Tumor Incidence Patterns and Nutrition in the Rat [1]

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ABSTRACT Levels of caloric and of protein intake were demonstrated to have a modifying influence on tumor incidence patterns in the male rat. The 5 uniform lifelong dietary regimens used differed only in allotments and intakes of protein (casein), carbohydrate (sucrose) or of total calories. Age-specific rate tables and tumor incidence ratios aided in assessment of the nutritional effects. Total tumor risk was directly and exponentially related to caloric intake, but time differences for development of each of the incidence patterns were related inversely to caloric intake. Among all the groups tumor incidences formed an exponential continuum when related to growth rate in early life and mature body weight. Within each dietary group, rats of heavier weight had greater tumor risk than lighter rats. Occurrence, the proportional incidence and the malignancy of certain tumors correlated with the level of protein intake. Malignant lymphomas were predominant in rats with high protein intake, whereas fibromas and fibrosarcomas predominated in rats with low protein intake. Tumor incidence patterns differed quantitatively, qualitatively or both. Thus, in 2 groups with identical caloric intake, risk for all tumor types was similar but the group with higher protein had a greater risk for malignancy. Rate patterns for benign tumors, but not for malignant tumors, were dependent upon the mortality rate patterns of their respective populations. Lowest incidence, greatest delay in time of occurrence, absence of malignant epithelial tumors and greatest life expectancy, were observed when intakes of protein, carbohydrate and total calories were low.

Different nutritional regimens affect the prevalence of many age-associated diseases, the life span of the individual and, as a result, mortality patterns of a population. A multidiscipline study, of which some aspects are presented here, was devised to assess the influence of diet upon degenerative disease and mortality in the rat. Particular emphasis was placed upon the chronological sequence of biochemical, biological and pathological events and processes over the entire life span. The interrelationships among diet, age and hepatic enzyme activity (1–6) suggested that the enzyme activity levels and patterns were related to processes of ageing and that modification of the rate of change of these and other tissue constituents by nutritional means would result in changing patterns of mortality and disease. It was learned that length of life was influenced not only by the degree of dietary restriction but also by change in the ratio of the protein and carbohydrate components of the diets (7). The shortest life expectancy was observed to be associated with the highest incidence of glomerulonephrosis, a condition noted commonly in rats fed a commercial diet ad libitum, whereas the greatest life expectancy and the lowest incidence of kidney lesions were found among rats whose intakes were low in protein, carbohydrate and calories (8).

The influence of long-term caloric restriction in reducing the incidence of tumors is well documented for the rat and the mouse (see reviews by Rusch (9), Tannenbaum and Silverstone (10), and White (11)). Experimental studies of the influence of diet on tumorigenesis have, for the most part, been concerned with induced or transplanted tumors, or with highly inbred mice predisposed to specific tumor types. In the rat, certain deficiencies have been shown to result in the appearance of specific tumors not normally observed in the strain of rats used, or to produce an increase in the incidence of specific tumors. The most notable of these

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involve the thyroid gland (12), forestomach (13, 14), maxilla (15), and liver (16).

Relatively few data are available on lifelong tumor incidence patterns of large closed or non-migrant populations of rats where non-food substances or known carcinogens have not been purposely added to the diet. It is the notable exception when pertinent information is cited in the literature regarding environmental conditions, size of population, or when statistical or actuarial analysis has been performed (17, 18).

This paper presents an evaluation using actuarial methods of analysis of the effects of 5 different lifelong, dietary regimens upon tumorigenesis and is particularly concerned with age-specific incidence, tumor type, and the quantitative contribution of these tumors to death patterns in the same population of rats reported upon earlier (7, 8).

MATERIALS AND METHODS

The rat. Approximately 1000 male rats of the Charles River S.D. strain were used in these studies. Breeders had been routinely vaccinated for *Salmonella enteriditis* and *Salmonella typhimurium* and the female breeders were never older than 7 to 8 months of age. The number in their litters averaged 12 to 14 at birth, but was reduced to, at most, 10 in the third day of life. Mortality among littermates, without respect to sex, was 5% during the first 20 days. On the twentieth day the animals were weaned, sexed and shipped; upon arrival on the twenty-first day they were immediately weighed and divided at random into 5 groups. The number of rats used in each of the 5 dietary groups, termed control, A, B, C and D, were 210, 210, 120, 210 and 195, respectively.

A detailed description of the care, environment and housing has been presented previously (7, 8).

Diet and feeding regimens. The "control" group was maintained with a commercial diet[2] ad libitum and consumption was measured daily. The 4 "experimental" groups were alloted purified diets (table 1) on a restricted basis. The components[3] of the purified diets were identical and the only variables were in intake of protein (casein), of carbohydrate (sucrose) and of total calories.

The dietary allotments permitted: Intakes high in protein — high in carbohydrate (dietary group A); intakes high in protein — low in carbohydrate (dietary group B); intakes low in protein — high in carbohydrate (dietary group C); intakes low in protein — low in carbohydrate (dietary group D).

The intake of protein was identical in rats of groups A and B, and in rats of groups C and D. In groups A and C, the individual daily intake was isocaloric. The carbohydrate intake was identical for rats receiving diets B and D. Rats of group D had the lowest caloric intake. The daily intake of all other constituents was identical. The feeding schedule was such that

[2] Purina Laboratory Chow, Ralston Purina Company, St. Louis.

[3] The authors acknowledge the generosity of the following organizations for their donations of ingredients used in the diets: sugar from the Sugar Research Foundation, New York; Mazola Oil from the Corn Products Company, New York; vitamins from Hoffmann-LaRoche, Inc., Nutley, New Jersey; Lederle Laboratories (Division of American Cyanamid Company), Princeton, New Jersey, and Merck Institute for Therapeutic Research, West Point, Pennsylvania.

