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Interactive environmental and genetic effects on longevity in the male rat: Litter size, exercise, electric shocks and castration

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Interactive Environmental and Genetic Effects on Longevity in the Male Rat: Litter Size, Exercise, Electric Shocks and Castration[1]

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The effects on longevity of manipulating litter size during rearing and of postweaning forced exercise were observed in intact and castrated male rats. Random-bred sib quintets of newborn littermates were reared in reduced litters or normal litters (5 or 6, or 10-12 young, respectively). The quintets were split into five postweaning treatments: (a) untreated (control), (b) exercised (forced to run by electric shocks), (c) shocked (without exercise), (d) castrated, and (e) castrated and exercised. Reduced litter size increased weaning weight from 46 to 56 g and diminished mean longevity from 724 to 620 days. The decrease in longevity due to reduced litter size was greater in the control (204 days) than in the other postweaning treatments (42 to 142 days). The postweaning treatments affected longevity only in the reduced-litter males; in these, exercise and mere shocks appeared to increase longevity. The variation in longevity among quintets was large and the expression of genetic longevity interacted with the treatments. The regression of individual on mean sib longevity was .89 in the castrates but only .10 in the controls; in exercised and shocked males it was .58 in normal-litter but only .09 in reduced-litter sibs indicating an interaction between pre- and postweaning treatments. The implications of the results on the interpretation of longevity studies are discussed.

The postweaning feed restriction of laboratory rodents prolongs their life span [for review, see 1]. The manipulation of litter size in rodents is a convenient technique to regulate neonatal feed intake from birth to 14 days, when the young subsist on milk alone. Reduction of litter size in rodents is commonly used to study the effects of obesity induced by neonatal overfeeding [10]. In man, the negative effect of obesity on life span is fairly well documented [20]. In rodents, however, the relationship between reduced litter size and concomitant obesity on the one hand, and longevity on the other, has not been established. Widdowson and Kennedy [21] compared the longevity of rats raised in litters of 3 and of 17 to 22 young; their results were equivocal. In a recent longevity study [2] comparing long-lived, hybrid female mice nursed (a) continuously in litters of five, or (b) in litters of nine and separated from the dams every other day after one week of age until weaning at 24 days, the survival of the latter was only very slightly better.

In a number of studies, forced or voluntary physical exercise has increased the longevity of rodents [6; 9; 11; 16]. However, the contention that exercise prolongs longevity requires qualification. It appears that exercise is effective only before a certain age and only on an ad lib feeding regime [for review see 12]. Furthermore, the effects of forced exercise may be confounded with those of the stimulus used to induce it.

Allegedly, early castration increases longevity in the rat [19] although it reduces voluntary activity [13] and produces obesity [6]. Therefore, in the rat the role of exercise in prolonging life span is controversial.

The main objectives of this study were to establish the effect on longevity of reducing litter size during rearing, and critically to re-evaluate the effects of forced exercise, in intact and in castrated male rats. Furthermore, the factorial design and the split-litter technique in a random-bred population, revealed unexpected environmental and genetic-environmental interactions.

METHODS AND PROCEDURES

Animals

The rats were obtained from our colony of closed but rather heterogenous, random-bred Norway rats. Information on their longevity and moribund pathology has been published [5; 6]. A total of 245 rats, representing 49 quintets of newborn male littermates (sibs) delivered by primiparous females, were used in this study. Our primiparous females successfully raise litters of about 11 young.

Experimental Design and Treatments

The study was designed as a 2×5 factorial with two preweaning and five postweaning treatments. The preweaning treatments were "reduced" and "normal" litter size. Forty-nine litters delivered within a few days, with at least five male young per litter, were selected on the day of parturition; female young were discarded. Twenty-two dams raised reduced litters, *i.e.*, only

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a sib group of 5 or 6 males, whereas 27 dams raised normal litters of 10 to 12 males consisting of a sib group of 5 or 6 males and 5 to 7 unrelated marked males discarded at weaning. At 25 days of age, 22 reduced- and 27 normal-litter quintets of sibs (245 males) were weaned. The males were individually ear notched and the quintets were distributed to five postweaning treatments by the split-litter technique, *i.e.*, each member of every quintet was assigned at random to a different treatment.

