supports the viewpoint that the nature of the restricted diet modifies the inhibitory effect on the formation of these two types of tumors in the mouse.

Graded caloric restriction using diets of different composition.—In Experiments 1 and 2 it was concluded that the composition of the restricted diet at a particular caloric level is a modifying factor for both the number of tumors and the average time at which they appear. In Experiments 3 and 4, which follow, the 3 types of restricted diets were studied at more than one caloric level.

The diets stemmed from a low-fat basic diet, 40, composed of Purina fox chow meal, skimmed milk powder, and cornstarch; this diet was sufficient in both essentials and calories to support good growth and health in the C3H male mice used in Experiment 3.

The types of diets were: (a) those constructed from basic diet 40 by restriction of cornstarch (calories) only; (b) those constructed from diet 40 but by restricting essentials (fox chow meal and skimmed milk powder) as well as cornstarch; (c) those comparable to the latter except that fat was substituted equicalorically for some of the cornstarch. The series may be briefly indicated as: (a) restricted in calories (carbohydrate) only; (b) restricted in calories and essentials (low in fat); (c) restricted in calories and essentials, but high in fat.

The diet and group numbers indicate the type of diet fed. Group numbers 40 and below received the carbohydrate-restricted diets; those of the 200 series were given the diets restricted in essentials as well as carbohydrates; the 100 series were fed the high-fat diets. The last 2 figures of the group or diet number indicate the caloric level of the diet. For example, diets 40 and 140 were equicaloric, as were diets 28, 128, and 228.

TABLE IIIA: COMPOSITION OF DIETS USED IN EXPERIMENTS 3 AND 4

Diet number	Fed to groups: In experiment 3 i-series	Fed to groups: In experiment 4 h-series	Constituents (grams per mouse per day): Fox chow meal	Skimmed milk powder	Cornstarch	Kremit	Total	Percentage composition of diet: Protein	Fat	Carbohydrate	Ash	Calories per mouse per day
40	i40		1.4	0.8	1.8		4.0	16	2	69	4	14.3
33	i33	h33a & b	1.4	0.8	1.1		3.3	19	2	65	4	11.7
28	i28	h28	1.4	0.8	0.6		2.8	22	2	60	5	10.0
26		h26	1.4	0.8	0.4		2.6	24	3	58	6	9.2
24	i24	h24	1.4	0.8	0.2		2.4	26	3	55	6	8.5
22		h22	1.4	0.8			2.2	28	3	52	7	7.8
228	i228	h228	1.2	0.7	0.9		2.8	19	2	64	5	10.0
226		h226	1.1	0.6	0.9		2.6	18	2	65	4	9.3
224	i224	h224	1.0	0.6	0.8		2.4	19	2	65	4	8.5
222		h222	1.0	0.5	0.7		2.2	19	2	64	5	7.8
140	i140		1.4	0.8	0.55	0.51	3.3	19	18	50	5	14.4
133		h133	1.4	0.8	0.10	0.42	2.7	23	18	45	5	11.9
128	i128	h128	1.2	0.7	0.05	0.35	2.3	23	18	45	5	10.1
126		h126	1.1	0.6	0.05	0.33	2.1	23	18	45	5	9.2
124	i124	h124	1.0	0.6	0.05	0.30	2.0	23	18	45	5	8.5
122		h122	1.0	0.5		0.28	1.8	23	18	44	6	7.8

The diets utilized in Experiments 3 and 4 are presented in Table IIIA.

Experiment 3.—This experiment was designed to test the effect of both graded caloric restriction and the composition of restricted diets on the formation of induced skin tumors. The nine groups were each composed of 50 C3H male mice (born between April 19 and July 17, 1943), and were equivalent as to age and weight of the mice. The diets were instituted when the mice were $5\frac{1}{2}$ to 16 weeks of age. Two

TABLE IIIB: MEAN WEIGHT IN GRAMS OF MICE OF EXPERIMENT 3

Group	Weeks after 1st application of carcinogen: −2 †	0	9	19	29	39	49	General * level
i40	24	27	31	34	34	34	35	34
i33	24	25	26	26	26	28	29	27
i28	24	22	23	23	25	26	24	25
i24	24	20	19	19	21	22	20	20
i228	24	22	23	23	23	24	23	23
i224	24	20	20	20	20	20	20	20
i140	24	28	31	34	34	33	37	34
i128	24	23	23	24	23	24	23	24
i124	24	21	20	19	20	20	21	20

* Approximate mean weight during course of experiment following last application of carcinogen.

