GROWTH RATES OF ORGANS OF MICROTUS OCHROGASTER WITH CHANGES IN NUCLEIC ACID CONCENTRATIONS

by

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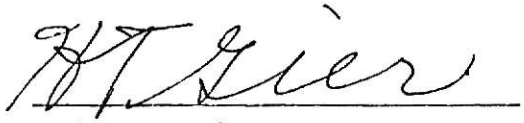

Major Professor

TABLE OF CONTENTS

LIST OF FIGURES

List of Figures (continued)

LIST OF TABLES

INTRODUCTION

With the increasing use of Microtus ochrogaster as a laboratory animal, the necessity for the establishment of growth patterns becomes evident. Hoffmeister and Getz (1968) and Gier and Cooksey (1967) reported growth changes in this animal; however, no parameters for organs during growth stages have been established.

By virtue of the fact that nucleic acids are involved with heredity and protein synthesis, they are associated with growth and development. In a continuing attempt to understand the basic relationships between nucleic acids and their functions, methods for measuring concentrations of DNA and RNA have become necessary. These concentrations have been utilized to compare tissues of experimental animals with normal animal tissues (Pond et al., 1969; Smith, 1953), animals of one age group with those of another (Barrows et al., 1962; Kurnick and Kernen, 1962) and diseased tissue with normal tissues (von Euler and Hahn, 1948; Schneider and Klug, 1946). Few researchers have systematically examined the tissue concentration levels of DNA and RNA during stages of rapid growth (Reddy and Cerecedo, 1951; Geschwind and Li, 1949). Although it is generally accepted that young growing tissues contain higher concentrations of nucleic acids than the corresponding tissue in an adult animal, no correlation between the DNA and RNA concentrations and growth rate of tissues has been derived.

For this study, experiments were devised to (1) determine the growth of Microtus ochrogaster from the embryonic stage to maturity, both in external characteristics and organ structure; (2) isolate and quantitate both DNA and RNA of several organs (liver, kidney, heart, brain and testes) from the day of birth to maturity; and (3) mathematically and graphically correlate DNA, RNA and growth of the various organs.

THIS BOOK CONTAINS NUMEROUS PAGES WITH THE ORIGINAL PRINTING ON THE PAGE BEING CROOKED.

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LITERATURE REVIEW

Numerous papers concerning microtine rodent growth are available (Foster and Peterson, 1961; Howell, 1924; Krebs, 1964; Martin, 1956; Snyder, 1954). However, papers dealing specifically with Microtus ochrogaster have been incomplete for the first 50 days after birth, during which time 90% of this animal's growth occurs (Hoffmeister and Getz, 1968).

Hoffmeister and Getz (1968) recorded measurements at weekly intervals during the first month and at two-week intervals during the second month. No weights nor measurements of internal organs were recorded. Gier and Cooksey (1967) reported growth measurements in terms of total length and total weight for animals up to 60 days of age, but measurements and weights of internal organs were lacking.

Nucleic acid concentrations of various tissues have been determined by many methods (Schmidt and Thannhauser, 1945; Webb and Levy, 1955; Burton, 1956; Schneider, 1945 and 1957; Seibert, 1940; Drury, 1948; von Euler and Hahn, 1948; Stumpf, 1947; Fiske and Subbarow, 1925; Lin and Schjeide, 1969; Ceriotti, 1955). Among these, maximum extraction of DNA and RNA is generally accomplished by utilizing Schneider's method (1945) as modified by Burton (1956), and for colorimetric detection of RNA and DNA, those procedures given by Lin (1969) and Burton (1956), respectively, seem to give the best results with minimum interference.

Much of the work concerning analyses of nucleic acid concentrations have been used in studying differences between groups of

animals or tissues. Embryonic tissues were found to have a higher content of both nucleic acids than those of the corresponding adult tissues (Davidson and Waymouth, 1944; Reddy and Cerecedo, 1951; Geschwind and Li, 1949). DNA and RNA concentrations and their ratios have also been used in studies of the differences between tissues of young animals and those of the very old (Barrows et al., 1962; Kurnick and Kernen, 1962; Falzone et al., 1959). Others have utilized nucleic acid contents to study the ways in which cancerous and normal tissues differ from each other (von Euler and Hahn, 1948; Brues et al., 1944; Schneider, 1945 and 1946; Schneider and Klug, 1946). An understanding of the relation of nucleic acids to proteins relies heavily on experimentation done with biochemical quantitations of DNA and RNA (Srivastava and Chaudhary, 1969; Pond et al., 1969; Murthy, 1966).

Some workers have correlated nucleic acid contents of tissues of rapidly growing animals with the age of the animal. Geschwind and Li (1949) used nucleic acid phosphorous as the basis for analysis of fetal and juvenile rat livers. They found that mg RNA-P per 100 g dry liver remained constant from the 16th to the 20th day of prenatal development, then gradually decreased to a low point at 40 days post partum. The DNA-P was constant until 19 days, then dropped more rapidly and to a lower level than the RNA-P giving an eventual RNA-P/DNA-P ratio of 2.9 at 40 days of age.

Reddy and Cerecedo (1951) reported a gradual decrease in mg pentose nucleic acid and deoxypentose nucleic acid per gram of

dry liver in the mouse from the 15th prenatal day to the 21st postnatal day; the eventual PNA/DNA ratio was approximately 1.9 at 21 days. These observations agree with those of Geschwind and Li (1949).

Srivasta and Chaudhary (1969) observed that mg RNA per gm of fresh mouse "hind leg muscle" decreased at a rapid rate during the first 30 days after birth and continued to drop at a more gradual rate thereafter. They also reported that the RNA/DNA ratio was greatest at 30 days, which preceded the period of maximum increase in the protein content. They concluded that the higher the RNA/DNA ratio, the higher the protein synthesis capacity per cell.

Several attempts have been made to minimize the variation of determinable nucleic acid concentrations in tissues, particularly in liver. Smith (1953) observed no significant differences in mg RNA and DNA per 100 g wet weight when rats were fasted for 12 and 24 hrs, nor when rats were adrenalectomized. These results were verified by Fukuda and Sibatani (1953) who reported that the amount of DNA per liver cell as a result of polyploidy could be correlated more with body weight than with the growth rate or the nutritional state of the animal. Wolfe (1965) found the estrous cycle produced no statistically significant differences in nucleic acid content of mouse liver.

It is generally known that high concentrations of nucleic acids are found in rapidly growing tissues; however, little has been done towards a systematic approach of correlating nucleic acid concentrations with growth rates. Draper et al. (1969)

determined quantities of RNA and DNA in lamb thyroid and plotted the ratios of these amounts against the growth rate (kg/day) of the entire animal, but found no correlation between the two variables.

MATERIALS AND METHODS

Study I

Material was obtained from a colony of Microtus ochrogaster maintained at Kansas State University for experimental purposes, as described by Gier and Cooksey (1967). Animals were sacrificed at known ages: 18 were taken between fetal day 12 and birth, and 110 between partuition and 50 days after birth. After sacrificing, the body weight and the following external measurements were recorded: total length, length of tail and length of hind foot. In addition, the following data were collected: weight of liver; length, width and weight of kidney, heart and brain; and length, diameter and weight of testes.

The total length of the vole was obtained by laying the animal on the ventral aspect with head and axial skeleton in a fully extended position. The measurements from the tip of the nose to the tip of the tail was obtained by using calipers. The tail length was recorded by measuring from the dorsal border of the anal sphincter to the tip of the tail. The measurement from the point of the hock (os calcis) to the tip of the longest digit (excluding the nail) was recorded for hind foot length.

The dimensions on the kidney were recorded by measuring the maximum longitudinal axis, and the width from the hilus to the point exactly perpendicular to this axis on its greatest curvature. The length of the heart was taken from the base of the atrium to the apex of the left ventricle, the width was obtained by measuring the widest ventricular diameter. The distance between

the anterior extremity of the olfactory lobes to the posterior edge of the cerebellum, in situ, is considered as brain length. The width was measured at the widest cerebral lateral borders.

Fetuses of known ages, determined by observed copulation, were recovered, weighed and crown-rump measured. Fetal organs were weighed at the earliest obtainable age to birth at 21 days gestation.

Study II

The nucleic acid extraction procedure found most applicable for the task at hand was basically that proposed by Schneider (1945) and modified for greater specificity by Burton (1956). For the colorimetric determination of RNA, Lin and Schjeide's procedure (1969) proved most favorable as it allowed for most accuracy and specificity with the least interference by non-RNA compounds. The procedure for the detection of DNA most acceptable was that proposed by Burton (1956), which again proved most specific and accurate with minimum interference colorimetrically.

Nucleic acids were extracted from 20-200 mg samples of liver, kidney, heart, brain (cerebrum) and testes of 100 known age animals. Each tissue was homogenized in a Potter-Elvehjem homogenizer with 10 volumes of 0.25 M $HC1O_4$. The homogenate was centrifuged for 5 min. at 1,000 rpm, the supernatant discarded and 4 ml of 0.5 M $HC1O_4$ added to the residue. The sediment was resuspended, heated for 20 min. at 70°C, then centrifuged for 5 min. at 1,000 rpm and the supernatant decanted into a graduated centrifuge tube. The sediment was re-extracted at 70°C for 20 min., centrifuged,

the supernatant added to the previously acquired extract and the total volume recorded. The ethanol extraction of lipoidal compounds as outlined by Schneider (1945) was found unnecessary.

Estimations of RNA were determined colorimetrically by the use of the cupric ion catalyzed orcinol reaction as presented by Lin and Schjeide (1969). Two ml of extract were pipetted into a test tube, and 2 ml of activated orcinol reagent added. Activated orcinol was prepared by adding 2 ml of stock orcinol (12.5 g orcinol made up to 25 ml with 95% ethanol) and 100 ml of cupric ion reagent (150 mg $CuCl_2 \cdot 2 H_2O$) dissolved in 100 ml of concentrated HCl). The tube containing the extract and the activated orcinol was heated in boiling water for 35 min., allowed to cool for 30 min, at room temperature, then the absorbance of the resulting color solution was read on a Bausch and Lomb Spectronic 20 at 665 mμ (with red filter). The amount of RNA was determined by the use of a previously prepared standard curve (Ribose Nucleic Acid, National Biochemicals Corporation). A control tube containing 2 ml of 0.5 M $HClO_4$ and two standards (one containing 50 μg RNA and one containing 100 μg RNA) were analyzed with each series of extractions. The concentrations of RNA (μg RNA/mg of tissue) were recorded along with the age of the animal from which the tissue was removed.

DNA content was quantitatively determined colorimetrically from the same extract by use of the diphenylamine reaction as modifed by Burton (1956). One ml of the extract was pipetted into a test tube and 2 ml of activated diphenylamine was added. Activated diphenylamine was prepared by adding 1% acetaldehyde

to diphenylamine reagent (3 g recrystallized diphenylamine in 200 ml glacial acetic acid and 3 ml concentrated H_2SO_4). The tube was then allowed to incubate for 16 hrs. at 30°C. The absorbance of the resulting color solution was read at 600 mμ on a Spectronic 20. The amount of DNA was determined by the use of a previously prepared standard curve (Calf Thymus DNA, Sigma-Na salt). A control containing only 0.5 M $HClO_4$ and a standard containing 50 μg of DNA were analyzed with each series of extractions. The concentrations (μg DNA/mg tissue) were recorded along with the age of the animal from which the tissue was removed.