The postweaning treatments were (a) control (untreated), (b) exercised, (c) shocked, (d) castrated, and (e) castrated and exercised. Castration under ether anesthesia was between 38 and 44 days of age, shortly before puberty. The exercised males (intact and castrated) were forced to run in a motor-driven compartmented drum. To induce running a stationary baffle delivered electric shocks of an intensity of 10,000 volts and 0.001 amp at 6 sec intervals. Exercise was started at 55 days of age and conducted 4 times a week, for 2 minutes, at a speed of 19 m/min, until the rats were 1 year of age. At that time the running speed was reduced to 9.5 m/min and the daily running time to 1 min. Running was discontinued when the rats were 18 months of age.

The shocked males were manually given three or four electric shocks, of the same intensity and at the same time, as the exercised males. The shocks were given without opening the cages. As in the exercised males, the shocks caused some defecation and urination which decreased with time, but little aversive behavior and no running.

General Conditions

A single stock diet which has supported adequate growth and reproduction for many years was offered *ad lib.* The subtreatment groups were housed separately, in groups of 5-6 per standard cage (45 × 50 × 15 cm) to permit the measurement of feed intake. Feed intake was measured as described previously [6]. All the rats were weighed at 2-week intervals throughout their lives. The location of the cages in the laboratory was randomized and changed from time to time. The temperature in the laboratory was kept at 22°C. Humidity was not controlled.

Body Composition

At 320 days of age, ten quintets preassigned at random, 6 from normal litters and 4 from reduced litters, were killed and their body composition was determined as described previously [5].

Life Span Data

The remaining 195 males, 39 quintets of sibs of which 18 were raised in reduced and 21 in normal litters, died of natural causes. The cages were inspected at least once a day and dead animals were removed. Occasionally a rat in coma was put to death.

Statistical Treatment

Coventional analysis of variance was applied to the longevity and feed consumption data. The differences were tested by multiple range test [8]. Linear regression was used to estimate the expression of genetic longevity in the subtreatments; the mean life span of four sibs was the independent variable. Covariance analysis according to principles outlined by Huitema [14] was applied to the life span data in order to adjust for genetic variation before the determination of pre- and postweaning interaction.

RESULTS

The mean weaning weight of 110 males reared in reduced litters and 135 in normal ones, was 56.2 and 46.1 g, respectively (S.E.M. = ± 1.0 g; $p<.001$).

The mean life span of the males in ten subtreatments and the pooled means for two preweaning and five postweaning treatments are presented in Table 1. The mean life span of all the males reared in reduced litters was 104 days shorter than that of the males reared in normal litters ($p<.001$). The survival rates of the males raised in reduced and normal litters are presented in Figure 1. The correlation coefficients between weaning weight and life span within the subtreatments were low and not statistically significant.

The differences in mean longevity among the postweaning treatments pooled for litter size were not significant. However, the differences between males reared in reduced and normal litters within postweaning treatments suggested significant interactions (Table 1). Control males raised in reduced litters had the shortest life span (544 days) among all the subtreatments although the control males from normal litters (with a mean life span of 748 days) outlived nearly all the other subtreatments. Thus, reduced preweaning litter size in the controls diminished the mean life span by 204 days, significantly more than in the exercised and shocked group, or in all the other treatments pooled ($p<.05$).

The variation in longevity among the quintets was highly

TABLE 1

Mean Life Span of Male Rats Reared in Reduced and Normal Litters, Submitted to Five Postweaning Treatments (Days ± S.E.M.)[1]

	Postweaning Treatments							
Preweaning Litter Size	*n*	Control	Exercised	Shocked	Castrated	Castrated & Exercised	*n*	Row Mean
Normal	21	748 ± 38^{a}	720 ± 27^{ab}	696 ± 42^{ab}	705 ± 44^{ab}	749 ± 40^{a}	105	724 ± 17^{x}
Reduced	18	544 ± 41^{c}	669 ± 53^{ab}	654 ± 47^{abc}	627 ± 45^{abc}	607 ± 50^{bc}	90	620 ± 18^{y}
Pooled for Litter Size	39	654 ± 28	696 ± 29	677 ± 31	669 ± 32	683 ± 32	195	676 ± 12

[1]The differences among pooled postweaning treatment means are not significantly different. Among unpooled subtreatments, means carrying at least one common superscript are not significantly different: a>b>c at $p<.05$. For row means: x>y at $p<.001$.

