APPARENT PROLONGATION OF THE LIFE SPAN OF RATS BY INTERMITTENT FASTING [1]

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ONE FIGURE

(Received for publication October 4, 1945)

INTRODUCTION

When a sufficient amount of choice food is available, laboratory rats, like many humans, eat enough to become more or less obese. As a consequence, the life span of rats feeding ad libitum, like the life span of their human counterparts, is presumably shortened. This inference is supported by the repeated findings of McCay and his associates ('42 a, b; '43) that the life span of rats can be considerably prolonged by a drastic restriction in their allowance of food. The findings of McCay and his associates practically constitute an experimental confirmation of the claims of Cornaro (Butler, '05) who attributed a considerable prolongation of his life to a rigid restriction of his food intake. However, since the time of Cornaro (1464–1566) no similarly prolonged and rigid voluntary restriction of the human food intake appears to have been recorded. Obviously, Cornaro's prolonged practice of food restriction has not been widely followed because a normal appetite tends to impel its more or less complete appeasement at reasonably frequent intervals, when sufficient palatable food is easily obtainable. Only short periods of food restriction, such as the religiously interdicted periods of food restriction or fasting of the past, would seem to be practical.

[1] This research was aided by a grant from Swift & Co., Chicago.

In the future however, a periodic practice of food restriction or fasting is likely to depend mainly on experimental evidence of its value. It therefore seemed of interest to determine whether periodic or intermittent fasting would serve to prolong the life span of rats or reduce or prevent the shortening of the life span which is presumably produced by feeding ad libitum.

A study was already made by Robertson, Marston and Walters ('34) of the effect of intermittent fasting on the life span of mice. In that study, twenty-four male and twenty-four female mice were fasted 2 successive days in 7. The average life span of the fasted males was found to be 745 days while that of twenty-four controls was 712 days. The average for the fasted females was 819 days while that of twenty-four control females was 773 days. However, the prolongation of life was not regarded as significant by Robertson and his associates. One criticism of their study is that littermate mice were not used as controls. Hence the individual life spans apparently varied too much to make the results seem significant. Another criticism is that no observations appear to have been made to determine whether the fasted mice remained free from peptic erosion or ulceration of the stomach and duodenum. This has been found to occur in some mice (and young rats) after single periods of starvation of 36 hours or more (Sun, '27; Hoelzel and Da Costa, '37).

The effect and after-effect of intermittent fasting on some aspects of growth and nutrition were also studied by von Seeland on chickens (1887), by Morgulis on salamanders ('13), by Kopec and Latyszwski on mice ('32) and by Kellermann on rats ('39) but the effect on the life span was not determined in any of these studies.

Observations previously made in this laboratory showed that rats fasted every other day and fed a diet low in protein between fasts developed peptic ulcers in the forestomach within about 2 weeks (Hoelzel and Da Costa, '32). However, rats fed a diet adequate in protein between single-day fasts usually remained free from peptic ulcers. With the use of a diet relatively high in protein, no complication with peptic ulceration was therefore expected to develop in rats fasted 1 day in 3 or 4 but some doubts were still entertained whether rats could be fasted 1 day in 2 during prolonged periods without peptic lesions developing. In man, ulceration of the stomach is far less likely to occur while fasting because of an apparently lower fasting gastric acidity and the absence of the forestomach. Fasting 1 day in 2 or 3 by man also is apparently not the equivalent of fasting 1 day in 2 or 3 by the rat. However, in personal experiments, one of us (H.) found it impossible to maintain normal energy or remain free from nutritional edema while fasting every other day during periods of 2 to 5 months (Hoelzel, '43) but fasting 1 day in 3 immediately after having fasted 1 day in 2 during 5 months led to a recovery of energy and disappearance of nutritional edema. It was also found possible to recover fully from a 33-day fast in less than 33 days (Hoelzel, '44). Under these circumstances, it was deemed advisable to try various amounts of fasting in determining whether intermittent fasting would prolong the life span of rats.