Study III

Data from the growth charts of liver, kidney, brain, heart and testes obtained in Study I were each partitioned into segments of 6-8 organ measurements per segment. A linear regression with X representing the age of the animal and Y representing the weight of the organ, was calculated on each partition with an Olivetti Underwood Programma 101 computer. A slope (b) and a mean weight value ($\bar{Y}$) were obtained for each partition. The slope was an estimate of the average absolute growth rate (change in weight/change in time) during a partition, and $b/\bar{Y}$ was an index of the relative rate of growth at a particular weight.

The RNA concentrations of the corresponding organs within each partition were averaged. This gave a mean value ($\bar{M}$) of the μg RNA/mg tissue for a given segment of the organ's growth curve. A linear regression was computed with X representing $b/\bar{Y}$ and Y representing $\bar{M}$ for all such points of an organ's growth curve.

A slope (b), correlation coefficient (r) and the standard deviation of the Y's in the population about their trend line were obtained. This procedure was repeated with DNA M̄'s substituted for RNA.

RESULTS

Study I

Total body weight of fetuses of Microtus ochrogaster (Fig. 1) was 8 mg at 12 days gestation, which was the earliest recovery of specimens of known age, to over 40 g at 50 days after birth. Growth rate was rapid between 12 and 15 days, with the fetus averaging 160 mg at 15 days gestation; they tripled this weight by 17 days gestation and attained approximately 3.0 g at partuition. Crown-Rump measurements averaged 3.8 mm at 12 days, 9.8 mm at 15 days and 24 mm by 19 days gestation (Fig. 1).

Weight and total length at birth (Fig. 2) were similar to those reported by Gier and Cooksey (1968); weight of the animals ranged between 2.8 and 3.2 g at birth and reached about 10 g by 10 days of age. Gain in weight averaged 1 g per day from 10 days to 35 days of age, after which time the rate of gain gradually declined. Total length at birth was 42-46 mm and reached 136 mm by 40-50 days (Table 1).

Length of the tail at birth averaged 6.3 mm and increased to 19.9 mm at 18 days and 28.4 mm at 37 days, at which time growth essentially ceased. At 50 days the tail averaged 28.1 mm (Fig. 3, Table 1).

At birth the hind foot averaged 6.9 mm in length. Increase continued at a rapid and steady rate to the maximum length of 17-19 mm as early as 20 days (Fig. 3, Table 1).

Measurements of internal organs were begun at 17 days gestation. The liver at this time weighed approximately 43 mg, in two

days the weight had increased to 145 mg. At birth, the liver weight was 153 mg and steadily increased to approximately 1 g at 20 days with little individual variation. Beyond 20 days, liver weight continued to increase, but with marked individual variation (Fig. 4, Table 2).

The combined weight of both kidneys (Fig. 4) at 17 days gestation could not be obtained because of the small size of the organs, but at 19 days these organs weighed 12 to 13 mg. At birth, the combined weight averaged 32 mg and steadily increased to approximately 300 mg by 20 days. Extreme individual variation was evident, ranging from as low as 320 mg to as high as 580 mg between 30 and 50 days. There was no apparent correlation between weight of kidney and age within this group of animals. The average length and width of this organ at birth was 4.3 mm and 2.3 mm, respectively; at 20 days, 9.0 mm by 4.2 mm, and 11.5 mm by 6.2 mm by 45 days (Table 2).

Brain growth as determined by weight was rapid. At 17 days gestation, the brain averaged 40 mg and by the 19th day it more than doubled to an average of 84 mg (Fig. 4), and at birth was approximately 145 mg. By the 21st day after birth, it had increased to 575 mg, which was almost maximum in that by 39 days the brains averaged 640 mg and rarely exceeded that weight after that time. At birth, brain length was 9.0 mm, width was 6.5 mm; by 21 days length had increased to 14.1 mm and width 9.8 mm; and at 39 days, 15.8 and 10.5 mm, respectively (Table 2).

The weight of the heart (Fig. 5, Table 2), which at 17 days gestation was 6.0 mg, was 9.0 mg at 19 days. The heart weighed

13.6 mg at birth, then increased steadily with little variation to 10 days when rate of gain began to decrease, so that by 30 days the stabilized weight of 170-190 mg was attained. The external dimensions of the heart at birth were 3.6 mm long and 2.4 mm wide across the top of the ventricles, increasing to 8.6 and 5.6 mm by 21 days, and 9.6 by 6.5 mm at 45 days.

The weight of the two testes combined averaged 3.7 mg at birth and at least doubled in each 10-day period up to 26 days, after which time the rate of weight gain tapered off. The average weight of a pair of testes at 10 days was approximately 20 mg, increased to 69 mg at 21 days and 280 mg at 37 days of age. At birth, the length of the testes was 3.6 mm and the greatest diameter was 1.6 mm. At 21 days the average length reached 5.2 mm and the diameter 3.4 mm; and by 39 days after birth, they were 9.5 mm long and 6.4 mm in diameter. After 21 days, the weights and dimensions of the testes were subject to considerable variation (Table 2).

Study II

RNA concentrations of the liver in units of μg RNA per mg of wet tissue were highly variable both within and between age groups. The concentrations ranged from 3.0 μg/mg liver in a 27-day old animal to 14.3 μg/mg liver in a new-born. Of the 77 livers analyzed, 54% contained between 6 and 10 μg RNA/mg tissue (Table 3). Of animals less than 13 days old, 75% contained more than 10 μg RNA/mg liver, and 66% of the livers containing less than 6 μg/mg were from animals 19 to 34 days of age (Fig. 6, Table 3).

The RNA content of kidneys (Table 3) was about as variable as that of liver, and ranged from 2.8 µg RNA/mg kidney in an animal 12 days old to 11.0 µg/mg kidney in an animal 9 days of age. Of the kidneys analyzed before the animals were 10 days old, 76% had concentrations of between 6.7 and 8.8 µg/mg and 79% of the kidneys from animals more than 20 days old had between 4.0 and 8.0 µg/mg kidney (Fig. 7).

Although the difference between the lowest and the highest RNA concentrations of the heart was greater than that found in the liver or the kidney, smaller variation within a more confined parameter was found. Of those hearts from animals younger than 13 days, 88% had concentrations greater than 4.0 µg/mg and 81% of those analyzed after 14 days had between 1.6 and 3.6 µg RNA per mg of heart tissue. A definite downward trend line could be established (Fig. 8, Table 3).

Concentrations of RNA in the cerebrum were lower per unit weight in the younger animals than in other tissues of the same age group with 80% of the 41 brains analyzed showing concentrations between 2.0 and 6.0 µg RNA/mg cerebrum. Most of the values obtained from animals less than 13 days of age were between 4 and 6 µg/mg; concentrations from animals 13 to 30 days old were generally less than 4 µg/mg, and most of those older than 30 days contained more than 4 µg RNA/mg (Fig. 9, Table 3).

Despite extensive variation (Table 3), a definite downward trend could be observed in the contents of testicular RNA plotted as a function of age (Fig. 10). Of animals less than 10 days old, 75% had more than 10.8 µg RNA per mg tissue; while, in those

younger than 15 days, 93% had more than 6.6 µg/mg. Of the values obtained from animals 15 to 50 days of age, 70% were between 3.0 and 6.0 µg/mg.

All of the data obtained from the analyses of DNA content indicated a gradual reduction in the amount of DNA per unit weight of tissue with increasing age of the animals. Concentrations greater than 6 µg DNA/mg liver were found in animals younger than 10 days of age. With age, these concentrations decreased until 25 days, at which time the concentrations stabilized. At 50 days, there was less than 1 µg DNA/mg liver (Fig. 11, Table 4).

While the relative values were much higher, DNA levels obtained for kidneys proceeded much like those of the liver. Concentrations of DNA in the kidney exceeded 15 µg/mg in animals less than 10 days old; these decreased considerably and stabilized at approximately 7.5 µg/mg from day 23 on (Fig. 12, Table 4).

The values of DNA from hearts followed the same downward trend in that the concentration was about 6 µg/mg in the younger animals. At 50 days, the concentration had gradually decreased to about 2.0 µg/mg (Fig. 13, Table 4).

The cerebrum contained much less DNA per mg of tissue than did other tissues. Most of the values obtained were between 0.5 and 2.0 µg/mg (Table 4) with the general trend, somewhat obscured by variation, being toward a decrease in µg DNA/mg brain with increasing age (Fig. 14).

The highest DNA concentrations found in any tissue were in the young testes. The content per unit weight exceeded 18 µg/mg in animals less than 12 days old (Table 4), and rapidly decreased

and stabilized at a level between 9.0 and 11.0 µg/mg by 35 days (Fig. 15).

Study III

The plot of $b/\bar{Y}$ versus µg of DNA and RNA per mg liver (Fig. 16) showed no correlation between the RNA concentrations as the correlation coefficient was not significant. The RNA and the DNA regression lines had positive slopes of 3.75 and 31.73, respectively, indicating a reduction in nucleic acid concentrations as the growth rate decreased even though the correlation was not significant. The position of the trend lines on the graph reflect the consistently higher RNA contents at all states of postnatal growth (Fig. 16).

The concentrations of RNA per mg kidney were highly correlated with the growth rate of that organ with an r value of 0.86, which was significant at the $\alpha = 0.01$ level. The DNA trend line was also significant; but because of fewer degrees of freedom, only at the $\alpha = 0.10$ level. These figures showed that both DNA and RNA had the highest concentrations in this organ during its most rapid growth. The slope of the DNA (49.49) indicated that its concentrations were affected by a decrease in growth rate more than was that of RNA, which had a slope of 8.84. As opposed to the condition found in the liver (Fig. 16), RNA concentrations never exceeded those of DNA (Fig. 17).

The heart RNA/growth rate trend line (Fig. 18) showed a significant correlation with $r = 0.86$ ($\alpha = 0.01$), and because of fewer degrees of freedom, the DNA/growth rate line was significant

at the $\alpha = 0.10$ level. The plotted lines were almost parallel, never separated more than a standard deviation as the slope of DNA is 23.50 and that of the RNA/growth rate regression was 24.26. Like those in the kidney (Fig. 17), DNA concentrations were greater; however, the difference in the heart is slight. Both trends indicated a decrease in concentrations of nucleic acids as growth rate of the heart decreased (Fig. 18).

Neither DNA nor RNA concentrations of the brain were significantly correlated with the growth rate of this organ. The trend lines were nearly parallel as the DNA and RNA trends had b's of 10.50 and 9.74, respectively. The lines indicated that the RNA concentrations were approximately 3 µg/mg higher than those of the DNA throughout all stages of postnatal growth (Fig. 19).

The coefficient of correlation (0.70) for the testes RNA/growth rate regression was not statistically significant, but was high enough that some conclusions could be drawn. The slope of this line indicated a definite decrease in RNA concentrations as the rate of growth decreased. The DNA/growth rate trend line with an r of 0.94 was significant, showed a positive slope of 54.89 and indicated that the relative concentrations of DNA were consistently greater than those of RNA at all rates of growth for the testes (Fig. 20).