TABLE 1

Composition of purified diets

	Diet A	Diet B	Diet C	Diet D
	%	%	%	%
Casein [1]	30.0	50.85	8.0	21.62
Sucrose	61.0	33.90	83.0	54.05
Corn oil [2]	5.0	8.47	5.0	13.52
Salt mixture (USP 12)	4.0	6.78	4.0	10.81
Vitamins and trace elements [3]				
Kilocalories/g	4.09	4.15	4.09	4.24
Kilocalories supplied by casein, %	29.3	49.0	7.8	20.4

[1] Vitamin-Free Test Casein, 89% protein nitrogen, General Biochemicals, Inc., Chagrin Falls, Ohio.
[2] Mazola Oil, Corn Products Company, New York.
[3] For vitamin and trace element content of diets, see reference (7).

the amount allotted differed according to the diet fed and to the age of the rat. The detailed feeding schedule and its rationale have been described earlier (5, 7).

The precise amounts of each ingredient consumed when maximal food allotments had been reached are given in table 2, and these amounts were maintained for the remainder of the rats' lives. The cumulative caloric intake for each of the groups is shown in table 3.

Pathological examination. Two hundred and ninety rats were chosen at random at 7 different age periods (between 100 to 995 days) and were killed for biochemical, pathological and cytological studies. All other rats were observed until their "natural" death dates.

A thorough necropsy was performed on every rat as soon after death as was practicable; pieces representing normal and diseased tissue (at least 25) were routinely taken from all animals and fixed in Bouin's fluid, as well as occasionally in buffered formalin. Histological sections obtained from the paraffin-blocked tissues were routinely cut at 6 μ and stained with hematoxylin and eosin, and special staining procedures were used as required. The gross necropsy observations accompanied the sections submitted to the histopathologist but data as to age and diet were withheld.

The nomenclature used conforms to the International Classification of Disease (19).

Analysis of data. Tumor data were organized so as to permit actuarial analysis according to the methods described by Dublin et al. (20), as well as to those

TABLE 2

Maximal food allotments of purified diets [1]

	Diet A	Diet B	Diet C	Diet D
	g/day/rat	*g/day/rat*	*g/day/rat*	*g/day/rat*
Casein	4.3	4.3	1.1	1.1
Sucrose	8.7	2.8	11.9	2.8
Corn oil	0.7	0.7	0.7	0.7
Salt mixture (USP 12)	0.6	0.6	0.6	0.6
Vitamins and trace elements [2]	—	—	—	—
Total food allotted	14.3	8.4	14.3	5.2

[1] Allotments from 685 days of age.
[2] Daily allotments of vitamins and trace elements identical for each rat.

TABLE 3

Cumulative caloric intake

Age of rats [1]	Dietary group				
	Commercial	A	B	C	D
days	*kcal*	*kcal*	*kcal*	*kcal*	*kcal*
99	5174	2344	1410	2344	898
199	13028	6690	4013	6690	2569
299	20828	11527	6907	11527	4422
399	28517	16894	10121	16894	6477
499	36256	22599	13505	22599	8669
599	43463	28325	16991	28325	10873
699	51159	34077	20447	34077	13090
799	58971	39925	23933	39925	15337
899	66262	45774	27419	45774	17584
999	72586	51623	30905	51623	19831
1099		57471	34391	57471	22079
1199		63320	37877	63320	24326
1299		69169	41363	69169	26573
1399		75018	44849	75018	28820
1499			48335		31067
1599					33315
1699					35562

[1] Exclusive of first 21 days.

methods recommended by our actuarial consultants.[4]

All data and derived computations are presented on the basis of the presence of a tumor at time of death. Tumor data obtained from the 290 animals killed were not used in the actuarial treatment, and population size was adjusted accordingly.

All age-specific tumor incidences were calculated on the basis of 100-day periods, using the number of rats having histologically confirmed neoplasia at time of death against the number of living rats entering that age period.

The relative tumor incidence ratios were derived because such values offer a valid basis for comparisons of incidence patterns of one dietary group of animals with those of others, particularly where initial population size differs among the groups or where, due to differences in mortality patterns, the size of the population at risk differs at different periods.

Computation of the tumor incidence ratio value (TIR)[5] was made by dividing the actual number of tumors observed at each age period in an experimental group by the expected number of tumors that would have occurred had that group experienced the same risk as a standard population. The age-specific tumor rates of the group of rats fed the commercial diet were used as "standard rates." Such ratios are determined for individual age periods to ascertain changes in trend with the passage of time; a broader age scale was also used where TIR values were calculated for the first 10 periods and for the remaining periods. To emphasize similarities or differences between total tumor incidence patterns of the different groups, however, ratios were obtained from the sum of the complete data for all age periods; namely, total, actual tumor incidence and total, expected incidence. This single value allowed quantitative expression of the difference in tumor incidence patterns as they are affected by different dietary regimens. All ratio values were expressed relative to the value for the control group which was 100. The "ratio of the corrected rates" used by Murray and Hoffman (21) were also applied.

Instantaneous relative growth rates were computed according to the method described by Brody (22) at age periods of 21 to 49, 98 and 189 days of age.

RESULTS

1. Tumor incidence in the total population. One hundred and forty-eight tumors were found in 138 rats among the 934 used for this study. Ten of these rats had more than one type of tumor. Of the 290 rats killed during the course of the experiment, six had tumors. Among those rats that were permitted to live out their lives, 132 rats (20.5%) showed histopathological evidence of neoplasia.

The largest number of cases, 25, occurred in the 900- to 999-day period, representing 19% of the total number of tumor-bearing rats (fig. 1).