In addition to various amounts of fasting, it also seemed advisable to try several diets. As a result, this study became somewhat complicated by the number of variables involved. The object of the present communication, however, is to report only the results of intermittent fasting on the life span of rats, independent of the specific effects of the different diets that were tried.

METHODS

In this study, 137 rats (60 males and 77 females), raised in the laboratory from rats obtained from The Wistar Institute, were used. These were all of the rats in seventeen litters with two or more of one sex or both sexes raised. The seventeen litters consisted of fourteen first litters and three second litters, with from two to thirteen raised rats in the individual litters. The rats were not weaned completely (separated from their mothers) until they were 35 days old.

Three omnivorous diets and one vegetarian diet were used. The omnivorous diets were a basic diet and two diets with 10% bulk-formers added to the basic diet. The basic diet consisted of 61.5% cooked and dried "whole veal," 31% corn starch, 2% powdered yeast, 1% cod liver oil, 1.5% inorganic salt mixture and 3% veal bonemeal. This diet provided approximately 35% proteins. The cooked and dried "whole veal" included practically all of the edible parts of calves, excepting excess fat and blood.[2] The first lot of this prepared veal contained 52% protein and 40% fat. Less fat was included in the preparation of subsequent lots but the composition of the original lot was approximated by adding fat when the diets were prepared. The second omnivorous diet consisted of the basic omnivorous diet plus 10% finely ground alfalfa stem meal. The third omnivorous diet consisted of the basic diet plus 5% psyllium seed husks and 5% specially prepared kapoc. The kapoc was mechanically cleaned, ground, boiled, washed, partly bleached, again washed and dried. The vegetarian diet consisted of 50% whole wheat flour, 10% peanut flour, 7% lima bean flour, 7% wheat gluten flour (containing 80% gluten), 7% corn gluten meal, 7% linseed meal, 5% powdered yeast, 5% alfalfa leaf meal and 2% NaCl. This diet provided approximately 30% proteins. Lettuce trimmings were supplied practically daily as a supplement to all of the diets. The control rats and the intermittently fasted rats while fed were kept continuously supplied with food.

Before the rats were 42 days old, all of them were supplied with the same food. This included some of each of the four experimental diets. When the rats became 42 days old, they were distributed so that some littermates of the same sex served in littermate tests of the effect of intermittent fasting or different amounts of intermittent fasting while other littermates served in tests of the effect of the different diets. Some rats with more than one littermate of the same sex consequently served as one of the littermates in 2 or more kinds of littermate tests. The intermittent fasting included fasting 1

[2] This was specially prepared for us by Swift and Co., Chicago.

day in 4, 1 day in 3 and 1 day in 2. The fasting was begun at the age of 42 days and was continued until the rats died.

RESULTS AND DISCUSSION

Effect of intermittent fasting on the life span

Figure 1 shows the distribution of the life spans of the individual control and fasted rats in 25-day periods. The life spans of rats with littermates only in the same group (but on different diets) are included. The average life spans of the

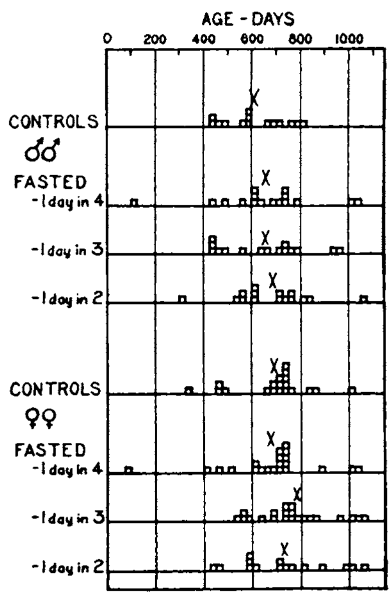

Fig. 1 Distribution of individual life spans of control and fasted rats in 25-day periods. Each square represents one rat. The average life span of each group is indicated by X. The life spans of rats with littermates only in the same group (but on different diets) are included.