DISCUSSION

Weights and dimensional measurements of external and internal structures of Microtus ochrogaster during growth indicated that the relative growth rate of this animal and its organs would fit well on a polynomial regression as obtained by Killian (1969) and Preston (1969).

Plots of the concentrations of DNA and RNA as a function of age (Figs. 6-15) showed general trends of decreasing amounts of nucleic acids per unit weight, with increasing weight, of the organ. Schneider and Klug (1946) and Smith (1953) dealt with RNA and DNA concentrations without reference to the age of the animals, so missed the age correlation. Determinations of nucleic acids should be done with control animals of the same age range as the experimental animals because concentrations of DNA and RNA change with age during the juvenile stages.

The relative growth rate rather than the age of the tissue or organ provides the basis for a more valid index of nucleic acid concentrations and their ratios. The concentrations of DNA and RNA in some organs have relatively stable parameters which are dependent upon the growth rate of those organs, and thus on the stage of development.

Because of the following analyses, it is perhaps necessary to state that the 0 point on the X-axis (Figs. 16-20) is the relative growth rate for the organs from animals studied 0 to 50 days of age and does not necessarily indicate that the organ growth has ceased. Likewise, this index of relative growth rate

would probably continue to increase to points greater than 0.35 if stages of fetal growth were plotted.

Reddy and Cerecedo (1951) plotted DNA and RNA concentrations of fetal mouse liver against age and reported a higher DNA content. In this study, a relative growth rate index of greater than 0.20 was consistently obtained during the time of fetal growth and the DNA concentration obviously exceeded that of RNA. At a relative growth rate index of 0.01, the RNA/DNA ratio was approximately the same as the 2.9 in the 40-day rat liver reported by Geschwind and Li (1949) who plotted the nucleic acid concentrations as a function of age. This study also verified Fukuda and Sibatani's (1953) work in that there was no proven correlation between growth rate and the DNA content of the liver (Fig. 16).

Although the liver values for growth rate were not statistically correlated with nucleic acid concentrations, there was a reduction to a much lower level of DNA concentrations with age than occurred in RNA concentrations.

As Srivastava and Chaudhary (1969) indicated, RNA can be considered an index of the capacity for protein synthesis and DNA as an approximation of cell number. Therefore, the RNA/DNA ratio provides a relative estimation of protein synthesis capacity per cell. This explains, as would be expected, the fact that the concentrations of RNA in the liver are higher than are those of DNA. Numerous enzymes are required for bile formation, carbohydrate metabolism, deamination with subsequent urea formation and for the destruction of RBC's with subsequent iron

storage. These require protein synthesis, which in turn, requires RNA. These activities explain the high RNA/DNA ratios seen in the liver.

The gradual decrease with age of DNA per mg liver tissue does not seem logical because of the reported increase in ploidy in liver cells with increasing age. However, appreciable polyploidization in rat liver does not occur until after the animal weighs over 25 g (Fukuda and Sibatani, 1953). Perhaps, polyploidization in Microtus during the first 50 postnatal days has not occurred.

In an attempt to explain the apparent decrease in liver DNA concentration, one could assume that in the rapidly growing liver, more cells per mg were analyzed in the S and G_2-period of the DNA cycle of mitosis than are "caught" in the more mature and less rapidly growing liver. This explanation could also serve as the reason other organs in this study have the same basic trend.

The greatest $b/\bar{Y}$ obtained for the kidney DNA regression (Fig. 17) was 0.112, and the concentration decreased rapidly from that point. One can visualize that, had the experiment been carried further in terms of acquiring older animals, the DNA concentration curve might have intersected the RNA regression line at a lower point because of a slower growth rate than that observed in this study. Two animals (not plotted), older than 5 months, had approximately 4.8 µg DNA/mg kidney; this concentration, by extrapolation of Fig. 17, would indicate that the growth of the kidney had not ceased at 50 days.

Since the kidney is not a secretory organ, and most of the ribose compounds (for energy transfer) in the kidney are small molecules that would be eliminated during the extraction procedure, the determined concentration of RNA should be low. The relative levels of DNA and RNA show that the protein synthetic capacity of the kidney is not great, as only maintenance of the organ is required of these constituents.

The heart regression lines of DNA and RNA (Fig. 18) are almost prallel and statistically inseparable. The indication here is that only enough RNA is produced at each stage of growth to be utilized in the synthesis of enzymes for aerobic metabolism and for the muscle proteins, actin and myosin. An interesting observation is that the asymptote of the heart weight curve (Fig. 5) is reached at approximately the same age as are the asymptotes of the nucleic acid concentrations for the heart (Figs. 8 and 13). The indication is that the decrease in the nucleic acid concentrations with age or with a decrease in growth rate is probably more a function of expanding myofibrils than of a decrease in the DNA cycle rate or mitotic activity.

The brain has, in Leblond's (1964) classification of cell types, been typed as static cell populations which are defined as "homogeneous groups of cells in which no mitotic activity can be detected" and in which "the total DNA remains constant". A characteristic of this type of cell population is the constant increase in cell volume that runs parallel to the growth of the organism (Levi, 1934; from De Robertis, 1965). The decrease in RNA and DNA concentrations is almost completely a function of an

inverse relationship with the volume of the brain cells. The relative concentrations of RNA and DNA obtained in this study are approximately parallel; the RNA concentration is about 3 μg/mg higher in all stages of growth (Fig. 19). When μg RNA/mg brain was plotted against age (Fig. 9), the plots followed a downward trend until about 25 days, at which time the concentrations increased. This increase occurred at approximately the same time that the animals were weaned; the pups nurse for 23 to 26 days unless another litter is born (Gier and Cooksey, 1967). RNA increase at weaning could be correlated with the learning processes of obtaining food, competing and agonistic behavior.

A striking similarity was found between the growth rate-nucleic acid regressions (Figs. 18-19) of heart and brain. Both organs are considered to be of the static cell populations variety. The trends obtained in this study could possibly serve as an index to this type of cell population.

Cells of the testes have been placed in the group of "renewing cell populations" (Leblond, 1964) as testes are "homogeneous groups of cells in which mitosis is abundant and exceeds that required for the total increase in DNA content". The large amounts of DNA found in the testes during all phases of growth can be correlated with the function of the organ, as well as with the structural changes that occur as maturity is reached. Seminiferous cords with tightly packed cells are found in the immature testes; but in the mature testis, there is a fluid-filled lumen in the seminiferous tubules. The mere presence of more cells in the same volume of tissue could account for more

DNA in the younger stages of testes growth. The DNA concentrations decrease rapidly with growth rate reduction; however, the lowest concentration is relatively high at the corresponding rate of growth and probably remains high throughout the animal's fertile life. One would expect to find high concentrations of DNA in the testes as the organ is little more than a DNA production factory.

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FIGURES

THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH ILLEGIBLE
PAGE NUMBERS
THAT ARE CUT OFF
OR MISSING.

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RECEIVED FROM
THE CUSTOMER.

Figure 1. Growth curves of Microtus ochrogaster fetal weight in mg and length of crown-rump in mm against time in days gestation.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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Fig. 1

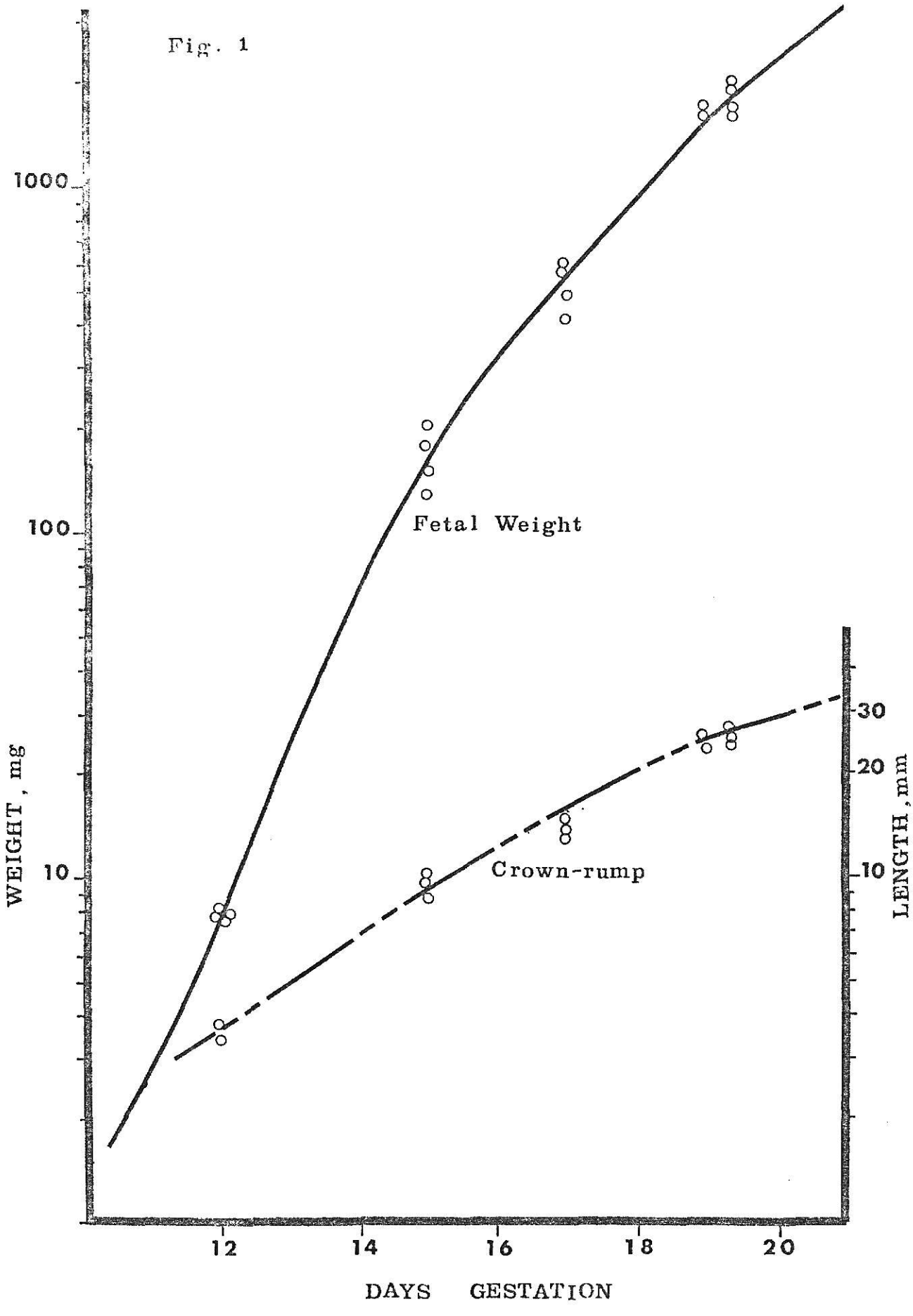

1000
100
10
WEIGHT, mg
Fetal Weight
Crown-rump
30
20
10
LENGTH, mm
12
14
16
18
20
DAYS GESTATION

Figure 2. Growth curves of Microtus ochrogaster total body weight in grams and total body length in mm against age in days. Points on the graph represent data in Table 1.