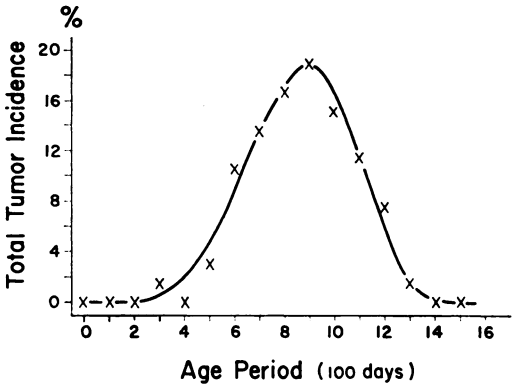

Fig. 1 Tumor incidence as percentage of total population (corrected for rats killed).

Except for the last period, the increase in age-specific incidence rate was exponential in character, with the acceleration being a function of the logarithm of age. Thus, to maintain the trend in acceleration of tumor incidence of this total population, progressively longer time-periods were required to maintain the same rate changes in chronologically older rats.

[4] Actuary Department, New York Life Insurance Company, New York.

[5] $\text{TIR} = \frac{(c_x)}{(c'_x)} \times 100$

where

$c'_x = (\bar{C}_{x_1} \times 1_{x_1}) + (\bar{C}_{x_2} \times 1_{x_2}) + (\bar{C}_{x_3} \times 1_{x_3}) \ldots\ldots$

c = actual number of tumor-bearing rats.

c' = expected number of tumor-bearing rats.

$\bar{C}_{x_{1,2,3}\ldots}$ = age-specific standard tumor rate, consecutive periods.

$1_{x_{1,2,3}\ldots}$ = exposure (i.e., number of living rats entering period), consecutive periods.

2. *Influence of diet upon tumor incidence.* A. Total incidence: the largest number of rats bearing tumors and the greatest number of tumors (10 rats had more than one tumor) were observed in that group whose intake was high in both carbohydrate and protein (group A), (see table 4). Considerably fewer tumors were noted in that group whose total intake was isocaloric to group A but which had a greatly reduced protein intake (group C). On the basis of an equivalent, initial population size, the smallest number of rats bearing tumors was found among that group whose intake was low in both protein and carbohydrate and which concomitantly had reduced intakes of calories (group D). Nearly twice as many tumors were found, however, in that group (B) whose intake of carbohydrate was similar to group D but whose intake of calories and protein was higher. Similar numbers of rats with tumors were found in that group of rats fed the commercial ration and in those of group C.

B. Age specificity: In each group the first tumor found was malignant, and the first tumor in any group was found at 345 days of age in a rat fed the commercial ration. In rats fed diets A and C, tumors were observed first in the 500- to 599-day period, whereas in rats fed diets B and D there was a delay in tumor appearance until the sixth period. Differences in time of occurrence among these groups became accentuated with advancing age, so that the time period in which 50% of the tumors in each group had been found was period 7, 8, and 9, and 10 for the control, A, B, C and D groups, respectively.

The distribution curves of deaths of rats with tumors at all age periods could be obtained only for the control, A and C groups and their maximal incidences occurred during the eighth, ninth and tenth 100-day periods, respectively. Because of the low incidence in tumors in groups B and D, significant distribution assessments cannot be made.

Figure 2 shows the tumor incidences arranged on an accumulated basis and adjusted for equal, initial population sizes and expressed as percentage of the total number of tumor-bearing rats. The individual curves were generally similar in contour but differed from each other in slope and in their displacement in time. The proportion of the total incidence was 22.5, 29.5, 15.8, 23.7 and 8.6% for the control, A, B, C and D groups, respectively.

The displacement in time for each of the curves (fig. 2) afforded an estimate of the time differences between groups in the development of each incidence curve: the curve for the control rats was used as a baseline, or standard, and was assigned a value of 1 (fig. 2); the remaining curves were adjusted so as to afford their superimposition upon the control curve by the use of the displacement factors 0.81, 0.72, 0.85, 0.62 for A, B, C and D, respectively. Although there was a large difference in the total number of tumor-bearing animals in groups A and C, the factors were nearly the same for the 2 groups, the members of which were fed on an isocaloric basis.

TABLE 4

Size of populations and total number of tumors

Dietary group	No. in initial population	No. in initial corrected population [1]	No. of rats killed	No. of rats bearing tumors	No. of tumors observed	No. of rats with 2 or more tumor types	No. of rats with metastatic tumors	No. of killed rats bearing tumors	No. of rats with tumors used in actuarial treatment of data
Commercial	210	208.6	50	32	34	2	11	1	31
A	210	209.3	60	49	53	4	19	2	47
B	120	119.4	60	10	10	—	2	1	9
C	210	208.4	60	36	40	4	11	2	34
D	195	188.7	60	11	11	—	5	–	11
Total	945	934.4	290	138	148	10	48	6	132

[1] Adjusted for accidental deaths and proportion of time alive in population.

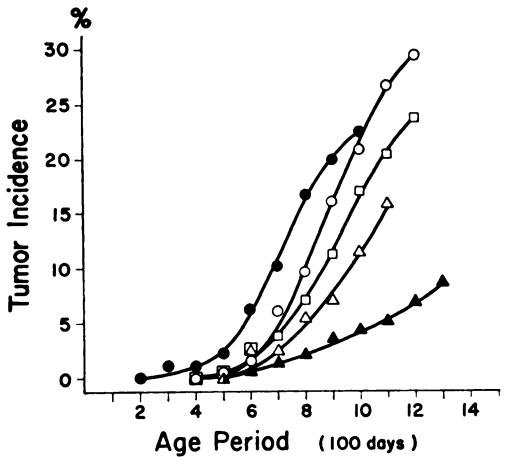

Fig. 2 Influence of diet on cumulative increase in tumor incidence with age, expressed as percentage of the total number of tumors. Key: ●, commercial laboratory chow; ○, high casein-high sucrose intake; △, high casein-low sucrose intake; □, low casein-high sucrose intake; ▲, low casein-low sucrose intake.