groups are also indicated in figure 1 and in table 1. The results of the different amounts of fasting on littermates alone, with littermate controls fed identical diets ad libitum, are presented in table 2. Figure 1 and tables 1 and 2 show that, with the exception of the females fasted only 1 day in 4, the average life spans of all of the groups of fasted rats exceeded that of the controls. Moreover, the data in table 2 indicate that the prolongation of life by fasting was practically proportional to the amount of fasting and that the life spans of the males were, on the average, increased more than the life spans of the females. However, this may merely mean that the life spans of the males were shortened more than the life spans of the females by feeding ad libitum.

The effect of fasting on littermate rats is also indicated by comparisons between the life spans of rats fasted 1 day in 4 or 3 and littermates fasted 1 day in 3 or 2, respectively. Data thus obtained and combined with the data in table 2 yield a

TABLE 1

Average life spans of control and fasted rats. These averages include the life spans of rats with littermates only in the same group but on different diets. Figures in parentheses indicate number of rats in each group.

SEX	AVERAGE LENGTH OF LIFE IN DAYS				
	Controls	Fasted			
		1 day in 4	1 day in 3	1 day in 2	All degrees
Males	612 (14)	658 (16)	653 (15)	683 (15)	664 (46)
Females	688 (19)	675 (21)	781 (22)	733 (15)	730 (58)

total of thirty male littermate comparisons and show that the average life span of the males was increased 90 days by fasting. Similarly obtained data on females show that the average life span in forty-five littermate comparisons was increased only 23 days by fasting. It seems noteworthy that, in spite of the substantial increase in the average life span of the males by fasting, the life span of the fasted males only approximated that of the control females (fig. 1 and tables 1 and 2).

A more detailed analysis of the results suggests that fasting 1 day in 4 and 1 day in 2 were complicated more by some extraneous factors than fasting 1 day in 3 or feeding ad libitum. Thus, figure 1 shows that the earliest male and female deaths occurred in the groups fasted 1 day in 4 and the impression was that some of the other rats fasted 1 day in 4 also did not fare as well as most of the rats fasted 1 day in 3 or the controls. Perhaps the amount of food consumed in 3 days of feeding, with increased voracity but without proportionately increased capacity after 1 day of fasting, constituted a greater physiological overstrain than the amount of food consumed by the controls or by the rats fasted 1 day in 3. Figure 1 further shows that the males and females fasted 1 day in 2 also began dying earlier than the rats fasted 1 day in 3. Evidently fasting 1 day in 2 and beginning this at the age of 42 days was too much fasting for some rats. One of the females fasted 1 day in 2 apparently died of a hemorrhage from a chronic duodenal ulcer. Nothing like this was seen among over 2000 rats in a study of the production of peptic ulcers (Hoelzel and Da Costa, '37). More or less erosion and ulceration of the stomach was observed in other rats in this study but in most cases the lesions merely seemed to be due to premortal conditions, chiefly starvation due to loss of appetite associated with, or produced by, respiratory infections. (In about half of the rats with respiratory infections, no erosion or ulceration of the stomach occurs in spite of a complete loss of appetite or starvation and the usual postmortem changes in the intestines also are not seen. In such cases, all digestive secretions seem to be suppressed, excepting the secretion of a little bile.) Some erosion of the stomach may have occurred as a direct result of the experimental fasting among the rats fasting 1 day in 2 while they were still young. Moreover, female rats were previously found to develop more severe gastric lesions than males during prolonged starvation and a complication of this type may therefore explain why the females in this study did not benefit as much as the males from repeated single-day fasts. However, individual rats vary in their susceptibility to peptic erosion and ulceration and even some that were fasted 1 day in 2 evidently remained entirely free from such lesions. Both the male and the female that lived longest among the 137 rats (1057 and 1073 days, respectively) were rats fasted 1 day in 2. The optimum amount of fasting for the average rat in this study nevertheless appears to have been fasting 1 day in 3 and the data in table 2 show that with this amount of fasting the life span of the males was increased about 20% and that of the females about 15%.