Fig. 2

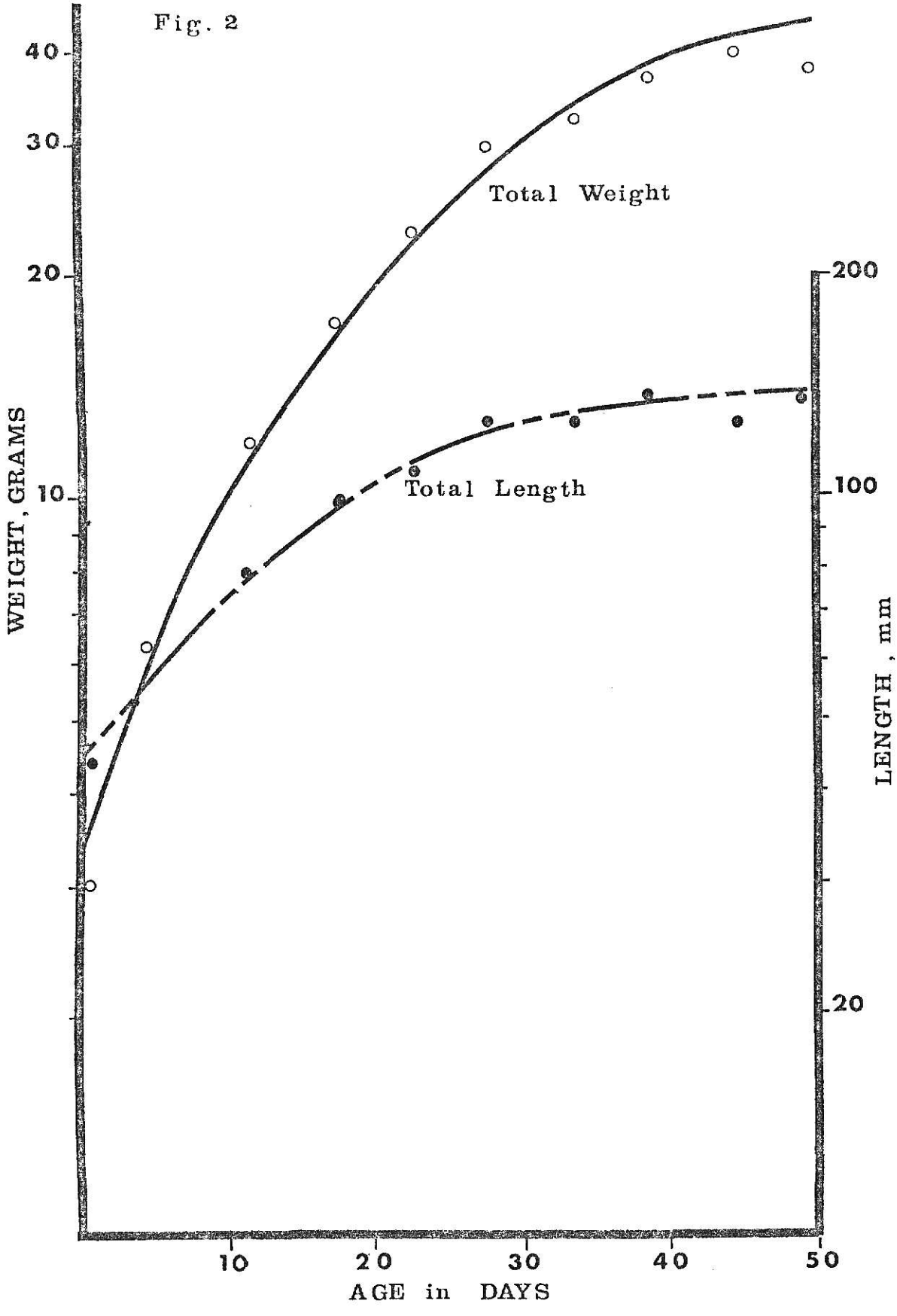

40
30
20
10
WEIGHT, GRAMS
Total Weight
Total Length
200
100
20
LENGTH, mm
10
20
30
40
50
AGE in DAYS

Figure 3. Growth curves of Microtus ochrogaster length of tail and length of hind foot in mm against age in days. Points on the graph represent data in Table 1.

Fig. 3

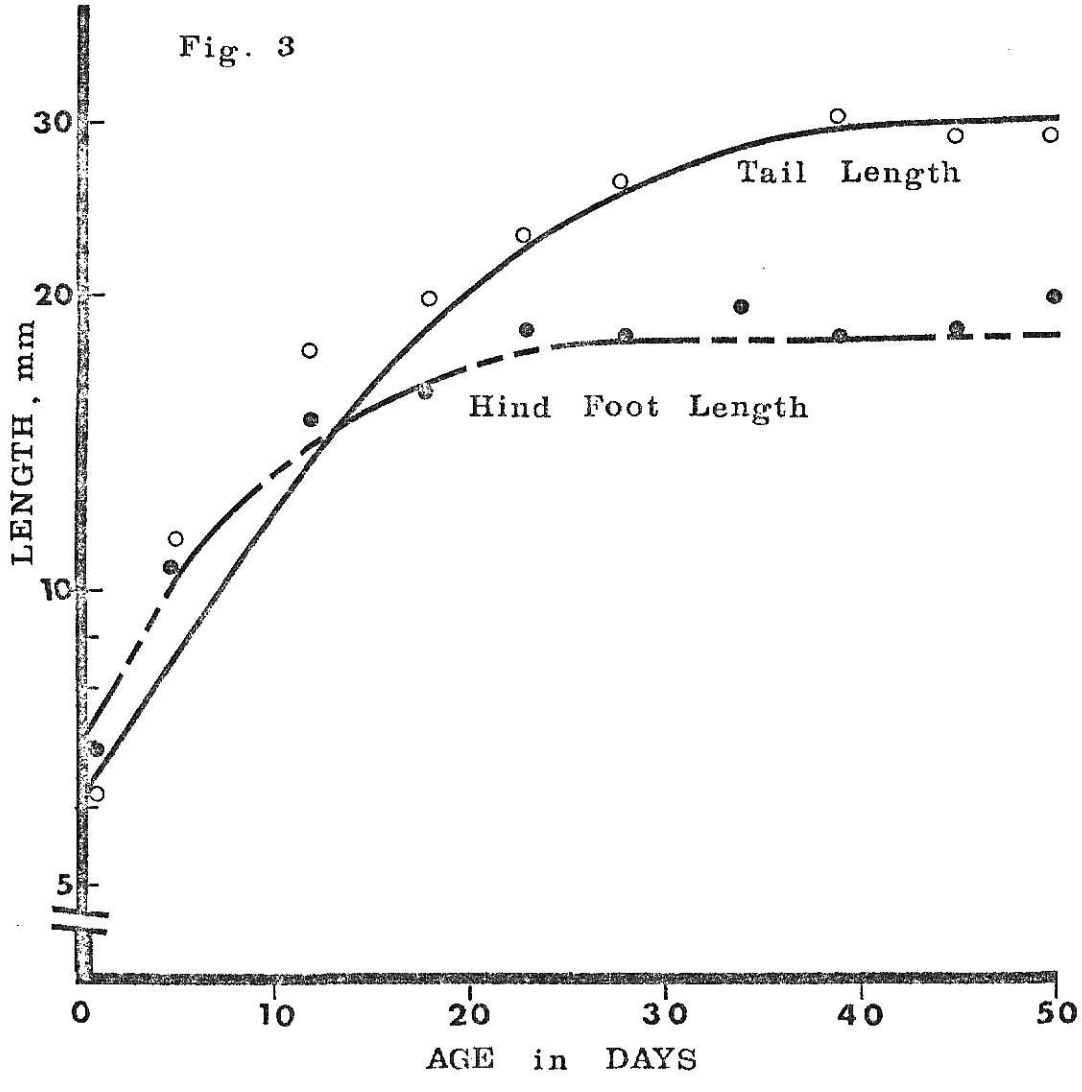

30
20
10
5
LENGTH, mm
Tail Length
Hind Foot Length
0
10
20
30
40
50
AGE in DAYS

Figure 4. Growth curves of Microtus ochrogaster weight of liver, kidney and brain in mg against age in days. Points on the graph represent data in Table 2.

Fig. 4

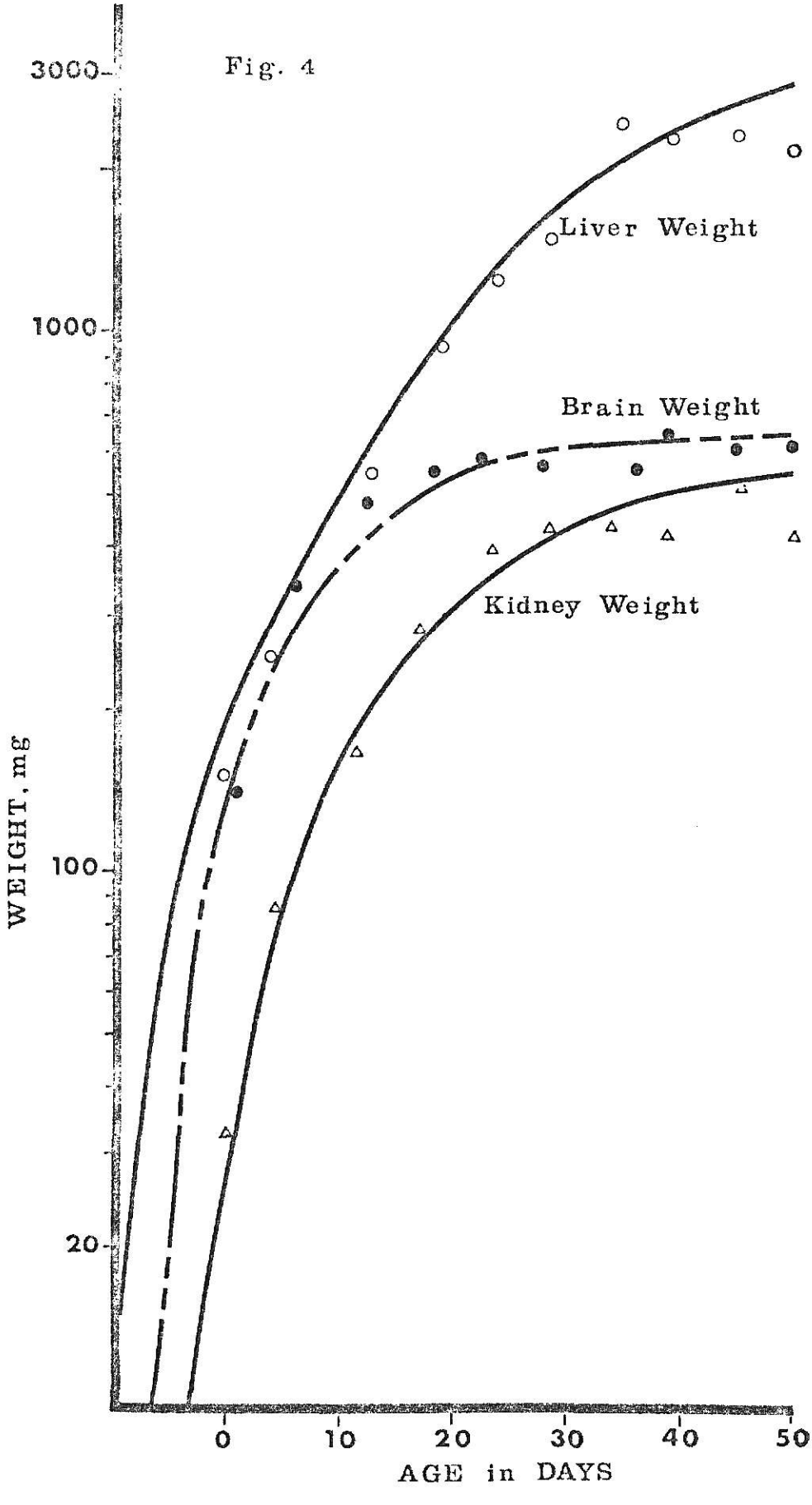

3000
1000
100
20
WEIGHT, mg
Liver Weight
Brain Weight
Kidney Weight
0
10
20
30
40
50
AGE in DAYS

Figure 5. Growth curves of Microtus ochrogaster weight of the heart and testes in mg against age in days. Points on the graph represent data in Table 2.