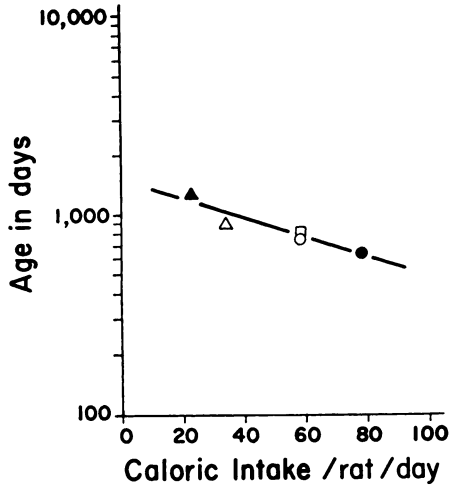

Fig. 3 Correlation between age when tumor incidences were identical, and caloric intake among the different dietary groups. Key: ●, commercial laboratory chow; ○, high casein-high sucrose intake; △, high casein-low sucrose intake; □, low casein-high sucrose intake; ▲, low casein-low sucrose intake.

However, 2 groups which have approximately the same number of tumor-bearing rats (controls and C) show a difference of about 15%. Not only did dietary group D have the smallest number of tumors, but it had the longest interval between time of occurrence of each tumor.

The age when each group contributed the same percentage (7.5%) to the total number of tumors in the entire population ranged from 630 days for the control group to 1260 days for group D. These times also correlated with the caloric intake of the animals as an inverse power function of age (fig. 3), namely, the lower the caloric intake the greater the delay in appearance of each tumor.

C. Age-specific tumor rate: Complete age-specific tumor rates are shown in table 5, and the tumor rate curves of all 5 groups were non-linear. The change in tumor rate for the control group increased exponentially and uniformly with age. The other tumor rate curves, by contrast, were not only displaced toward later ages but each of these groups had its own characteristic and significantly different tumor rate pattern. With the exception of period 9 for group C, at no single time-period was there an age-specific rate among the experimental groups higher than that observed in the control group, even though the largest total number of tumors was found among the rats fed diet A.

D. Caloric intake relationship: There was an exponential relationship between tumor incidence ratio (TIR) values (all periods) and the daily caloric intake of rats in each of the 5 groups. Rats of group D consumed approximately one-third of the total calories consumed by those rats fed the commercial diet but their TIR values were only 7% that of the latter group. The relative increase in tumor risk, therefore, became progressively larger with increasing levels of caloric intake. The similarity of the relationship between TIR values for all periods and caloric intake was also evident in the relationship between the TIR values and caloric intake for the first 10 age-periods (table 6).

Although there were nearly 40% more tumors in rats fed diet A than in rats fed diet C, the TIR values for these 2 groups was almost the same. The larger number of tumors of group A is explained by the difference in life expectancy. This is shown in table 7 where the population of group C was expanded at each age period to equal that of group A. This recalculation resulted in an expected total number of tu-

TABLE 5

Diet and age-specific tumor rates ($\bar{C}_x$) [1]

Age periods	Dietary group				
	Commercial	A	B	C	D
days	$\bar{C}_x$	$\bar{C}_x$	$\bar{C}_x$	$\bar{C}_x$	$\bar{C}_x$
21–99	0	0	0	0	0
100–199	0	0	0	0	0
200–299	0	0	0	0	0
300–399	0.012	0	0	0	0
400–499	0	0	0	0	0
500–599	0.014	0.006	0	0.006	0
600–699	0.050	0.013	0.027	0.021	0.008
700–799	0.065	0.055	0	0.024	0.008
800–899	0.191	0.052	0.041	0.053	0.010
900–999	0.267	0.124	0.026	0.104	0.025
1000–1099	0.500	0.143	0.100	0.250	0.020
1100–1199	—	0.276	0.143	0.267	0.027
1200–1299		0.444	0	0.800	0.091
1300–1399		0	0	—	0.250
1400–1499		—	0		0
1500–1599			—		0
1600–1699					0
1700–1799					—

[1] $\bar{C}_x$ (spontaneous tumor rate) is the ratio of the number of rats demonstrating neoplasia at death to the number of living rats entering that period. The exposure is corrected for accidental deaths and those killed. Dash indicates entire population dead.

TABLE 6

Diet and relative tumor incidence ratios (TIR)

Dietary group	TIR	
	First 10 periods	All periods
	(× *100*)	(× *100*)
Commercial	100	100
A	37.9	35.8
B	17.6	13.9
C	37.3	37.9
D	7.2	7.0

mor-bearing rats nearly equivalent to that observed for group A. The TIR values of each dietary group did not correlate consistently with the percentage level of, the proportion of, or the intake of any dietary constituent consumed by the rats in that group.

The relationship between tumor risk and caloric intake can be extended to tumor risk and body weight parameters. The complete growth pattern for each group from day 21 of life of all rats, to the death of the last rat in each group, is shown in figure 4. Cessation of growth occurred at approximately 33 weeks of age for rats fed the commercial diet. Average mature weight at this time was 530 g and was maintained until the fiftieth week. Subsequently, the animals became more obese until their average weight reached a maximum of 612 g at 85 weeks. Body weights as high as 800 g were recorded for individual rats that did not have tumors. Food intake diminished for this group of rats after 85 weeks of age and roughly paralleled weight loss.

The growth rates of each of the experimental groups were different over a selected, early period of time (table 8) but the rate of change of these values also differed with increasing age. The growth curve for rats in group C was nearly linear for more than the first 60 weeks. Although consistent differences between the average body weight of rats in groups A and C were evident, these differences became much less after 145 weeks of age.

