

# Failure To Inhibit the Formation of Mammary Carcinoma in Mice by Intermittent Fasting\*

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Chronic restriction of caloric intake inhibits the formation of various types of tumors in the mouse. This inhibition is evidenced both by a decrease in the proportion of mice developing tumors and by a delay in the time at which the tumors appear (4-6, 8, 11, 15, 16). The magnitude of the inhibitory effect is dependent on the degree of caloric restriction (1, 10, 14), the type of tumor (11), the dosage or potency of the carcinogen (12), and on whether the restricted caloric intake is maintained during the stage of initiation or the stage of development of the tumor (9). In all these studies the daily dietary restriction of caloric intake was continued for months—in some instances for almost the whole life span of the mice. The question arose as to whether continued daily caloric restriction is necessary to inhibit the formation of tumors or whether comparable effects might be observed in animals subjected to intermittent fasting. The following experiment was planned to determine the effect of fasting for 24-hour periods twice weekly, with no other limitation of food intake.

## EXPERIMENTAL

One hundred and four female mice of the dba inbred strain, raised in our laboratory, were divided into two equal groups by random selection. The mice were 34 weeks of age, and each had delivered and raised one litter at about 3 months of age. They had been fed Purina laboratory chow since weaning. For the study they were housed in sets of five, in cages with solid bottoms.

The diet employed was composed of Purina Fox Chow meal, 50 per cent; skimmed milk powder, 25 per cent; and cornstarch, 25 per cent. Drinking water was available at all times. The mice of the control group, A 101, were fed *ad libitum* daily. Those of the experimental group, A 102, were

given the same diet, except that the food was withheld from them every Monday and Thursday (in addition, the cages were cleaned at about 9:00 A.M. on those mornings); on Tuesday and Friday mornings the mice of this group were given a double weight of the ration. Although the mice of group A 102 fasted 2 days during each week, their food intake was *ad libitum* on a weekly basis.

Each mouse was numbered and its course recorded separately. At biweekly intervals the animals were weighed and inspected for general appearance and neoplasms. All mice underwent autopsy at death or at the termination of the experiment. Tumors were recognized grossly; for confirmation, microscopic sections were made of a considerable proportion of the mammary carcinomas, as well as of the few metastases and other lesions found.

The experiment proceeded smoothly and was terminated when the few remaining mice were 113 weeks old.

The average daily food consumption during the study was 3.2 gm. per mouse for group A 101 and 3.1 gm. for group A 102. The over-all effect of the periodic fasting regimen on the mean body weight of the mice was a slight depression, as shown in Table 1.

TABLE 1  
EFFECT OF INTERMITTENT FASTING  
ON BODY WEIGHT OF MICE

GROUP*	AGE OF MICE, WEEKS					
	34†	40	50	60	70	80
	Mean body weight, gm.					
A 101	27	27	29	31	31	30
A 102	27	27	28	30	29	28

\* A 101, fed *ad libitum* daily; A 102 fasted twice each week for 24 hr.

† Experiment began when mice were 34 weeks old.

At various times during the experiment the mice of group A 102 were weighed just preceding the fasting period, at the end of the 24-hour fasting period, and again 24 hours after refeeding. The 24-hour fast resulted in an average weight loss of

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3 gm. (approximately 10 per cent of body weight). Within the first 24 hours of realimentation the mice approximated their pre-fasting body weight.

The effect on the mice of fasting twice weekly for 24 hours is shown in the cumulative tumor curves (Fig. 1); other comparative factors of tumor formation are given in Table 2.

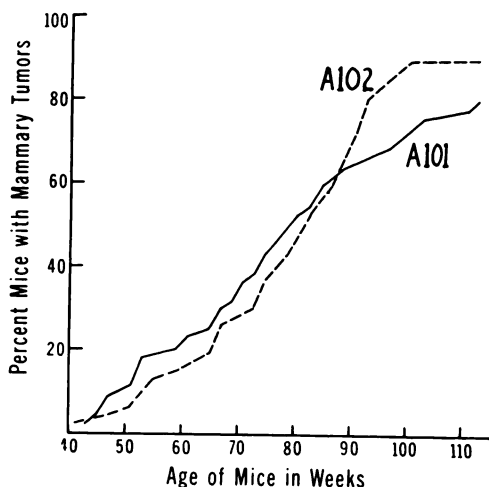


FIG. 1.—Cumulative curves showing formation of spontaneous mammary carcinoma. Group A 101 fed *ad libitum* daily; group A 102 fasted twice weekly for 24 hours and fed *ad libitum* between fasting periods.