Fig. 5

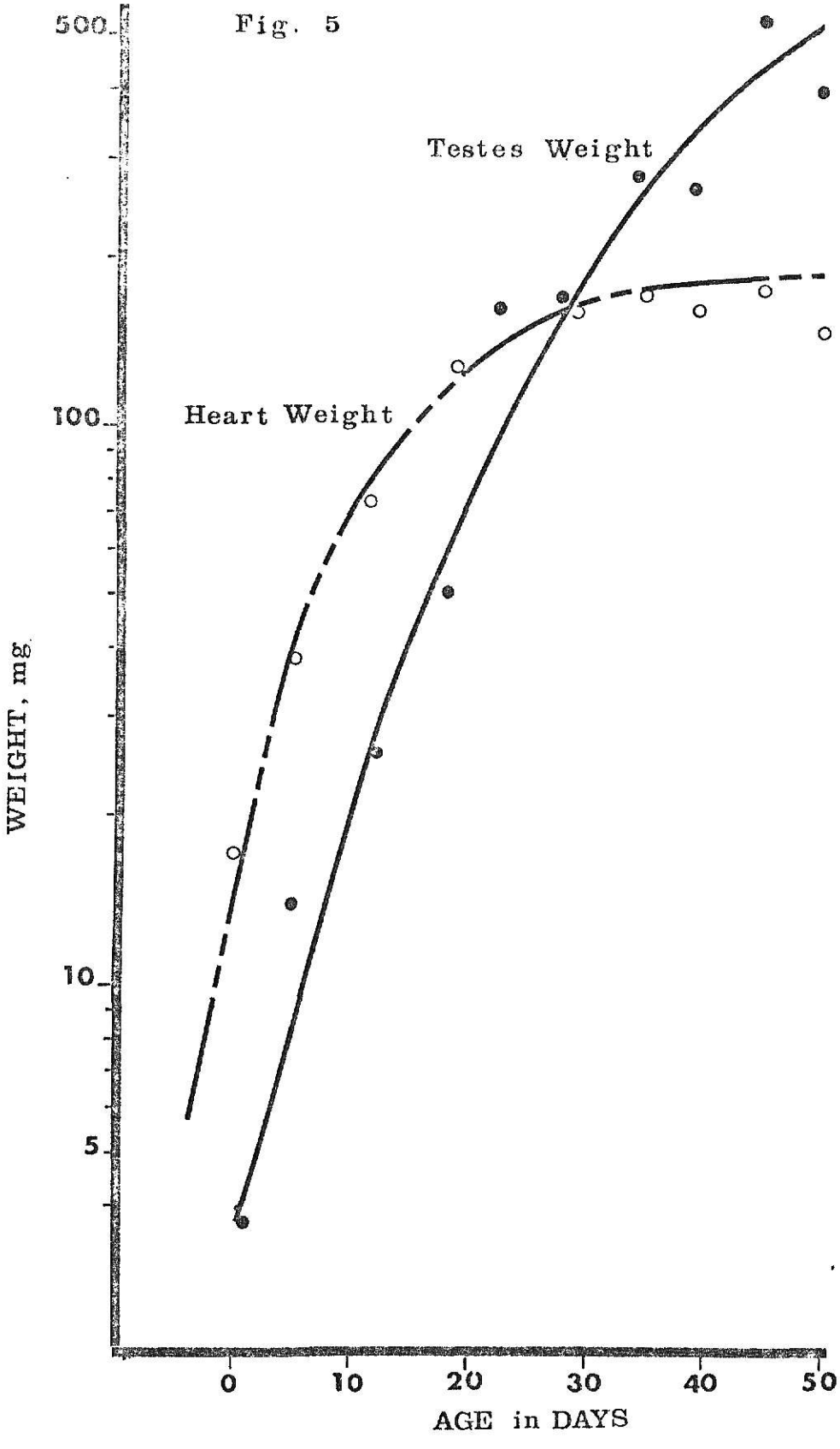

500
100
10
5
WEIGHT, mg
Testes Weight
Heart Weight
0
10
20
30
40
50
AGE in DAYS

Figure 6. Concentrations of µg RNA per mg liver of *Microtus ochrogaster* against age in days.

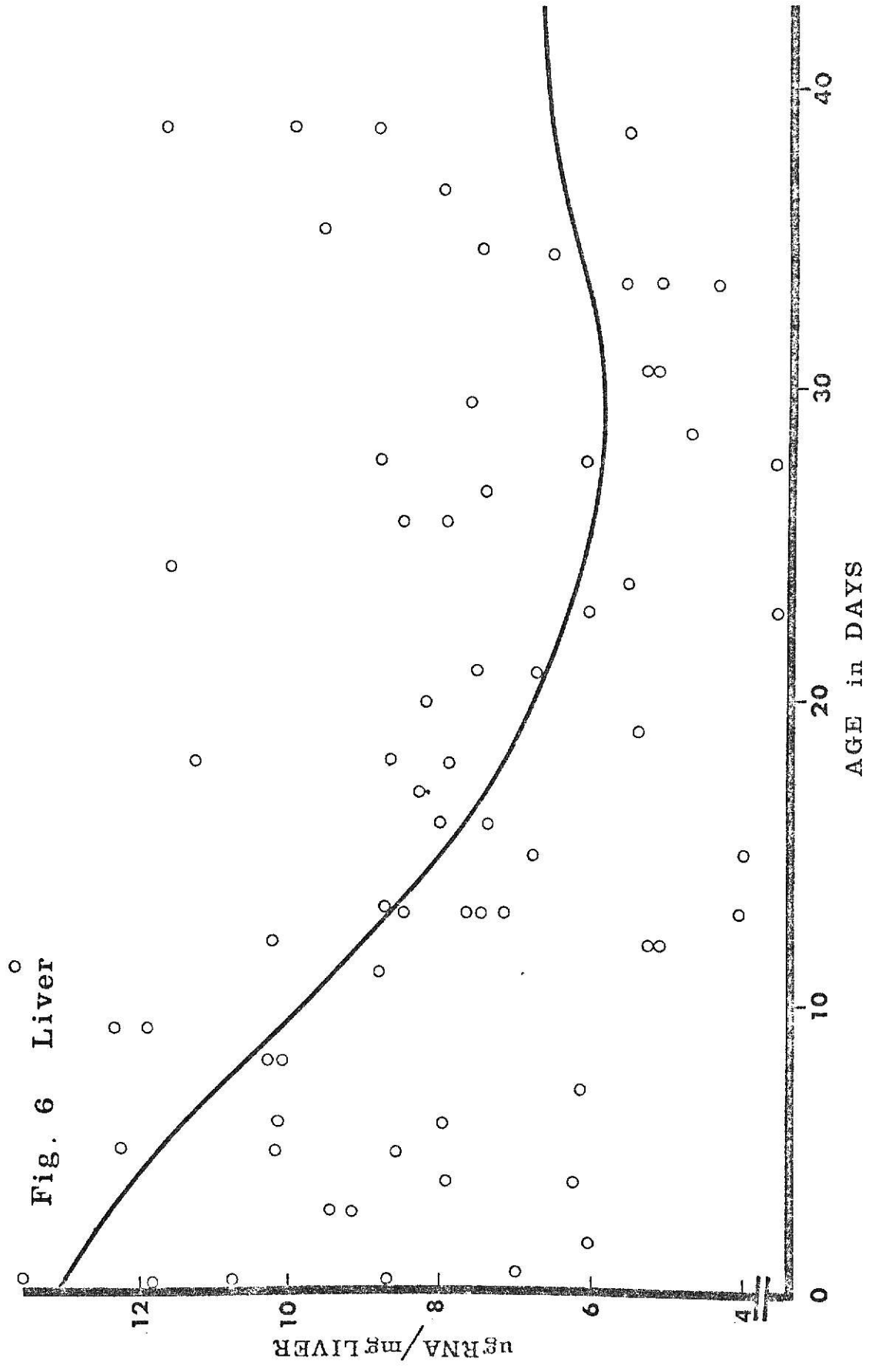


Fig. 6 Liver

Figure 7. Concentrations of µg RNA per mg kidney of *Microtus ochrogaster* against age in days.