† Mean weight when experimental diets were instituted; 2 weeks before first application of carcinogen.

In each of the 3 series, tumor incidence is progressively decreased with progressive caloric restriction of the diet. There is also a tendency for the tumors to appear later, on the average, as restriction is increased. In addition, at a particular level of caloric restriction, the high-fat diet (i140, 128, or 124) permits the formation of a higher percentage of tumors than its low-fat counterpart (i40, 228, or 224). While the difference at each level is not statistically significant, the augmentation is consistent and in agreement with the results of other experiments. The lack of difference in this experiment between the effect of diets restricted in carbohydrate only (i24 and 28) and the corresponding equicaloric diets restricted in all components (i224 and 228) will be discussed later. These points are more readily seen in Fig. 3, in which the incidence of

TABLE IIIC: FORMATION OF METHYLCHOLANTHRENE SKIN TUMORS AS AFFECTED BY COMPOSITION OF DIET AND CALORIC INTAKE

Group	Diet characteristics: Mean caloric intake	Relative * amount of "essentials"	Per cent fat	Number † of mice (effectual total)	Mice developing skin tumors by end of experiment (55 weeks): Number	Per cent	Time of appearance (weeks) ‡: Range	Mean	Per cent mice with carcinomas	Mice tumor-free and alive at end of experiment
i40	14.3	1	2	50	43	86	5-55	32 ± 1.9	60	4
i33	11.7	1	2	50	29	59	11-53	37 ± 2.4	38	17
i28	10.0	1	2	50	17	34	21-55	41 ± 2.4	22	24
i24	8.5	1	3	46	7	15	23-55	38 ± 4.5	14	24
i228	10.0	0.86	2	49	18	37	19-53	39 ± 2.1	22	23
i224	8.5	0.73	2	45	9	20	13-53	41 ± 4.1	16	26
i140	14.4	1	18	50	46	92	11-55	29 ± 2.1	78	3
i128	10.1	0.86	18	49	27	55	17-55	40 ± 2.1	41	19
i124	8.5	0.73	18	48	14	29	17-55	41 ± 2.8	23	25

* Relative amount of protein, vitamins, and minerals: i40 = 1.

† Number of mice alive at fifth week of experiment.

‡ Weeks after first application of carcinogen.

weeks later the first of 10 weekly applications of carcinogen was given: one drop of a 0.3 per cent solution of methylcholanthrene in acetone applied with a dropping pipette to the interscapular area.

The experimental diets have been discussed, and are listed in Table IIIA. The control diets, low-fat (i40) and high-fat (i140), contained equal amounts of essential components, and were approximately equicaloric. During the major part of the experiment most of the mice fed these 2 diets ate all the daily ration. The restricted mice consumed all their daily ration throughout the experiment.

At the beginning of the experiment the mean weights of the 9 groups ranged from 23.7 to 24.4 gm. The mean weight of the mice at various times of the experiment is shown in Table IIIB.

The experiment was terminated 55 weeks after the first application of the carcinogen. The data regarding the incidence and mean time of appearance of skin tumors are given in Table IIIC.

tumors as a function of both caloric intake and composition of diet is graphically presented.

An incidental finding is the data on hepatomas, which develop spontaneously in the males of strain C3H. The first one was seen in a 45 week old mouse of the i140 group. Of 34 mice of i140 group (high-fat control) sacrificed between 45 and 73 weeks of age, 12 (35 per cent) were found to have hepatomas. In contrast, hepatomas were found in only 3 of 32 mice (9 per cent) in the i40 group (low-fat control) sacrificed during the same interval; the first hepatoma observed in this group was in a mouse 65 weeks old. In the 7 restricted groups, 158 mice were sacrificed between 62 and 73 weeks of age; only 2 had hepatomas. Apparently, as in the case of the induced skin tumor and spontaneous mammary tumors, the formation of a spontaneous hepatoma in C3H male mice is inhibited by caloric restriction, and augmented by a high-fat diet.