Effect of intermittent fasting on growth

Table 2 shows that the average weights of the intermittently fasted rats at 300 days were always lower than the average weights of their littermate controls but no drastic retardation of growth was produced by the fasting. In some cases, the average femoral lengths of the fasted rats at death were greater than, or equal to, those of the controls and, in other cases, the rats were only a little smaller. In short, intermittent fasting seems to make it possible to increase the life span to some extent without stunting the rats. Tests are in progress

TABLE 2

Showing the effect of different amounts of fasting on the weight, size (length of femur at death) and life span of littermate rats. Littermate controls fed identical diets ad libitum.

AMOUNT OF FASTING	NUMBER OF PAIRS OF LITTERMATES	AVERAGE WEIGHT (GM)				AVERAGE FEMORAL LENGTH AT DEATH (MM)		AVERAGE LENGTH OF LIFE (DAYS)		
		At 42 days		At 300 days						
		Controls	Fasted	Controls	Fasted	Controls	Fasted	Controls	Fasted	Difference due to fasting
Males										
1 day in 4	7	149	149	449	413	39.1	39.3	681	768	+ 87
1 day in 3	7	133	133	397	339	38.7	36.7	557	667	+ 110
1 day in 2	4	118	127	356	265	38.1	36.0	527	666	+ 139
All degrees	18	136	138	408	357	38.7	38.1	599	706	+ 107
Females										
1 day in 4	12	117	123	280	248	34.6	35.1	721	685	− 36
1 day in 3	10	118	120	291	258	34.8	35.2	708	814	+ 106
1 day in 2	5	113	114	276	234	34.5	34.1	614	768	+ 154
All degrees	27	117	120	823	249	34.7	35.0	696	748	+ 52

to determine whether the size of fasted rats can be more fully maintained and life can still be prolonged by beginning the fasting at 100 or 200 days, instead of at 42 days, and fasting the rats no more than 1 day in 3.

Are the results of fasting due, in whole or in part, to increased activity?

Tests made with rotary cages by Wald and Jackson ('44) revealed that rats run more when deprived of food, and McCay and his associates ('41) found that exercised rats lived longer than non-exercised rats. The activity of our fasting rats was largely limited to gnawing at the cages in attempts to escape. Many of the rats that were fasted 1 day in 4 or 3 seemed to become adapted to the fasting and remained at rest most of the time. The greatest unrest was manifested by some of the rats that were fasted 1 day in 2 but the life spans of the most restless rats were not the longest. The explanation seems to be that the greatest unrest or gnawing was manifested by the most voracious rats — the rats that apparently ate the greatest amounts of food between the days of fasting. The amount of food eaten may therefore have more than offset any possible benefit from increased exercise. In any event, the finding of McCay and his associates that rats subjected to forced exercise lived longer than non-exercised rats did not prove that the exercise per se increased the life span. The periods of forced exercise may merely have served to prevent the rats from eating as much as the controls in relation to their respective physiologic needs.