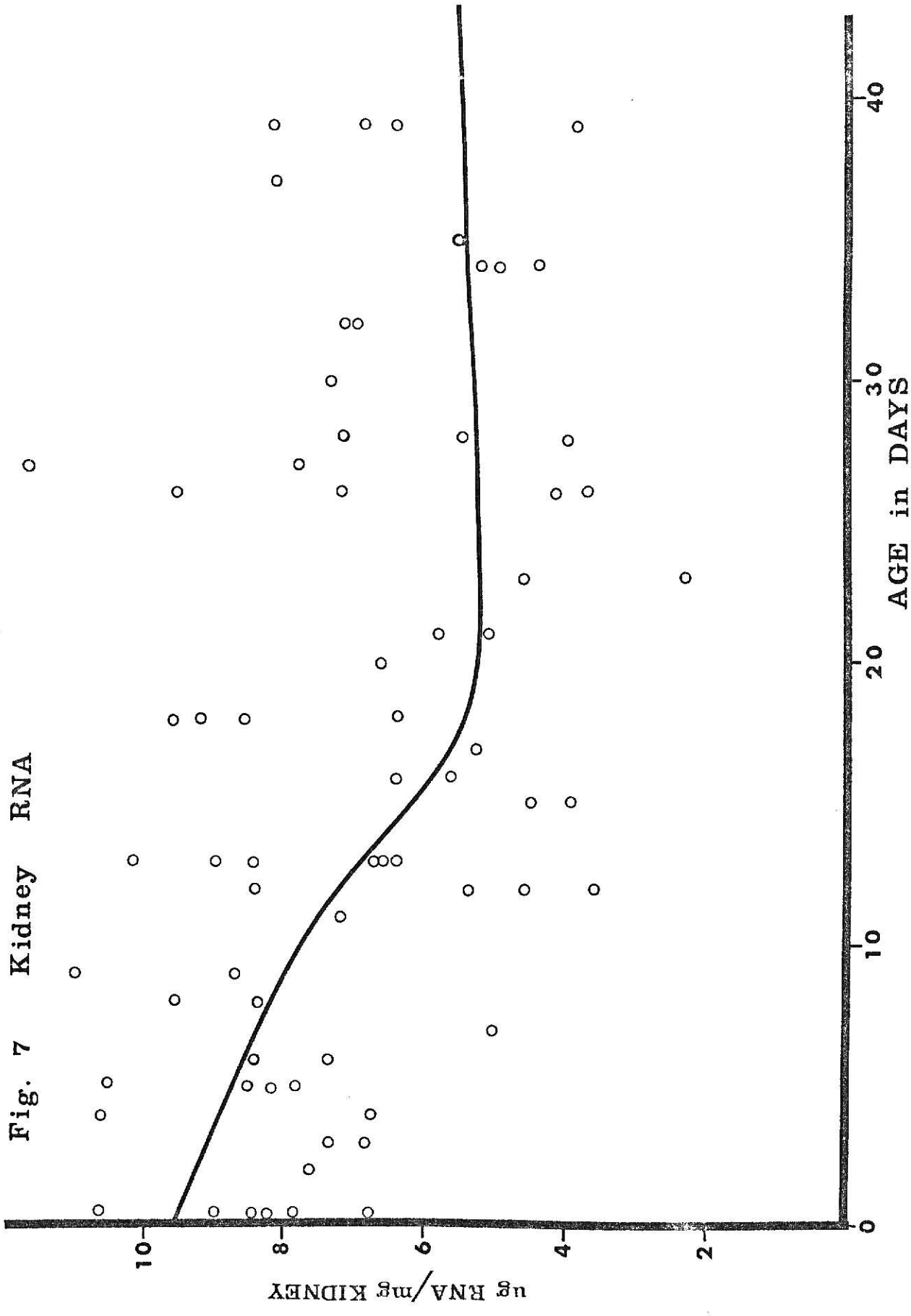


Fig. 7 Kidney RNA

Figure 8. Concentrations of μg RNA per mg heart of *Microtus ochrogaster* against age in age.

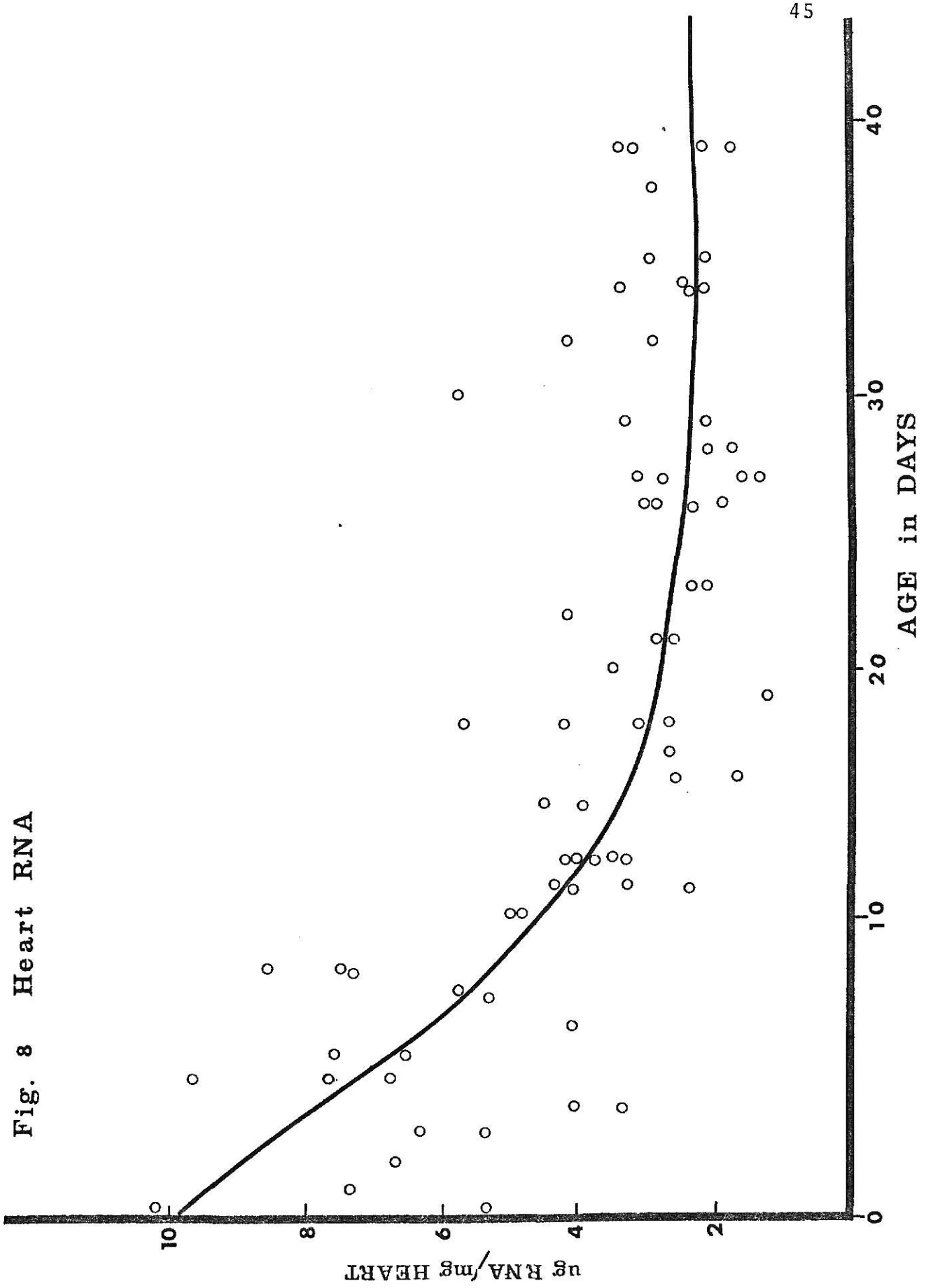


Fig. 8 Heart RNA
µg RNA/mg HEART
10
8
6
4
2
0
10
20
30
40
AGE in DAYS

Figure 9. Concentrations of μg RNA per mg brain of *Microtus ochrogaster* against age in days.

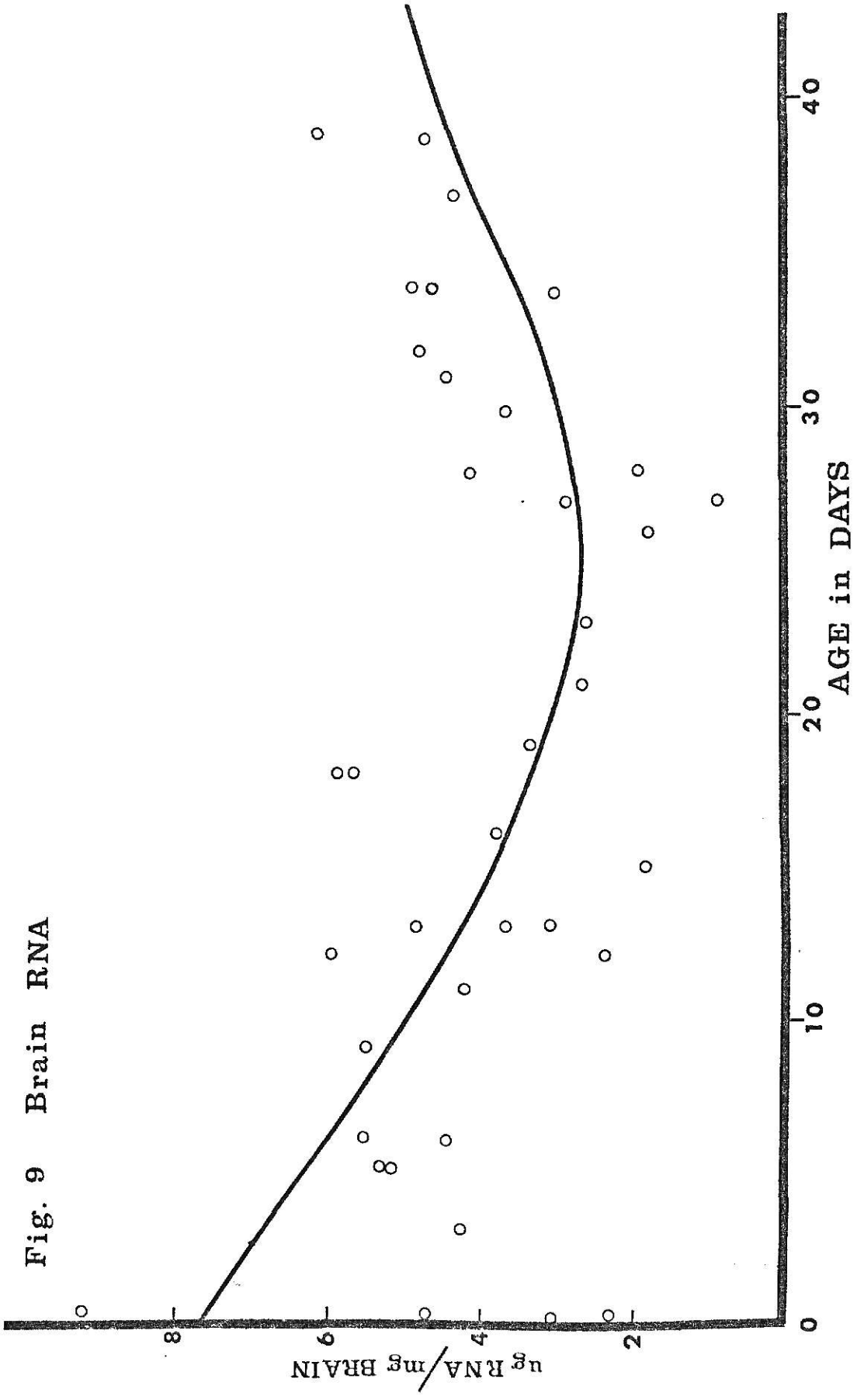


Fig. 9 Brain RNA

Figure 10. Concentrations of µg RNA per mg testes of Microtus ochrogaster against age in days.