Experiment 4.—This experiment was designed as a companion investigation to Experiment 3, but in this

instance spontaneous mammary tumors were studied. The 15 groups were each composed of 30 virgin C3H females (born between April 19 and July 17, 1943) and were equivalent as to age and weight. The experimental diets were instituted when the mice were from 5 to 15 weeks of age. As in Experiment 3, the 3 diet types were studied at several caloric levels (Table IIIA). In this experiment diet 33 served as control, since it is at the *ad libitum* level for C3H female mice; throughout the major portion of the experiment most of the mice being fed this diet consumed all the daily ration. The mice receiving the

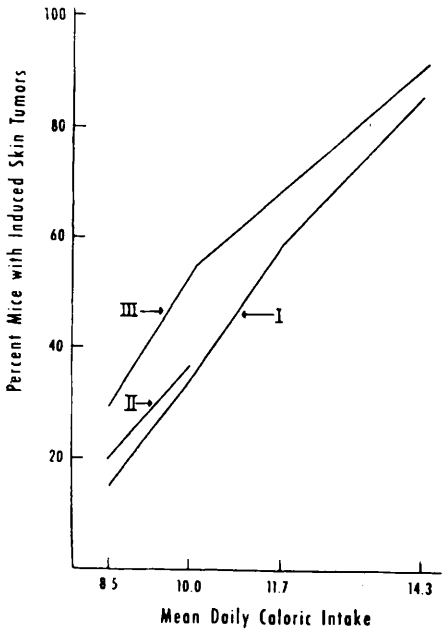

FIG. 3.—Incidence of methylcholanthrene skin tumors: dependence on composition of diet and caloric intake. Curve I—diets varying in carbohydrate content (caloric restriction *per se*); Curve II—diets varying in all components (with low-fat content); Curve III—diets varying in all components but with high-fat content.

restricted diets consumed all their daily ration throughout the experiment.

Because of inherent difficulties in constructing 15 uniform groups, two separate groups (h33a and h33b) were used as controls and were fed diet 33. This was done in order to obtain an impression, at least, of the variation to be expected between any 2 groups when such variation arises because of the manner of selecting mice for the individual groups of the experiment and other random factors.

Again, as in the previous experiments, graded caloric restriction produced graded weight levels. The mean weight of the mice of the various groups is shown in Table IVA.

Laboratory conditions prompted the termination of the experiment when the youngest mice were 64 weeks of age. The percentages of tumors formed by the 64th week, on an effectual total basis, are given in Table IVB. Although further extension of the experiment would have increased these percentages in some groups, previous experience (including the results of Experiment 2) suggests that the main differential results would be maintained.

The difference in tumor formation between the two control groups, h33a and h33b, is well within the expected limits, suggesting that with respect to the potentiality of tumor formation the 15 groups were relatively uniform at the beginning of the experiment.

As in Experiment 3, it can be noted that the greater the degree of caloric restriction in each of the 3 series,

TABLE IVA: MEAN WEIGHT IN GRAMS OF MICE OF EXPERIMENT 4

Group	Age in weeks						General * level
	10	20	30	40	50	60	
h33a	21	24	27	26	30	31	28
h33b	20	25	27	27	30	32	28
h28	20	21	22	23	24	24	23
h26	21	20	21	22	24	25	22
h24	21	20	19	20	20	21	20
h22	21	18	17	18	18	18	18
h228	21	21	23	23	25	25	24
h226	21	20	21	21	23	24	23
h224	21	20	19	21	22	22	21
h222	21	18	17	18	17	17	17
h133	21	25	27	26	27		27
h128	21	22	23	23	25	25	24
h126	21	21	23	23	25	25	24
h124	20	20	19	20	21	22	20
h122	21	18	18	19	20	21	19

* Approximate mean weight between 20 and 60 weeks of age.

the greater the inhibition of tumor formation. Again, at the levels of caloric intake studied, the high-fat diets (100 series) caused the formation of more tumors than did the corresponding equicaloric low-fat diets (200 series).