At any age period, individual weights of rats in each of the 5 dietary groups varied sufficiently to produce overlaps between adjacent groups. Although the amount of food offered was held constant for a considerable period of time after the sixtieth week, accumulation of body mass continued. Subsequently, nearly every individual rat in all 5 groups showed a decrease in weight and a linear proportionality was found among the 5 groups between the maximal weight and weight at death.

TABLE 7

Tumor incidence on an adjusted population basis

Age periods	Group A		Group C				
	No. of rats	No. of rats with tumors	No. of rats	No. of rats with tumors	Age-specific tumor rate	Adjusted [1] no. of rats	Expected no. of tumors
days					(× 100)		
21–99							
100–199							
200–299							
300–399							
400–499							
500–599	176	1	165	1	0.06	176	1.1
600–699	157	2	140.1	3	2.1	157	3.3
700–799	144.5	8	123.5	3	2.4	144.5	3.5
800–899	116	6	95.0	5	5.3	116	6.2
900–999	89.1	11	67.2	7	10.4	89.1	9.3
1000–1099	49	7	28.0	7	25.0	49	12.3
1100–1199	29	8	15	4	26.7	29	7.7
1200–1299	9	4	5	4	80.0	9	7.2
1300–1399	1	0	0	0	0	1	—
Total		47		34			50.6

[1] Adjusted to number of rats in group A.

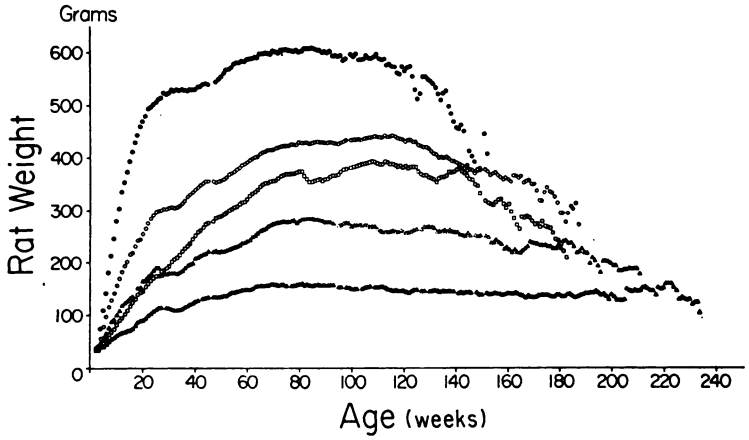

Fig. 4 Influence of diet on growth of rats and subsequent body weight changes with age. Key: ●, commercial laboratory chow; ○, high casein-high sucrose intake; △, high casein-low sucrose intake; □, low casein-high sucrose intake; ▲, low casein-low sucrose intake.

When the average weights of each of the experimental groups had reached a maximum (table 8) they were found to be related linearly to the cumulative caloric intake of that group although the time required to reach these weights differed. An exponential relationship existed between the maximal body weight of the group and its tumor prevalence.

The tumor incidence data were reorganized so that "new groups" were formed using the maximal body weights of the rats as the basis of segregation rather than dietary history. Age-specific tumor rate tables were derived, arbitrarily using that group of rats whose maximal weights ranged between 400 and 499 g as the standard for calculation of TIR values. The risk of tumor formation for the rats in the lowest weight class was less than 2% that of those in the heaviest weight class (fig. 5). Rats with maximal body weights approxi-

TABLE 8

Diet and body weight

Dietary group	Avg initial wt	Avg wt, 119 days	Growth rate [1]	Maximal avg wt [2]
	g	*g*	*K*	*g*
Commercial	39.4	429	0.024	610
A	37.5	232	0.019	440
B	36.6	145	0.014	282
C	38.2	130	0.013	394
D	39.3	80	0.007	167

[1] Computed as: Instantaneous relative growth rate (K)

$$K = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \text{ where}$$

W = weight in grams
t = age in weeks ($t_2 = 17$, $t_1 = 3$).

[2] Excluding rats that were ill and showed marked and moribund weight loss at that time.

mately 12% less than that of the heaviest group had a tumor risk at least 50% lower.

In addition, since rats within each group, consuming a single diet, attained different weights, they were divided into 2 subgroups on the basis of body weight, namely, a heavier and a lighter subgroup (at a time when maximal weight of each group had been reached). TIR values were calculated, using the age-specific rates of the entire control group as a standard. In every group but that fed diet C, the heavier subgroup had a greater TIR value than the lighter subgroup (table 9). Although each set of TIR values was exponentially related to the corresponding mean body weight of the subgroup, the slopes of these curves differed; the lighter members of each dietary group showed greater rate of increase in risk with increase in body weight than did the heavier members. That there was variation in response to the different dietary regimens was also evident (table 9)

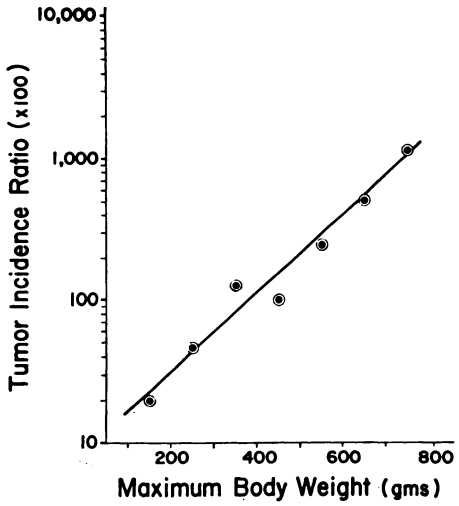

Fig. 5 Correlation between maximal body weight and tumor incidence ratio. (Rats with maximal body weights, 400 to 499 g, used as standard population for computation of TIR values.) Compared with the standard population, TIR values lower than 100 indicate the degree of beneficial effect and more than 100, the degree of deleterious effect.

since the 2 subclasses of group C showed no significant difference in TIR values while there was more than 109% difference in TIR values between the heavier and lighter members of group D.