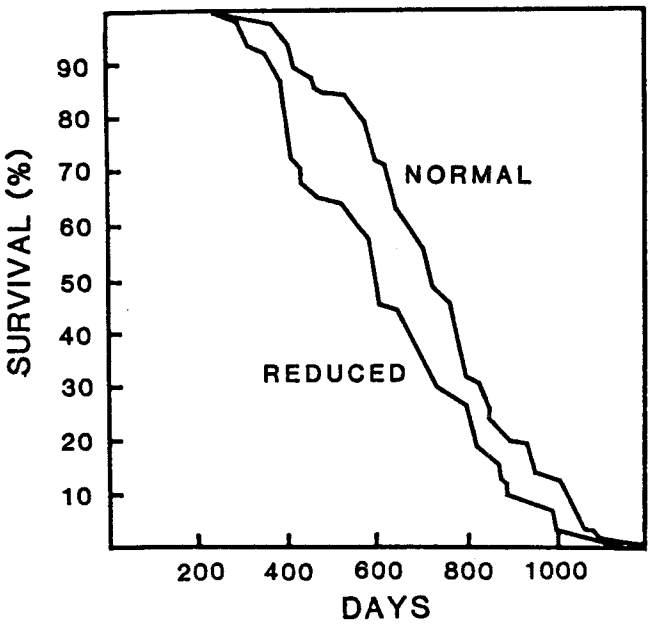

FIGURE 1. Survival of male rats raised in reduced litters of 5 or 6 young and in normal litters of 10 to 12 young.

significant ($p<.001$) and the range was large. The coefficient of variation of mean quintet longevity was 18% and the ranges in days were 420 to 816, and 528 to 994, for 18 reduced- and 21 normal-litter quintets, respectively. However, the regressions of individual on mean quintet longevity calculated for the several postweaning treatments, indicated great differences among treatment-groups, suggesting strong environmental-genetic interactions in the study. In order to test this hypothesis, ten linear regression equations were calculated of actual individual life span (Y) on the mean life span of four respective sibs (X). The latter was preferred because the use of mean quintet life span as the independent variable would have produced exaggerated and misleading "part on whole regressions." The regression equations and the correlation coefficients (r) in the reduced-litter males, were:

Control:	$Y = 502 + (0.06 \pm 0.33)X$;	$r = 0.05$
Exercised:	$Y = 615 + (0.09 \pm 0.44)X$;	$r = 0.05$
Shocked:	$Y = 601 + (0.09 \pm 0.39)X$;	$r = 0.06$
Castrated:	$Y = 172 + (0.74^a \pm 0.42)X$;	$r = 0.40^a$
Castrated and exercised:	$Y = -36 + (1.03^a \pm 0.51)X$;	$r = 0.44^a$

For the normal-litter males the regressions and correlations were:

Control:	$Y = 645 + (0.14 \pm 0.27)X$;	$r = 0.12$
Exercised:	$Y = 336 + (0.53^c \pm 0.21)X$;	$r = 0.50^b$
Shocked:	$Y = 253 + (0.61^a \pm 0.33)X$;	$r = 0.39^a$
Castrated:	$Y = 99 + (0.83^b \pm 0.37)X$;	$r = 0.46^b$
Castrated and exercised:	$Y = 65 + (0.95^b \pm 0.39)X$;	$r = 0.49^b$

The superscripts a, b and c indicate coefficients significant at the probability levels of .10, .05 and .02, respectively. Covariance analysis of the postweaning treatments produced regressions and correlations which were significant ($p<.01$) for the unexercised and exercised castrated males, but not significant for the other three groups. In order to present the differences in genetic expression among the treatments clearly, in a way which obviates computation in order to reach conclusions, the regression lines for the reduced- and normal-litter groups of rats were superimposed in Figures 2 and 3,