The questionable existence of a difference in tumor formation between groups restricted only in carbohydrate and the corresponding groups restricted in all components will be taken up in the discussion.

In Fig. 4 the incidence of mammary tumors as a function of both caloric intake and composition (type of restriction) is graphically represented.

DISCUSSION

The results of Experiments 3 and 4 confirm the finding (11) that the inhibitory effect of caloric restriction *per se* (carbohydrate only) on the formation of spontaneous mammary and induced skin tumors of the mouse is dependent on the actual degree of restriction.

TABLE IVB: FORMATION OF SPONTANEOUS MAMMARY TUMORS (C3H VIRGIN FEMALE MICE) AS AFFECTED BY COMPOSITION OF DIET AND CALORIC INTAKE

Group	Diet characteristics: Mean caloric intake	Diet characteristics: Relative * amount of "essentials"	Diet characteristics: Per cent fat	Number † of mice (effectual total)	Mice developing mammary tumors by 64 weeks of age: Number	Per cent	Age at appearance (weeks): Range	Age at appearance (weeks): Mean	Mice tumor-free and alive at 64 weeks of age
h33a	11.7	1	2	30	16	53	35-59	48 ± 1.5	14
h33b				30	19	63	35-64	45 ± 1.6	9
h28	10.0	1	2	30	8	27	41-63	52 ± 2.6	18
h26	9.2	1	2	30	2	7	45-47	46 ‡	25
h24	8.5	1	2	30	0	0			19
h22	7.8	1	2	29	0	0			22
h228	10.0	0.86	2	30	12	40	46-61	54 ± 1.4	18
h226	9.3	0.77	2	28	4	14	29-63	50 ‡	20
h224	8.5	0.73	2	29	0	0			24
h222	7.8	0.68	2	28	0	0			19
h133	11.9	1	18	30	27	90	22-54	42 ± 1.6	2
h128	10.1	0.86	18	30	20	67	27-64	50 ± 2.3	10
h126	9.2	0.77	18	30	18	60	33-64	50 ± 2.5	10
h124	8.5	0.73	18	29	5	17	41-63	53 ‡	18
h122	7.8	0.68	18	28	1	4	37		24

* Relative amounts of protein, vitamins, and minerals: h33 = 1.
† Number of mice alive in group when first tumor appeared in experiment: mice 22 weeks old.
‡ Little significance can be attached to these means because of the small number of tumors.

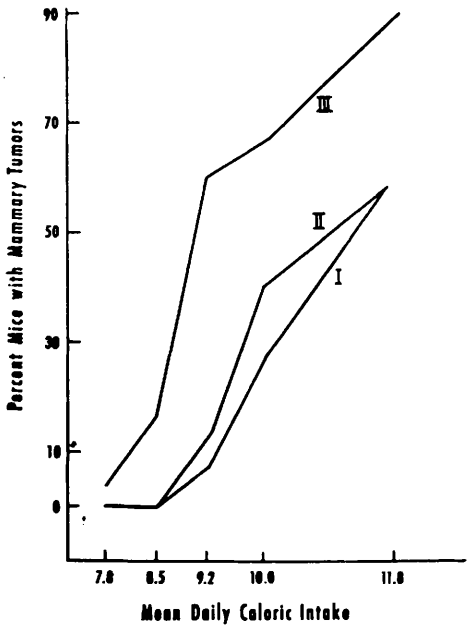

FIG. 4.—Incidence of spontaneous mammary tumors in C3H virgin female mice (at 64 weeks of age): dependence on composition of diet and caloric intake. Curve I—diets varying in carbohydrate content (caloric restriction *per se*); Curve II—diets varying in all components (with low-fat content); Curve III—diets varying in all components but with high-fat content.

This relationship obtains whether the calorie-restricted rations are: (a) restricted only in the amount of carbohydrate (caloric restriction *per se*); (b) restricted in essentials (protein, vitamins, and minerals) as well as in carbohydrate (low-fat content); or (c) restricted in essentials and carbohydrate, but with high fat content. In addition, the average time of appearance of the tumors is also dependent, in general, on the degree of restriction: The greater the caloric restriction the later the tumors appear.