Tumor prevalence was related to growth rate (fig. 6). When longer periods (first 200 days) were used in computing growth rates the deviation of group C in this relationship became negligible, even though these rats had attained only 50% of their growth.

TABLE 9

Body weights [1] and tumor incidence ratios (TIR) [2]

Dietary group	(1) Lighter wt subgroup		(2) Heavier wt subgroup		Increase in TIR values of subgroup 2 over 1
	wt range	TIR	wt range	TIR	
	g	(x 100)	*g*	(x 100)	%
Commercial	316–596	74.4	597–842	107.0	44
A	255–425	30.4	426–543	35.8	18
B	146–278	11.4	279–324	18.8	65
C	146–383	38.9	384–498	38.4	−1 [3]
D	100–158	4.7	159–192	9.8	109

[1] Division of groups made on the basis of individual rat weight at a time when average maximal weight of all rats had been reached.
[2] Computed, using age-specific tumor rates of the commercial dietary group as "standard rates."
[3] Negligible.

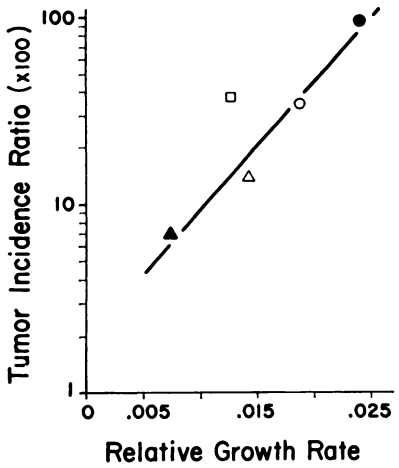

Fig. 6 Correlation between growth rate in early life and tumor risk (TIR) in later life. Key: ●, commercial laboratory chow; ○, high casein-high sucrose intake; △, high casein-low sucrose intake; □, low casein-high sucrose intake; ▲, low casein-low sucrose intake.

3. *Influence of diet upon tumor type.* Among the 148 tumors found in the total population, including the 6 observed in the animals killed, there were 28 taxonomic types (table 10). Malignant lymphoma was the predominant tumor found, with fibrosarcomas, fibromas and pancreatic islet cell tumors found in decreasing numbers in that order.

The major observations which indicated that nutrition has a marked influence upon tumor type, were: 1) occurrence in certain dietary groups of significant numbers of tumor types not observed in the other groups; 2) difference in "proportional" incidence of tumor types between groups; 3) difference in proportion of malignant-to-benign tumors; 4) difference in age-specific incidence patterns of specific tumor types.

Among the experimental groups, the greatest variety of tumor types was observed in those rats whose caloric intake was highest (groups A and C). Of all 5 groups, primary tumors of the lung were noted in rats of group A only, and accounted for 9.4% of the total number of tumors occurring in this group. Those groups with intakes high in carbohydrate (control, A and C) had malignant epithelial tumors to the extent of some 12% of the total number of tumors. However, malignant epithelial tumors were not found in groups B and D.

Malignant lymphomas predominated in the 3 groups of rats having high intakes of protein, whereas in the 2 groups with low intakes of protein (C and D), tumors of connective tissue, fibromas and fibrosarcomas were predominant (table 11). In addition, the incidence of pancreatic islet cell tumors was significantly higher in those groups of rats whose intake of protein was high.

Malignant lymphomas, tumors of subcutaneous tissue, pancreatic islet cell tumors and primary tumors of the lung showed an increase in rate with increasing age in those groups in which there were sufficient data to permit determination of age-specific rates. In the control group, malignant lymphomas, tumors of the subcutaneous tissue and pancreatic islet cell tumors were consistently displaced to earlier ages than in the other groups. The malignant lymphoma rate patterns of group C were displaced to later ages than that of group A, but for tumors of the subcutaneous tissue, this relationship was reversed.

The rate pattern for each tumor type conformed to a general trend within each dietary group with the exception of malignant lymphomas of rats in group A. The age-specific rate pattern for malignant lymphomas in these rats was significantly displaced to earlier ages compared with the patterns of the other tumor types of this group of rats.

A relatively high tumor risk for tumors of connective tissue (accounting for approximately 50% of the total TIR value for that group) was observed among rats with low protein intake, regardless of caloric intake (table 12). A relatively high tumor risk for malignant lymphomas (accounting for between one-third to one-fourth of the total TIR value) was noted for those groups (control, A and B) whose intake of protein was high regardless of the caloric intake. Tumors of the pancreas account for approximately 20% of the total risk incurred by those groups whose caloric intake was low regardless of the intake of protein.