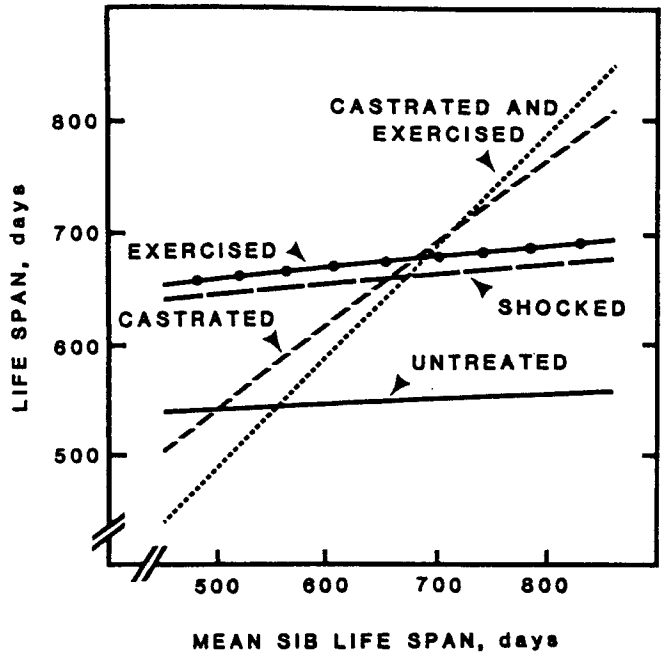

FIGURE 2. Regression lines of life span on mean sib life span for five postweaning treatments in male rats raised in reduced litters of 5 or 6 young.

respectively.

From Figures 2 and 3 it is evident that the regressions of the control males were nearly unrelated to those of their sibs in both reduced- and normal-litter males. In sharp contrast, the life span of all the castrated male groups was consistently related to that of their sibs. The regressions of the exercised and the shocked males suggested a further interaction: in the reduced-litter males (Figure 2) their regressions were close to zero and very much

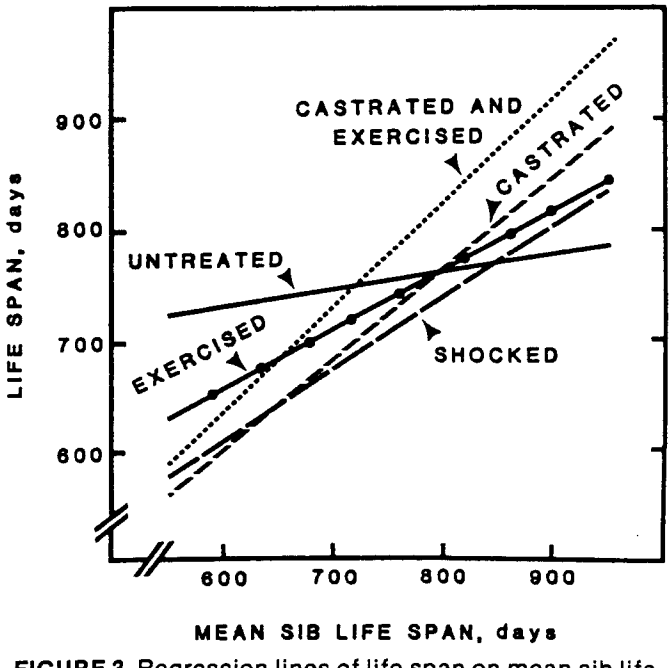

FIGURE 3. Regression lines of life span on mean sib life span for five postweaning treatments in male rats raised in normal litters of 10 to 12 young.

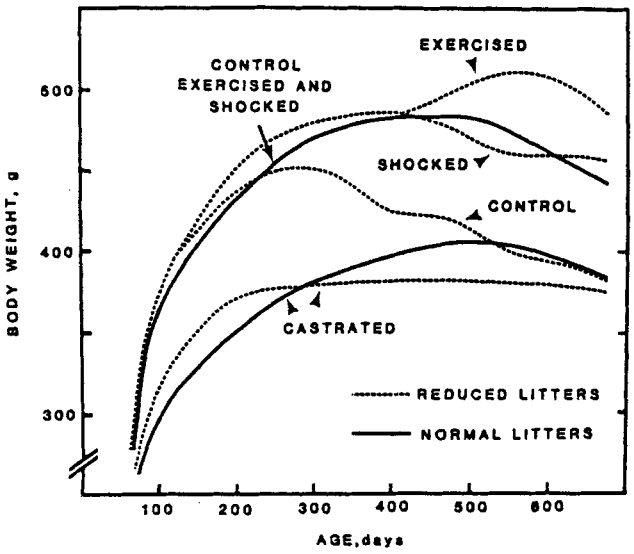

FIGURE 4. Growth curves of male rats raised in reduced litters of 5 or 6 young and in normal litters of 10 to 12 young, submitted to postweaning treatments: control, exercised, shocked and castrated.

like that of the untreated males; on the other hand, in the normal-litter males (Figure 3) they showed significant regression on mean sib life span approaching that of the castrates.