In Experiment 4 the curves (Fig. 4) showing the relationship between tumor formation and caloric intake for the 3 different types of diets appear to have a modified ∫ shape. This is in general agreement with the previous conclusions regarding caloric restriction *per se* (11). On the other hand, the comparable curves of Experiment 3 (Fig. 3) appear to be linear, but this is probably due in part to the fact that fewer levels of caloric intake were studied.

Increasing the fat content of an *ad libitum* ration enhances the production of spontaneous mammary and induced skin tumors of the mouse. In this communication (Experiments 2, 3, and 4) it is further shown that there continues to exist an augmenting effect of a high-fat diet even at restricted levels of caloric intake.

Lavik and Baumann (1) investigated the differential effects of low-fat and high-fat diets at 2 levels of caloric intake, normal and restricted, on the production of skin tumors by methylcholanthrene. The incidence at each level of caloric intake was somewhat higher on the high-fat diet than on the corresponding low-fat diet. They interpreted this increase as possibly due to local contact of the fatty rations with the skin. Furthermore, they suggested that "much of the systemic or cocarcinogenic activity of dietary fat, if not all, is exerted through the medium of a voluntarily increased

intake of calories on diets high in fat." Our experiments do not support this view.

In earlier publications (9, 10) we have stated that the ingestion of a high-fat diet augments the formation of certain types of tumors mainly through a cocarcinogenic action on the developing tumor cell. Mice fed high-fat diets consumed more calories, possibly because of the greater compactness of the high-fat diet, and the augmentation in tumor formation may have been partly dependent on the increase in caloric intake. However, in the present experiments, such situations were obviated by the use of equicaloric diets which were fed at restricted levels where the mice consumed all food given them. The equicaloric consumption of the diets is evidenced by the rough equality in mean weight of corresponding groups. In every case where equicaloric diets were fed, the animals receiving the high-fat ration developed a higher incidence of tumors. Moreover, in the case of spontaneous mammary tumors, where mechanical contact with the fatty ration can not logically be assumed to be a factor, the augmenting effect of increased dietary fat is most pronounced; so great, in fact, that the groups in which the mice were fed diets approximately 85 per cent of the *ad libitum* caloric intake, but with a high-fat content, developed as many tumors as, or more than, the control groups on the *ad libitum* low-fat diet (T15 and T2; h128 and h33a or b).

Thus the evidence confirms our previous concept, that the enhancing effect of a high-fat diet on the formation of spontaneous mammary and chemically induced skin tumors is due mainly to some direct property of fat. What is important is that there exists an action of fat independent of a general caloric effect that might be produced by the added consumption of the whole ration, carbohydrate, or fat.

In contrast with the definite increase in tumor formation produced by increasing the dietary fat at various caloric levels, there is only a questionable difference between the inhibitory effect of diets prepared by limiting essential components (as well as calories) and that produced by diets limited only in the carbohydrate component (caloric restriction *per se*). In Experiments 1, 2, and 4 it appears that caloric restriction *per se* (restriction in carbohydrate only) is more effective in inhibiting tumor formation than is the restriction of essentials as well as calories. Even though Experiment 3 does not support this viewpoint and, in general, the differences are not of great magnitude, it is probable that a difference does exist. This is seen more definitely in the experiments on spontaneous mammary tumors than in those utilizing induced skin tumors. In all our studies on the consequence of caloric restriction or high-fat diets on the formation of tumors we have observed that the effects were of greater magnitude with spontaneous mammary tumors than with induced skin tumors.

There are probably many modifying factors in experiments designed to test whether diets restricted in carbohydrate only or in essentials as well as calories have differential effects on tumor formation. Possible factors are: the type of tumor studied; the carcinogenicity of the inciting agent, and dosage; the composition of the control *ad libitum* diet; and the extent of the differences in essential components. In Experiments 1 and 2, for example, where larger differences in tumor formation were observed, the diets restricted in essential components contained 40 per cent less essentials than did the carbohydrate-restricted diets; on the other hand, in Experiments 3 and 4 the differences in essential components were smaller: 14 per cent less at the 10 calorie level, and 27 per cent less at the 8.5 calorie level.