Influence of individual constitutions on the life spans

Constitutional similarities and differences among the individual rats, as determined by genetic factors and pre-experimental nutritional conditions, were obviously important factors determining the specific life spans. In the first place, a high degree of genetic uniformity in the Wistar strain evidently explains the death of 67% of the rats between the ages of 550 and 850 days and the death of 85% between the ages of 400 and 900 days in spite of the use of four different diets and four different regimens of feeding or fasting. Some littermate rats, after having been kept from 400 to 1000 days on widely differing nutritional regimens, died within 24 hours or a few days of one another. Four of the twelve rats that lived to be over 1000 days old belonged to one of the seventeen litters. The view that rats in small litters are likely to be in a superior condition is supported by the finding that two males that composed one of the two smallest litters not only lived longer than eight (all) other males on similar nutritional regimens but also lived longer than fourteen (all) females on similar nutritional regimens. In contrast to this, the average life span of thirteen rats that composed one of the three largest litters was the lowest of any of the seventeen litters. However, independent of the size of the litters from which the rats came, the life span was found to be influenced more or less by the pre-experimental nutritional status or weight attained by the age of 42 days, when the rats were started on the specific experimental regimens. That is, the rats that were heaviest at the age of 42 days tended to live longest among the rats on the same regimen but this again was less true of the rats that were fasted 1 day in 3 or 2 than of the control rats or those that were fasted only 1 day in 4. That is, the prolongation of life due to fasting 1 day in 3 or 2 tended to outweigh the apparent handicap of a poorer nutritional start in life, as indicated by a lower than average pre-experimental weight. The data in table 2 show clearly that the heaviest group of male control rats lived longer than the lighter male controls and that their fasted littermates had correspondingly long life spans.

Influence of intermittent fasting on the development of disorders leading to death

Saxton ('45) showed that the development of inflammatory, neoplastic and degenerative diseases was delayed by the restriction of calories which increased the life span of the rats in the experiments of McCay and his associates. Similarly, intermittent fasting seems to delay the development of the disorders which lead to death. Table 3 shows that a retardation of the development of mammary tumors, proportional to the amount of fasting, occurred in this study. These results support the observations previously made by Tannenbaum

TABLE 3

Development of mammary tumors in control and fasted rats.

	CONTROLS	FASTED		
		1 day in 4	1 day in 3	1 day in 2
Number of females developing tumors	7	6	8	1
Per cent of females developing tumors	37%	29%	36%	7%
Earliest age at which a tumor began developing	437 days	458 days	675 days	775 days
Average age at which tumors began developing	628 days	613 days	783 days	..
Average life span of females with tumors	760 days	760 days	871 days	977 days
Weight of largest tumor	462 gm	220 gm	140 gm	26 gm
Average weight of tumors	193 gm [1]	67 gm [1]	36 gm	..
Average rate of growth of tumors (gm gained per 100 days)	134 gm [1]	48 gm [1]	42 gm	13 gm

[1] The weights of the tumors in 2 control rats and in 1 rat fasted 1 day in 4 were not recorded and the weight of a tumor in another rat fasted 1 day in 4 was excluded because the tumor was dehydrated. It became dehydrated because it was torn loose by the rat after it apparently began to interfere with defecation.

('40; '42) concerning the relation between the food intake and the development of tumors in mice. However, genetic factors may explain the occurrence of mammary tumors in all of the (six) females in one of the seventeen litters and the occurrence of ten of the other sixteen tumor cases in five pairs of littermates. The larger tumors became responsible for the deaths of some of the females because of the tendency of the large tumors to ulcerate or become obstructive. The development of other types of tumors was apparently also retarded by intermittent fasting. The only tumor in the digestive tract, a fibrosarcoma in the forestomach, was found in the oldest control rat (a female 1012 days old). The tumor in the stomach was probably secondary to a tumorous condition of the uterus. Ulceration of the tumorous uterus evidently was responsible for the development of a fistula between the uterus and the ileum in this rat. Nothing comparable to this was seen among the fasted rats.

SUMMARY

Tests in which a group of thirty-three rats were allowed the same food ad libitum and groups of thirty-seven, thirty-seven and thirty rats were fasted 1 day in 4, 3 and 2, respectively, after the age of 42 days, showed that the apparent life span was increased by the intermittent fasting. The optimum amount of fasting appeared to be fasting 1 day in 3 and this increased the life span of littermate males about 20% and littermate females about 15%. However, the pre-experimental condition of the individual rats was also found to be an important factor determining the life spans.

No drastic retardation of growth was produced by the intermittent fasting but the development of mammary tumors was retarded in proportion to the amount of fasting.

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