Among the 142 tumors observed in rats permitted to live out their lives, 62 were

TABLE 10

Diet and tumor type

Tumor type	Total	Number of tumors				
		Dietary group [1]				
		Commercial	A	B	C	D
Malignant lymphoma						
Lymphosarcoma	4 } 34	—	3	—	—	1
Reticulum cell sarcoma	30 }	11	11	2	5	1
Connective tissue						
Fibrosarcoma	28	3	11	1	9 [2]	4
Fibroma	22	8 [2]	2 [2]	1	9	2
Skin						
Papilloma	2	1	—	—	1	—
Squamous cell carcinoma	5	—	3	—	2	—
Appendix	1	1 [3]	—	—	—	—
Pancreas						
Islet cell tumor	18	4	8	3 [2]	1	2
Adenocarcinoma	1	—	—	—	1	—
Adrenal cortical adenoma	3	—	—	2	—	1
Thymoma	2	—	—	—	2 [2]	—
Lung						
Adenoma	3 } 5	—	3 [2]	—	—	—
Carcinoma	2 }	—	2	—	—	—
Salivary gland adenocarcinoma	1	—	1	—	—	—
Liver						
Hepatoma	3	1	1	—	1	—
Cholangioma	1	—	1	—	—	—
Stomach adenocarcinoma	1	—	—	—	1	—
Kidney cortical adenoma	1	—	1	—	—	—
Testes	7	3	3	—	1	—
Mammary fibroadenoma	1	1	—	—	—	—
Retinoblastoma	1	—	—	1	—	—
Glioma	1	—	—	—	1	—
Lipoma	2	1	—	—	1	—
Rhabdomyosarcoma	2	—	2 [4,5]	—	—	—
Lymphangioma	3	—	1 [4]	—	2	—
Hemangioma	1	—	—	—	1 [6]	—
Unknown origin						
Anaplastic carcinoma	1	—	—	—	1	—
Carcinoma	1	—	—	—	1	—
Total	148	34	53	10	40	11

[1] Number of tumors not adjusted for differences in initial population size or for the number of rats killed at various ages.
[2] One case found in a rat that was killed.
[3] Hair follicles or hair matrix.
[4] Mesentery.
[5] Voluntary muscle.
[6] Mesenchymal tissues.

benign whereas 80 were malignant. The difference in proportional incidence of malignant to benign tumors (table 13) was most striking between groups A and C; there were twice as many malignant tumors as benign in rats of group A, whereas the incidences were equal in group C. Furthermore, the age-specific rate pattern of malignant tumors of group A was nearly consistently displaced to earlier ages than the benign pattern of this group or the malignant or benign pattern of group C. Since the numbers of benign tumors in each of these 2 groups were nearly equal, this difference in ratio emphasizes the greater malignant tumor risk suffered by

TABLE 11

Proportionate tumor incidence [1] and diet

Dietary group	Malignant lymphoma		Tumors of subcutaneous tissue	
	No. specific/ total tumors		No. specific/ total tumors	
		%		%
Commercial	10/33	30.3	11/33	33.3
A	14/51	27.5	12/51	23.5
B	2/9	22.2	2/9	22.2
C	5/38	13.2	17/38	44.7
D	1/11	9.1	6/11	54.6

[1] Tumor data from animals permitted to live out their lives.

TABLE 12

Tumor incidence ratios for specified tumor type

Tumor type [1]	Tumor incidence ratios [2]				
	Dietary group				
	Commercial	A	B	C	D
	(× 100)	(× 100)	(× 100)	(× 100)	(× 100)
Malignant lymphoma	35.6	10.7	3.1	5.6	1.3
Tumors of subcutaneous tissue	32.3	9.1	3.1	19.0	3.8
Pancreatic islet cell tumors	12.9	6.1	3.1	1.1	1.3
Primary tumors of lung	0	3.0	0	0	0
Others	25.9	9.9	4.6	16.7	0.6
Total	106.8	38.8	13.9	42.4	7.0

[1] All tumors included if more than one type was present in a rat.
[2] "Standard rates" = age-specific tumor rates of all tumor-bearing rats in the commercial dietary groups permitted to live out their lives.

TABLE 13

Influence of diet on proportion of malignant to benign tumors

Dietary group	No. of tumors [1]		Tumor incidence ratio [2]		Tumor incidence ratio
	Benign	Malignant	Benign	Malignant	Malignant-to-benign
			(× 100)	(× 100)	
Commercial	16	17	51.6	54.8	1.06
A	17	34	12.9	25.9	2.00
B	5	4	7.7	6.2	0.81
C	19	19	21.2	21.2	1.00
D	5	6	3.2	3.8	1.19
Total	62	80			

[1] In rats permitted to live out their lives.
[2] New age-specific rate tables organized for each group on the basis of malignancy. Tumor incidence ratio values computed using age-specific rates of entire commercial dietary group as standard rates. Values may exceed 100 since standard rates are based on incidence of tumor bearing animals, whereas these data consider every tumor type.

rats of group A. The TIR values obtained for the benign tumors and for the malignant tumors of each of these groups not only confirm this observation but also show a considerable decrease in relative benign tumor risk. When age-specific rates of the benign tumors and of malignant tumors of group C were used as the standard rates in deriving the corresponding TIR values of group A, the value obtained for the benign tumors of group A was less than that of group C by 57%, whereas the malignant tumor TIR value of group A was higher than that of the group C rats by 24%. The age-specific rate patterns of the benign tumors show an exponential trend related to age. For malignant tumors, however, the rate trends for groups A and C are directly and linearly related to age.

DISCUSSION

The exponential character of the relationship between tumor incidence (as expressed in TIR values) and caloric intake, indicates that the level of incidence for rats on any dietary regimen forms a continuum. A separation, therefore, cannot be made between the level of caloric intake at which caloric over-consumption, with its attendant, deleterious effects ends, and the level at which caloric restriction with its beneficial effects begins. The extremes in caloric restriction required under the conditions of the this study rule out the possibility that increasing beneficial effects could be expected by further restriction. As also reported by others (23, 24) the reduction in incidence achieved by caloric restriction is accompanied by a displacement of the tumor incidence pattern to later ages. It appears that these salutary effects upon tumor incidence are, in a large measure, attributable to a delay in time of onset of the incidence pattern to ages which chronologically approach or are well within the senescent period of life.