Thus the *composition* of the restricted diet as well as the *degree* of restriction is of importance in determining the extent of inhibition of tumor formation produced through caloric restriction. A restricted diet with a high-fat content definitely results in the least inhibition of tumor formation in comparison with either (a) an equicaloric diet that differs only in that it is low in fat, or (b) an equicaloric diet containing more protein, vitamins, and minerals than (a). Until more evidence is presented, one may accept the view that between the latter 2 types of restricted diets, there is a difference in the effects, often very slight.

The results reported previously (11) combined with those recorded in this communication suggest the tentative curves given in Fig. 5, indicating the relationship of tumor formation to both the *degree* of restriction and the *composition* of the restricted diet.

These curves have been drawn for spontaneous mammary and chemically induced skin tumors of the mouse. They may be valid for other types of tumors also, but certainly not for all. For example, it is known (9) that a high-fat diet has little or no effect on the formation of spontaneous lung tumors of the mouse. Therefore the 3 curves (Fig. 5) for that particular type of tumor may, in reality, be reduced to only 2 separate curves or even one. It has been suggested also (9) that the induction of sarcomas by hydrocarbons may actually be inhibited by a high-fat diet; in this case the 3 curves may have entirely different relationships.

It is realized that both the experiments and interpretations regarding the effects of the degree of caloric restriction and the composition of the restricted diet refer to the effects produced by the *ingestion* of the various diets. The consequent differences in absorption, interconversion of fats and carbohydrate, sparing mech-

anisms,[3] and intermediary metabolism in general, are not sufficiently defined at present to afford an explanation for the observed effects on tumor formation.

At this time it seems advisable to summarize some of the evidence regarding a possible role of nutrition in the prevention of human cancer. Included are: (a) the inhibiting effect of caloric restriction on the formation of induced skin tumors (1, 5, 8), induced sarcoma (5, 8), spontaneous mammary tumors (5, 8, 12, 13), spontaneous lung tumors (5), and induced and spontaneous leukemia (4, 13) of the mouse, and spontaneous tumors of the rat (3); (b) the fact brought out in this communication, that some inhibition of the formation of induced skin and spontaneous mammary tumors is obtained even with reasonable degrees

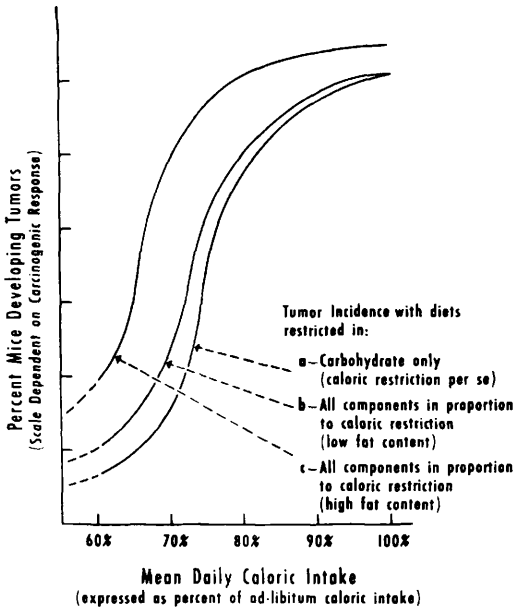

FIG. 5.—*Tentative composite curves* showing relationship of tumor incidence (spontaneous mammary and chemically induced skin tumors) to both level of caloric intake and composition of diet. Based on experiments in this communication and those of preceding paper (11).

of caloric restriction; (c) the inhibiting effect of a low-fat diet on the formation of induced skin tumors (1, 9), spontaneous mammary tumors (9), and spontaneous hepatoma (this paper); (d) the existence of a direct relation between the frequency of mammary carcinoma of the mouse and the average weight, the latter being greater in tumor-bearing mice (2); and (e) the data obtained from a study (6) of insurance statistics, indicating that overweight persons have a greater tendency to develop cancer.

[3] Phenomena like the action of carbohydrate in reducing the need for protein.

These observations support our previous conclusions (6, 7, 8, 10) that the avoidance of overweight through calorie-restricted and low-fat diets may aid in preventing many types of cancer in man, or at least delay the onset.