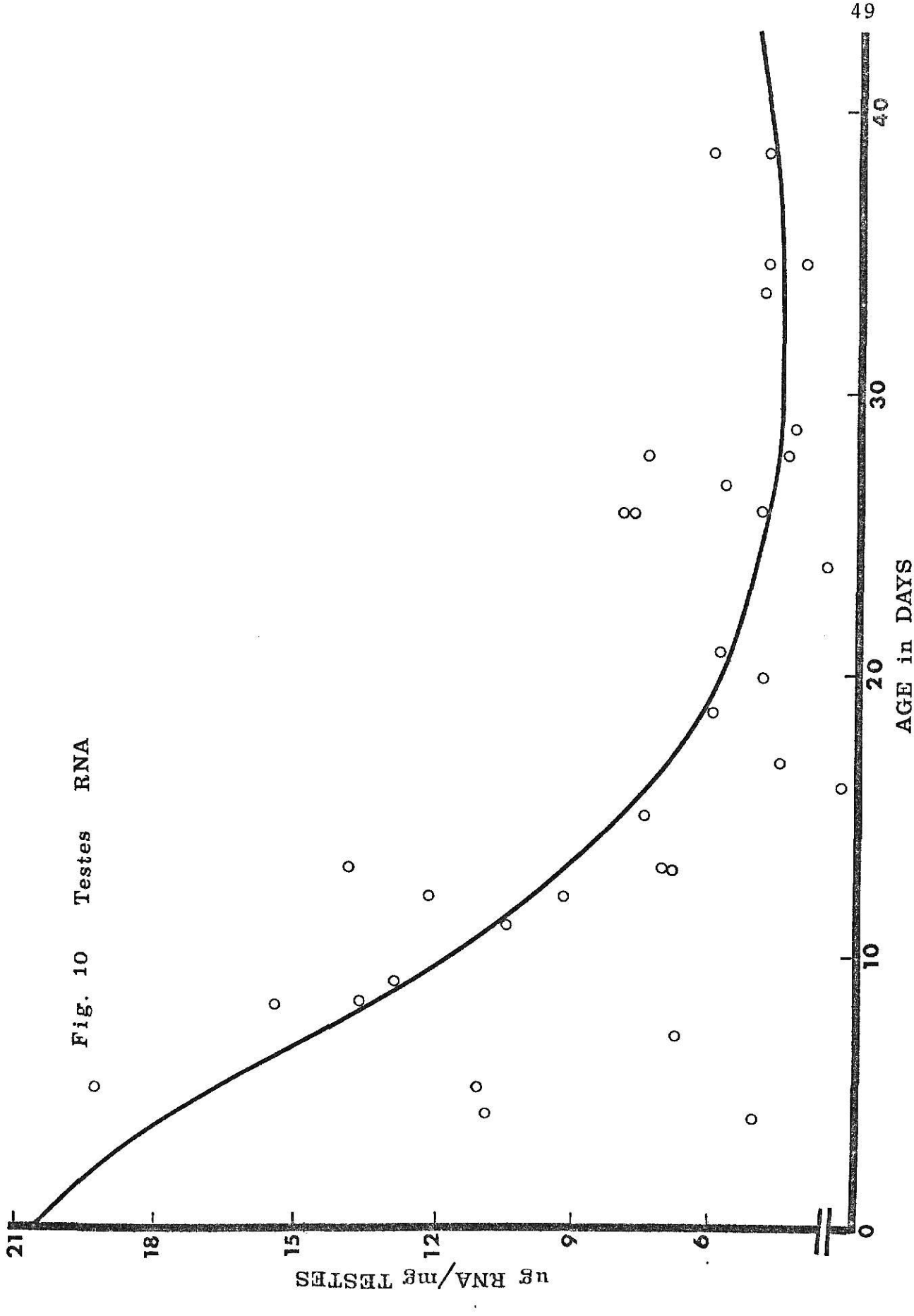


Fig. 10 Testes RNA

Figure 11. Concentrations of µg DNA per mg liver of Microtus ochrogaster against age in days.

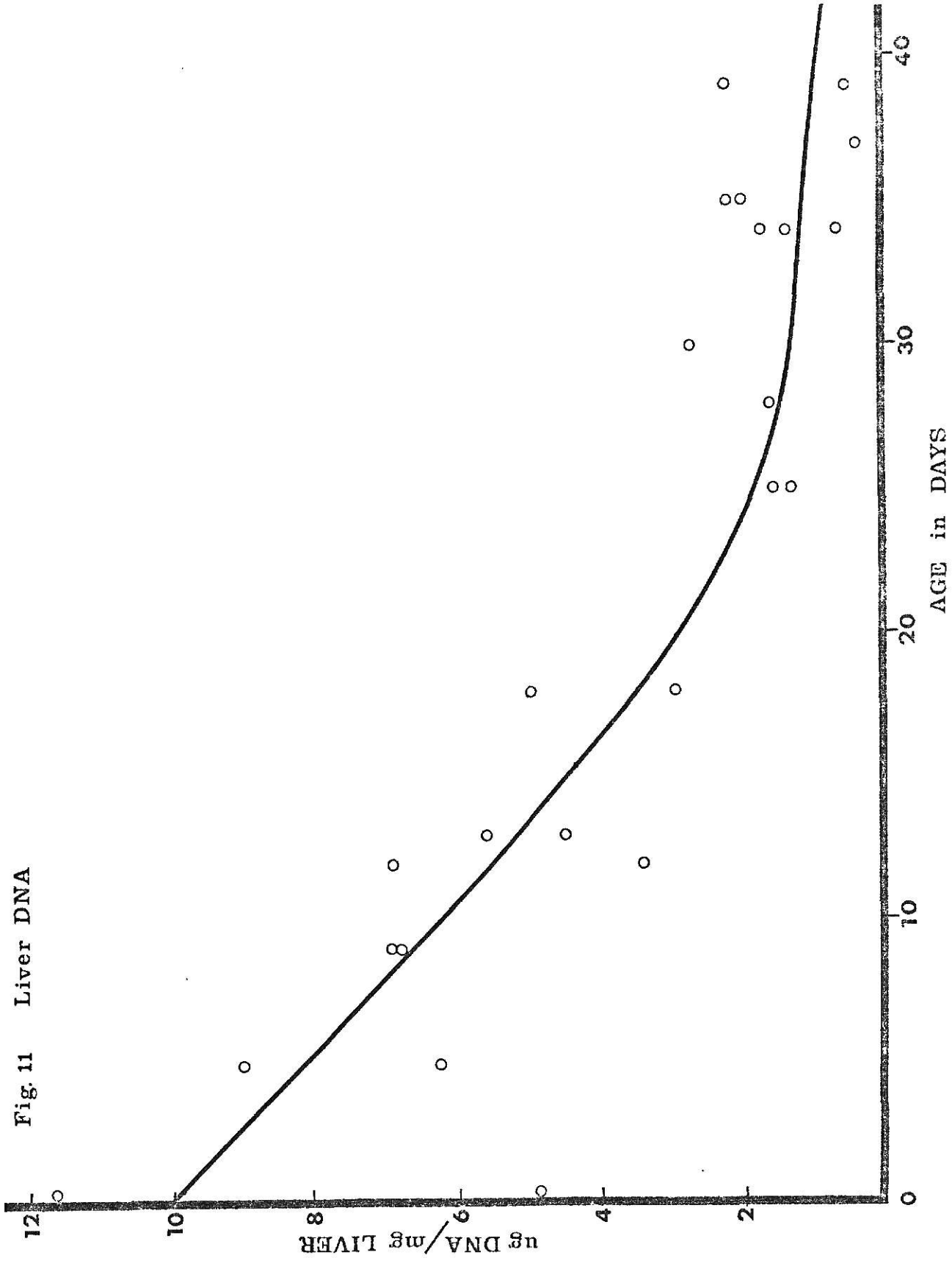


Fig. 11 Liver DNA

Figure 12. Concentrations of µg DNA per mg kidney of *Microtus ochrogaster* against age in days.

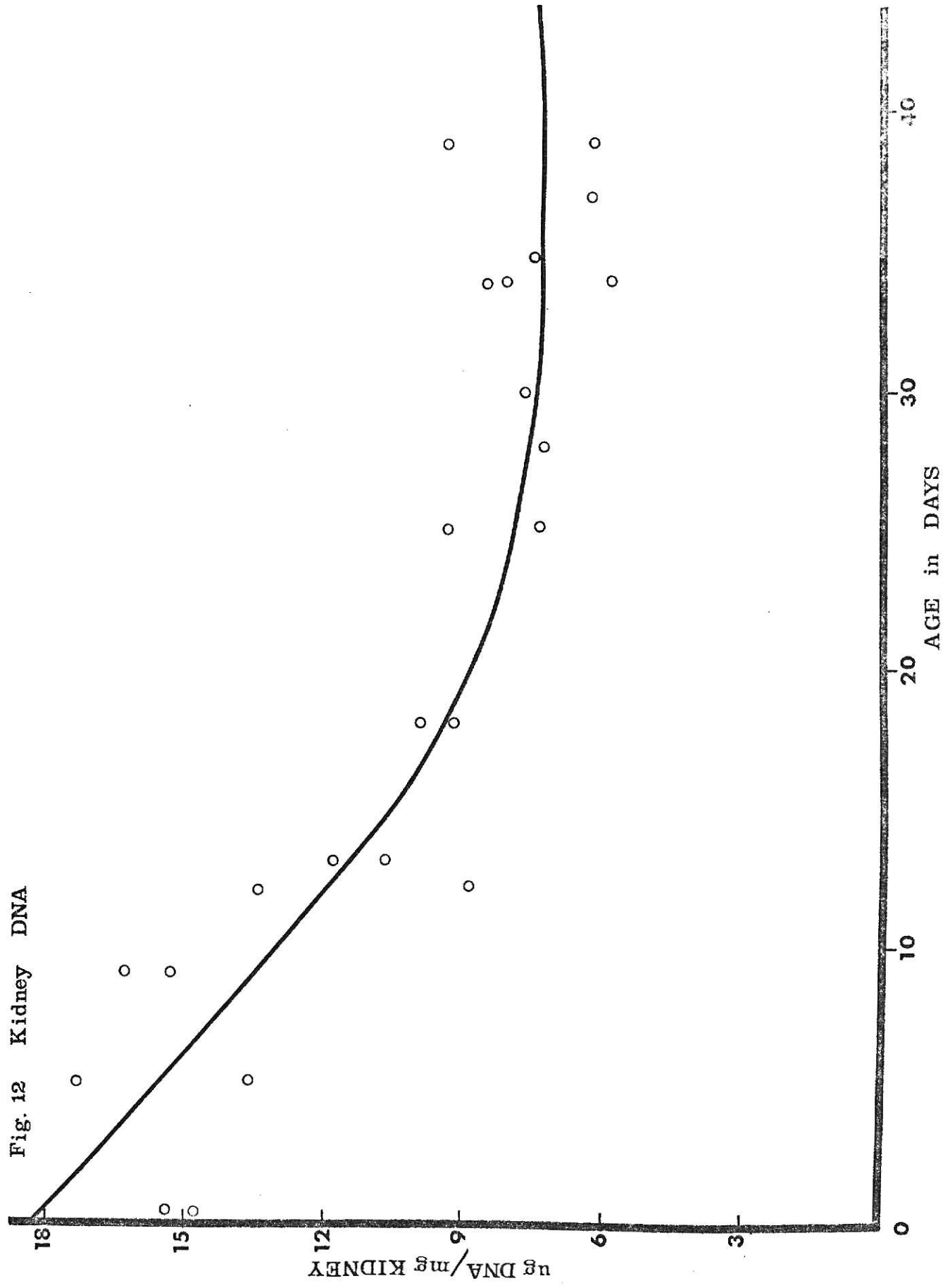


Fig. 12 Kidney DNA

Figure 13. Concentrations of μg DNA per mg heart of *Microtus ochrogaster* against age in days.