TABLE 2

EFFECT OF INTERMITTENT FASTING ON INCIDENCE AND TIME OF APPEARANCE OF MAMMARY CARCINOMA IN DBA MICE

GROUP*	NUMBER OF MICE	MICE FORMING MAMMARY CARCINOMA		AGE AT TUMOR APPEARANCE, WEEKS		NUMBER OF MICE TUMOR-FREE AND ALIVE AT 113 WEEKS ‡
		(Number)	(Per cent)	(Range)	(Mean)	
A 101	44	35	80	43-113	74.5 ± 3.2	2
A 102	46	41	89	41-101	77.7 ± 2.4	2

\* A 101, fed *ad libitum* daily; A 102 fasted twice each week for 24 hr.

† There were 52 mice in each group initially; the number of mice indicated represents a calculated adjustment to correct for the deaths of nontumor mice during the experiment (2).

‡ End of experiment.

Both indices of tumor formation—per cent of mice forming mammary carcinoma and the mean time of appearance of the tumors—indicate that periodic fasting, under the experimental conditions outlined, had no inhibitory effect upon the formation of spontaneous mammary tumors. There was also no effect upon the proportion of tumor-bearing mice that developed multiple mammary tumors—31 per cent and 29 per cent for groups A 101 and A 102, respectively.

The mice that developed tumors were continued

on their particular dietary regimen, and the tumors were measured at 1-week intervals to determine their rate of growth. The periodic fasting regimen (imposed on the mice of group A 102) had no effect on the rate of growth of the tumors nor on the time of survival of the animals after tumor appearance (Table 3).

TABLE 3

EFFECT OF INTERMITTENT FASTING ON GROWTH OF MAMMARY CARCINOMA AND SURVIVAL OF TUMOR-BEARING MICE

GROUP*	GROWTH INDICES OF MAMMARY CARCINOMA †		SURVIVAL TIME, WEEKS ‡	
	(Range)	(Mean)	(Range)	(Mean)
A 101	1.4-13.5	6.2 ± 0.49	4-17	9.1 ± 0.62
A 102	1.2-15.4	5.8 ± 0.55	0-17	8.5 ± 0.66

\* A 101 fed *ad libitum* daily; A 102 fasted twice each week for 24 hr.

† Mean daily increment of sum of major and minor axes of tumor in units of 0.1 mm.; 32 tumor-bearing mice measured in A101; 35 in A 102.

‡ Interval between detection of the tumor and death of the animal.

## DISCUSSION

Mice that fasted for 24 hours twice weekly (Monday and Thursday)—eating *ad libitum* on the other days of the week—developed spontaneous mammary tumors in about the same percentage as mice fed *ad libitum* daily. This was not unexpected, inasmuch as the dietary regimen resulted in only a slight decrease in the over-all caloric intake and a slight retardation in the weight of the mice (13).

Actually, the group that fasted intermittently developed a slightly higher percentage of tumors than the controls, and this occurred through an increased rate of formation in the mice of group A 102 after they were 70 weeks old (Fig. 1). It is not concluded from this single experiment that this augmentation is real. Yet, the augmentation might have occurred through the stress on the tissues, particularly the liver, or because the animal's caloric requirement during the fasting period was obtained through the use of fat from its depots—in this period the mice might be considered to be on a high fat diet (7). Further experimentation, starting with younger mice, would be needed to clarify this possibility.

A study related to the present experiment is reported by Carlson and Hoelzel (3), who investigated the effects of intermittent fasting on the prolongation of life in rats. They concluded from incidental findings with relatively few animals that "the development of mammary tumors was retarded in proportion to the amount of fasting." However, while there appears to have been an effect in the rats that fasted 1 day in 2, tumor formation in those that fasted 1 day in 3 or 4 did not differ significantly from that in the controls.

On the basis of the present experiment and previous reports, it is our opinion that, while chronic caloric restriction strikingly inhibits tumor formation, intermittent or periodic caloric restriction does not have this effect unless the dietary regimen is such that the body weight of the animals is significantly less than that of the *ad libitum* controls.

#### SUMMARY

Intermittent caloric restriction—mice fasting twice weekly for 24 hours, followed by *ad libitum* feeding between fasting—did not materially affect the mean food consumption or mean body weight of dba female mice. There was no inhibitory effect on the incidence or rate of formation of spontaneous mammary carcinoma or on their subsequent growth.

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