SUMMARY

The present experiments on spontaneous mammary and induced skin tumors in mice were performed to gather more data regarding the effect on tumor formation of the *degree* of caloric restriction, and at the same time to ascertain whether or not there is a differential effect due to the *composition* of the diet (at various caloric levels).

Further evidence was obtained that caloric restriction inhibits the formation of tumors, and that a high-fat diet enhances the formation of the types of tumors studied. This finding refers not only to the incidence of tumors but also to the average time at which they appear. As an incidental finding it appears that the spontaneous hepatoma of C3H male mice is affected by both caloric restriction and a high-fat diet, in a manner similar to the other tumors studied.

It is confirmed that the formation of tumors (incidence and average time of appearance) is dependent on the degree of caloric restriction *per se*. Evidence is presented that this is true also for diets restricted in essentials as well as calories (high or low in fat).

At a particular restricted caloric intake, a high-fat diet is less inhibitory than a diet with a low-fat content. It is also possible that a diet restricted in carbohydrate only inhibits slightly more than an equicaloric diet containing less essential components. Thus tumor formation is dependent on the *composition* of the diet, as well as on the *degree* of caloric restriction.

Evidence is presented that the enhancing effect on tumor formation of a high-fat diet, *ad libitum* or restricted, is due mainly to some specific action of fat. This action is independent of a general caloric effect that might be produced by the added consumption of the whole ration, carbohydrate, or fat.

REFERENCES

1. LAVIK, P. S., and BAUMANN, C. A. Further Studies on the Tumor-Promoting Action of Fat. Cancer Research, **3**:749-756. 1943.
2. LOEB, L., SUNTZEFF, V., BLUMENTHAL, H. T., and KIRTZ, M. M. Effect of Weight on the Development of Mammary Carcinoma in Various Strains of Mice. Arch. Path., **33**:845-865. 1942.
3. MCCAY, C. M., ELLIS, G. H., BARNES, L. L., SMITH, C. A. H., and SPERLING, GLADYS. Chemical and Pathological Changes in Aging and after Retarded Growth. J. Nutrition, **18**:15-25. 1939.
4. SAXTON, J. A. JR., BOON, M. C., and FURTH, J. Observations on the Inhibition of Development of Spontaneous Leukemia in Mice by Underfeeding. Cancer Research, **4**:401-409. 1944.

5. Tannenbaum, A. The Initiation and Growth of Tumors. Introduction. I. Effects of Underfeeding. Am. J. Cancer, **38**:335-350. 1940.
6. Tannenbaum, A. Relationship of Body Weight to Cancer Incidence. Arch. Path., **30**:509-517. 1940.
7. Tannenbaum, A. Nutrition and Cancer. Bull. Am. Soc. Control Cancer, **23**:3-4. 1941.
8. Tannenbaum, A. The Genesis and Growth of Tumors. II. Effects of Caloric Restriction per se. Cancer Research, **2**:460-467. 1942.
9. Tannenbaum, A. The Genesis and Growth of Tumors. III. Effects of a High-Fat Diet. Cancer Research, **2**: 468-475. 1942.
10. Tannenbaum, A. The Dependence of the Genesis of Induced Skin Tumors on the Fat Content of the Diet during Different Stages of Carcinogenesis. Cancer Research, **4**:683-687. 1944.
11. Tannenbaum, A. The Dependence of Tumor Formation on the Degree of Caloric Restriction. Cancer Research, **5**:609-615. 1945.
12. Visscher, M. B., Ball, Z. B., Barnes, R. H., and Sivertsen, I. The Influence of Caloric Restriction upon the Incidence of Spontaneous Mammary Carcinoma in Mice. Surgery, **11**:48-55. 1942.
13. White, Florence R., White, J., Mider, G. B., Kelly, Margaret, G., and Heston, W. E. Effect of Calorie Restriction on Mammary-Tumor Formation in Strain C3H Mice and on the Response of Strain dba to Painting with Methylcholanthrene. J. Nat. Cancer Inst., **5**:43-48. 1944.

The Dependence of Tumor Formation on the Composition of the Calorie-Restricted Diet as Well as on the Degree of Restriction

Albert Tannenbaum

Cancer Res 1945;5:616-625.