The regressions also indicated that the differences in longevity among the treatments used in this study depended on the genetic component of longevity (as indicated by mean sib life span) and may be in *opposite directions* in stock widely differing in genetic potential.

The generalized growth curves of the subtreatment groups, based on the bi-weekly individual body weights of all the surviving rats are presented in Figure 4. The mean body weights of the normal-litter control, exercised and shocked males were not significantly different at any time and are represented by a single curve. These three subtreatments produced a normal growth curve leveling off at 350 days. In contrast to these, the intact reduced-litter males presented first two and later three different curves. The control, with the shortest life span among all the subtreatments, peaked earlier than the others. The exercised and the shocked groups produced normal growth curves to 400 days. From then on the surviving exercised males continued to grow to 550 days, while the shocked group declined. Exercise had no effect on the growth curves of the castrates; therefore, only two growth curves are shown. The reduced growth rate and mature weight of castrated males are well documented [6]. However, the difference in initial growth between the reduced- and normal-litter castrates was similar to that in intact males; all the reduced-litter animals grew faster and leveled off earlier. The differences in body weight between reduced- and normal-litter animals at 200 days were significant ($p<.05$).

The mean daily feed intake from 90 to 650 days of the surviving males and the body fat content of a sample of males at 320 days are presented in Table 2. Feed intake declined uniformly in all the subtreatments, but no differences in feed intake were observed at any time between the corresponding groups of reduced- and normal-litter males. Among the postweaning treatments, four distinct levels of intake were observed. Exercise and electric shocks increased feed intake in both intact and in castrated males.

DISCUSSION

In physiological studies in which litter size is manipulated, there is commonly a large disparity in the number of young. Litters of 4 to 20 pups have been used [7; 10]. Although strains of rodents differ in this respect, in litters of 14 pups or more, weanling rats usually become stunted, their voluntary feed intake may be reduced and they tend to succumb early to infectious diseases [21]. In the study of Cheney et al. [2] feed restriction throughout life was necessary in female mice to enhance the life prolonging effect of preweaning restriction. In the present study, using moderately disparate litter sizes and not more than 12 young per litter, no stunting occurred and subsequent feed intake was neither manipulated nor was it affected by lit-

TABLE 2

Feed Intake and Body Fat Content of Male Rats Reared in Reduced or Normal Litters, Submitted to Five Postweaning Treatments[1]

	Feed Intake[2] (g/d)		Body Fat Content[3] (%)	
Postweaning Treatment	Reduced Litter Size	Normal Litter Size	Reduced Litter Size	Normal Litter Size
Control	16.67[a]	17.60[b]	21.8[b] ($n=4$)	20.5[b] ($n=5$)
Exercised and Shocked[4]	18.87[a]	18.60[a]	28.0[ab] ($n=7$)	22.6[b] ($n=10$)
Castrated[5]				
Not Exercised	14.28[d]	14.46[d]		
Exercised	15.26[d]	15.82[c]	37.2[a] ($n=7$)	28.2[ab] ($n=9$)
S.E.M.	0.28		2.65	

[1]Means not carrying at least one common superscript are significantly different ($p<.01$).
[2]Means of 13 equally spaced one-week observations of replicates (cages) from 90 to 650 days of age of all the survivors (45 or more observations per subtreatment).
[3]At 320 days of age, the difference between all the reduced and normal litter males was significant ($p<.02$).
[4]There were no significant differences between exercised and shocked males; hence the data were pooled.
[5]Pooled for body fat content due to small number of observations; exercised castrates had nominally higher fat content than others.