Group D rats not only had the greatest life expectancy (7) and the lowest tumor incidence, but had the greatest delay in time of development of the age-specific incidence pattern. Despite this, however, far fewer total calories were consumed by this group by the time the first tumor appeared than by any other group. In fact, all the tumors observed in this group had occurred when the cumulative caloric intake was less than that of the control group or of the 2 experimental groups with high caloric intake at the time when the first tumor death in any of these 3 groups was recorded. Those tumors that did occur in rats of group D may have had an altered dependency on caloric intake, or the caloric intake may have been so severely reduced that the effect of other "factors" became dominant.

The inseparable effect of long-term restriction in caloric intake upon body weight and upon tumor incidence has prompted the suggestion (24, 25) that inhibition of tumorigenesis may be mediated through mechanisms which are intimately involved with growth. Our results lend further emphasis to this correlation in that the TIR values were related exponentially to maximal body weight attained by each dietary group. The relationship between tumor incidence and maximal body weight exists irrespective of distinct dietary groups. In fact, within all dietary groups, except group C, higher tumor risks were noted for the heavier rats than for the lighter rats even though their caloric intake was identical. The rate of increase in risk with increasing caloric intake accelerates more rapidly for the lighter weight series than for the heavier series. Each subgroup with its different tumor risk may conceivably represent a somewhat different portion of the genetic spectrum of the entire group (26); the variation in their mature body weights suggests different abilities early in life to convert food substances into body mass. These variations in body weight characteristics, however, need not be solely of genetic origin since it has been shown that the nutritive condition in utero (27) and during the suckling period (28) has a marked effect on postweaning rate of development and on body weight.

The modification in variety and incidence of specific tumor types by the level of protein of the diet consumed is definite and striking with no two dietary groups having similar patterns. It is remarkable, however, that change in total number of specific tumor types and their TIR values is uniquely balanced and conforms

strictly to limits set by the total caloric intake only. The modification, by dietary means, of the incidence and type of specific tumors has also been shown for the rat in the data of Saxton et al. (24), and of Gilbert et al. (18); and for the mouse in the data of White and Andervont (29), Tannenbaum and Silverstone (30), and Silverstone and Tannenbaum (31).

The changes in incidence of different degenerative diseases, including specific tumor types brought about by a single measure, caloric restriction, can best be explained by postulating the existence of a common underlying mechanism:

Different, lifelong, dietary regimens have been shown to influence age-specific mortality rates differently at different age periods (7). The factors required to adjust the life expectancy pattern of each of the experimental groups to that of the control group were nearly identical to the factors necessary to make the same adjustment in total tumor risk patterns. Within any dietary population there is a signal uniformity in the sequence and proportion of time required for biological, biochemical and pathological events to occur (5–8), and the numerical factors in actuality must represent a quantitative expression of the difference in physiological conditions of the populations. Thus, the major differences at any one chronological age period, for each of these parameters, are most likely the result of the different rates of physiological ageing.

The contribution made by tumors toward mortality risk may be assessed by comparing the number of deaths of rats bearing tumors with total number of deaths or of number of rats dying without tumors. Differences in actual number of tumors may, in part, be due to: (a) the consequence of a "true" reduction in prevalence of tumors, (b) the result of a delay in time of appearance which resulted in a concomitant, subsequent reduction in prevalence, or (c) the result of more members of a population reaching older ages where the risk of incurring tumors was greater.

The increase in age-specific tumor rate was found to be proportional, logarithmically, to the existing mortality rate of that population (6). The patterns were nearly identical for four of the dietary groups but not for group D. Thus, the displacement of mortality patterns in time, and the differences in population size, led to differences in number of tumors but not in tumor probability at death.

In comparison of groups A and C. both having the same tumor risk, rats of group A with their longer life span, allowed more of its members a longer period of time in which to develop tumors. Similarly, when group C is compared with the control group there is a significantly lower risk for the longer-lived rats in group C even though numbers of tumors were equivalent between groups and tumor rate patterns had the same dependency upon mortality rate patterns.

Even though rats of group B were relatively long-lived, there was an apparent reduction in prevalence of tumors due to delay in time of onset, a subsequent difference in age-specific tumor rates and a difference in the duration of time in which tumors were found.

However, rats of group D that had the longest life expectancy, showed only one-third the tumor probability at death of the other groups. This may have been the result of time differences in rate of tumorigenesis. The relatively large number of deaths due to trichobezoars in this group, however, may have so altered this relationship that, had these rats survived, the entire group might have suffered tumor morbidity rates that were similar to those of the other groups.

The benign tumor morbidity rate increases strictly as a function of the mortality rate for each of the 5 groups. Each of these coincides one with the other. Ultimately, regardless of diet, all rats suffer a benign tumor risk of the same magnitude, although at different times. The discrete and consistent effects of the level of caloric intake of each group, therefore, disappears when the calendar is replaced as a basis of calculation by the age-specific mortality rate pattern. This also indicates that animals bearing benign tumors do not generally have their life spans shortened any more than the other members of their group.

Age-specific, malignant tumor morbidity rate, however, differs markedly in its rela-

tionship to age-specific mortality rate for each of the 5 groups, with the greatest malignant tumor risk at death in rats of group A and the lowest in rats of group D. Thus, with increasing mortality rate, after a rate of 0.1 has been reached, the corresponding increase in malignant morbidity rate is more than 3 times greater for rats of group A than that of rats in group D. Unlike the observations based upon specific rates or relative tumor incidence, that rats fed the control diet suffered the most deleterious effects, the above relationship places these rats in a position of risk of dying with malignant tumors, intermediate to those of groups C and D. This deviation cannot be explained on the basis that these control rats maintained their malignant tumors and their lives for longer periods of time than did the rats fed the other diets, since the age-specific morbidity-mortality rate pattern is consistently displaced to earlier ages. Regardless of the method of expression, the basis of calculation, the type of tumors that occur, or the degenerative disease under consideration, a regimen low in total calories, carbohydrate and protein consistently showed the most beneficial effects of any of the dietary groups.

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