ter size. Therefore, the most obvious effect in this study was the consistent and significant difference in survival between males raised in normal and reduced litters. Their respective mean weaning weights (56 to 46 g) are poor indicators of the effects of restriction during the nursing period. At 14 days of age rats begin to consume dry feed and largely compensate for any preceding restriction. Although in this study the body weights at 14 days were not recorded, very extensive data from our laboratory indicate that young raised in litters of 5 or 6, and of 10 to 12, reach 14-day body weights of approximately 33 and 22 g, respectively. Therefore, the relative differences in neonatal growth rate between reduced- and normal-litter males were probably greater than suggested by the weaning weights. Accelerated neonatal growth rate in rats may increase the number and size of adipose tissue cells [15], elevate serum insulin [3] and modify the activity of hepatic enzymes involved in lipid and carbohydrate metabolism [7]. In man, neonatal overfeeding may lead to obesity, impaired health and reduced life span [20]. Presumably, in this respect, there is some analogy between evidence from human and animal studies [18]. However, the relationship between reduced litter size and life span in rodents may be more complex.

The results of this study lend limited support to the hypothesis that neonatal overfeeding favors obesity. At 320 days, the body fat content of all the reduced-litter males was significantly greater than that of normal-litter males ($p<.02$; Table 2). In the control males this effect was small probably because reduced-litter males declined in health, and consequently in weight, earlier than other groups; this is evident from their short life span (Table 1) and their growth curve (Figure 4). Nevertheless, all the males from reduced litters retained consistently more energy from weaning to 200 days of age. During this period they did not consume more feed than normal-litter males, but grew more rapidly and became relatively obese. Since the rate of absorption of energy is rather constant, the relative obesity of reduced-litter males may be due to reduced basal metabolic rate and/or to reduced voluntary activity. Endocrine or neuroendocrine changes may be postulated in either case.

Unlike in normal-litter males, in those raised in reduced litters the exercised group outlived the control, but this requires qualification. The reduced-litter control group had the shortest life span among ten subtreatments (Table 1) and its decline in the body weight began very early (Figure 4). Cutler [4] expressed concern that the purported control conditions in life span studies are "unnatural" and accelerate aging in laboratory anaimals fed ad lib in small cages. This view is confirmed with emphasis on neonatal overfeeding rather than other factors. Furthermore, the shocked but unexercised males lived nearly as long as the exercised males. This suggests that shocks, rather than running per se prolonged the life span of the exercised males. In our previous study [6] intact males exercised under similar conditions outlived the control group by 132 days ($p<.01$). Litter size was not controlled in that study but two-thirds of the males were raised in litters with a mean of 6.6 young, and the rest in litters of 9 or 10. Mean litter size was 7.6 vs. 5.5 and 11.0 in this study. This appears to resolve the apparent conflict between our present and previous reports. Therefore, exercise or electric shock only counteracted the reduction in life span due to reduced litter size. Likewise, castration tended to prolong lifespan only in reduced litters.

The great variation in the mean life span among quintets of sibs may be attributed to genetic and/or maternal effects. However, longevity is rather heritable in the rat [17], and probably in a random bred colony of laboratory rats, the variation is largely genetic. The significant differences in the expression of genetic longevity as indicated by the greatly disparate regressions of actual on estimated genetic life span, suggest important practical conclusions concerning longevity studies. First, different inbred lines may produce conflicting results, even to the point of being diametrically opposed, and therefore of questionable applicability to a random-bred population. Figures 2 and 3 indicate that castrates from genetically long-lived families outlived the controls, whereas genetically short-lived castrates died earlier than the controls regardless of litter size. To a lesser extent, the same holds for exercised and shocked males raised in normal litters. Only in the reduced-litter males were the differences within control, exercised and shocked males unaffected by genetic potential. Secondly, the use of the split-litter technique may increase rather than decrease the experimental error. It is obvious from Figures 2 and 3 that variation within the quintets of sibs increased as mean sib life span deviated from the overal means (620 days in reduced- and 724 days in normal-litter males). The split-litter technique would reduce the experimental error only for treatments having rather similar regression coefficients of actual on genetic life span, i.e., similar expression of genetic longevity. Nevertheless, in this study, the use of the split-litter technique with a random-bred population produced a great amount of information which would have been lost in a randomized design.

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