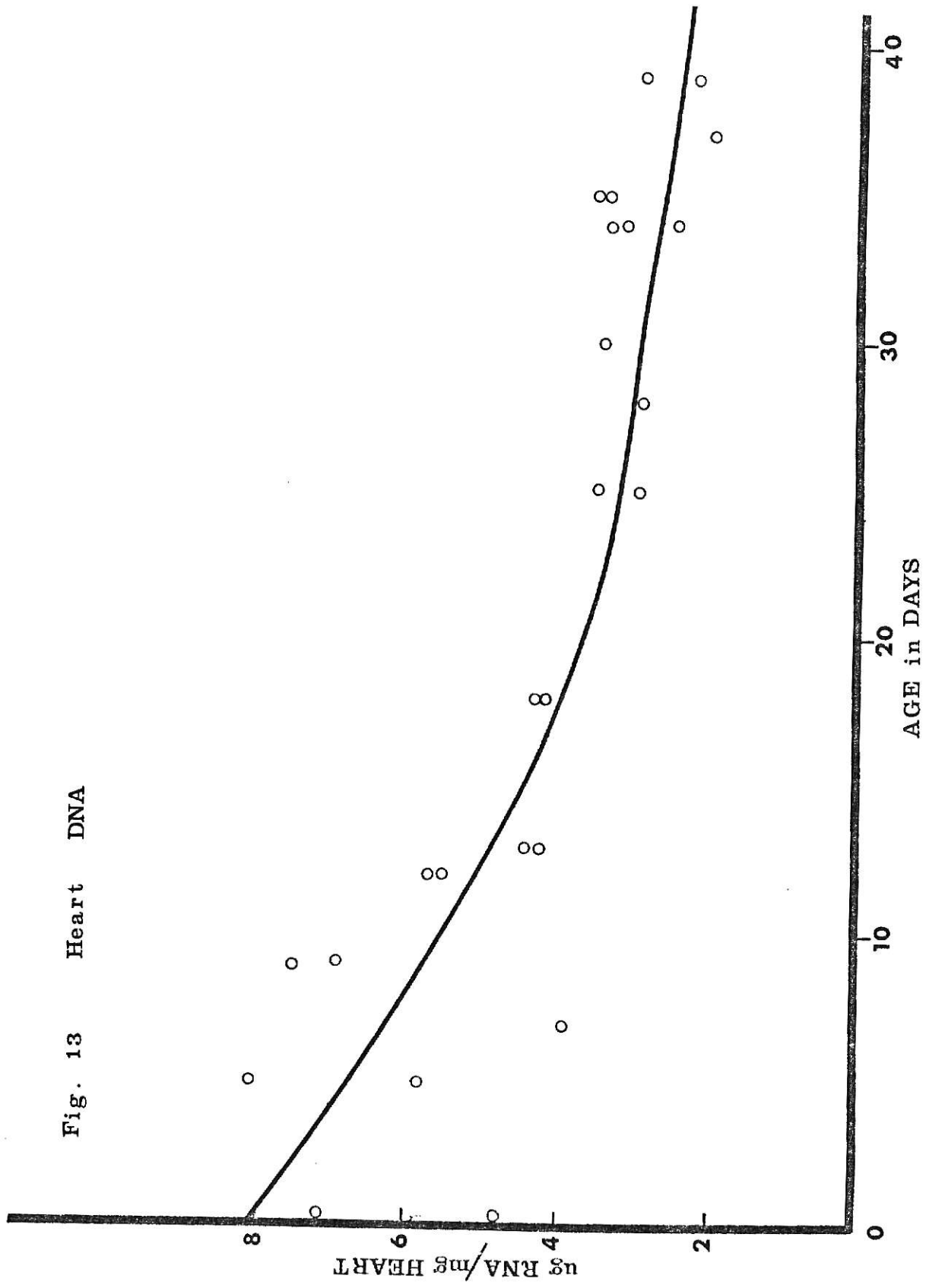


Fig. 13 Heart DNA

Figure 14. Concentrations of µg DNA per mg brain of *Microtus ochrogaster* against age in days.

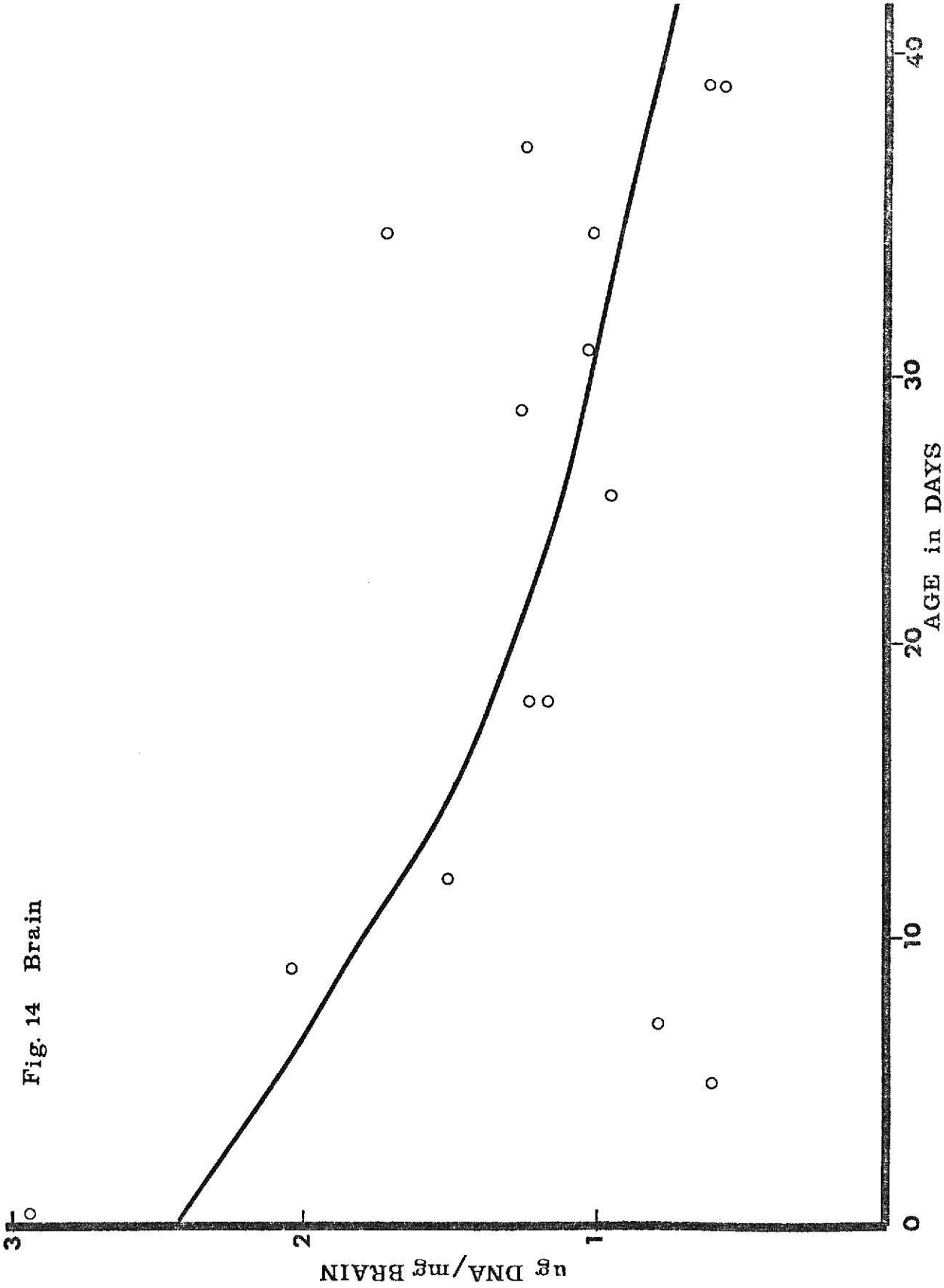


Fig. 14 Brain

Figure 15. Concentrations of µg DNA per mg testes of Microtus ochrogaster against age in days.

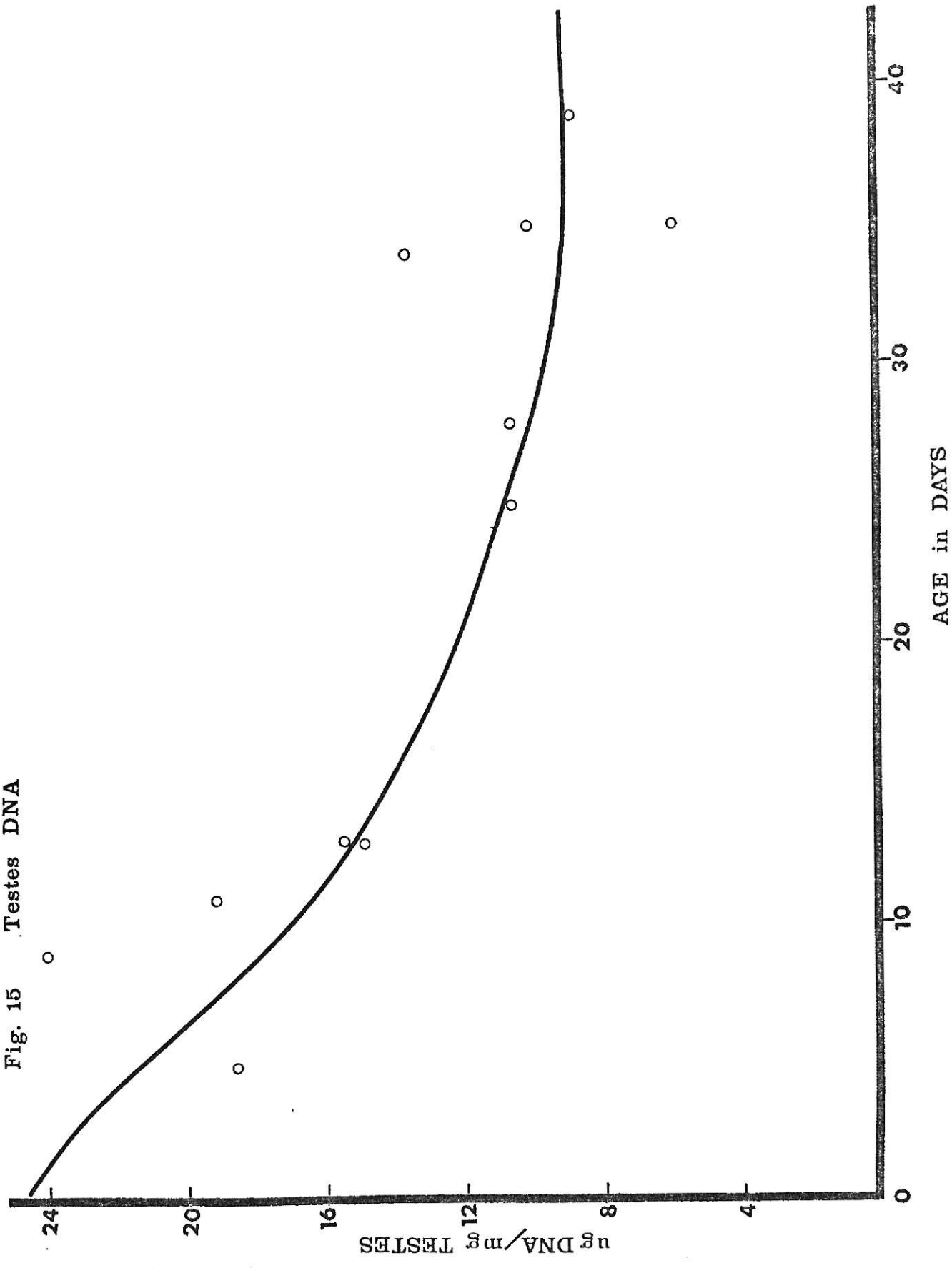


Fig. 15 Testes DNA

Figure 16. Concentrations of μg DNA and RNA per mg liver of Microtus ochrogaster against the relative growth rate of the liver.

Fig. 16 Liver

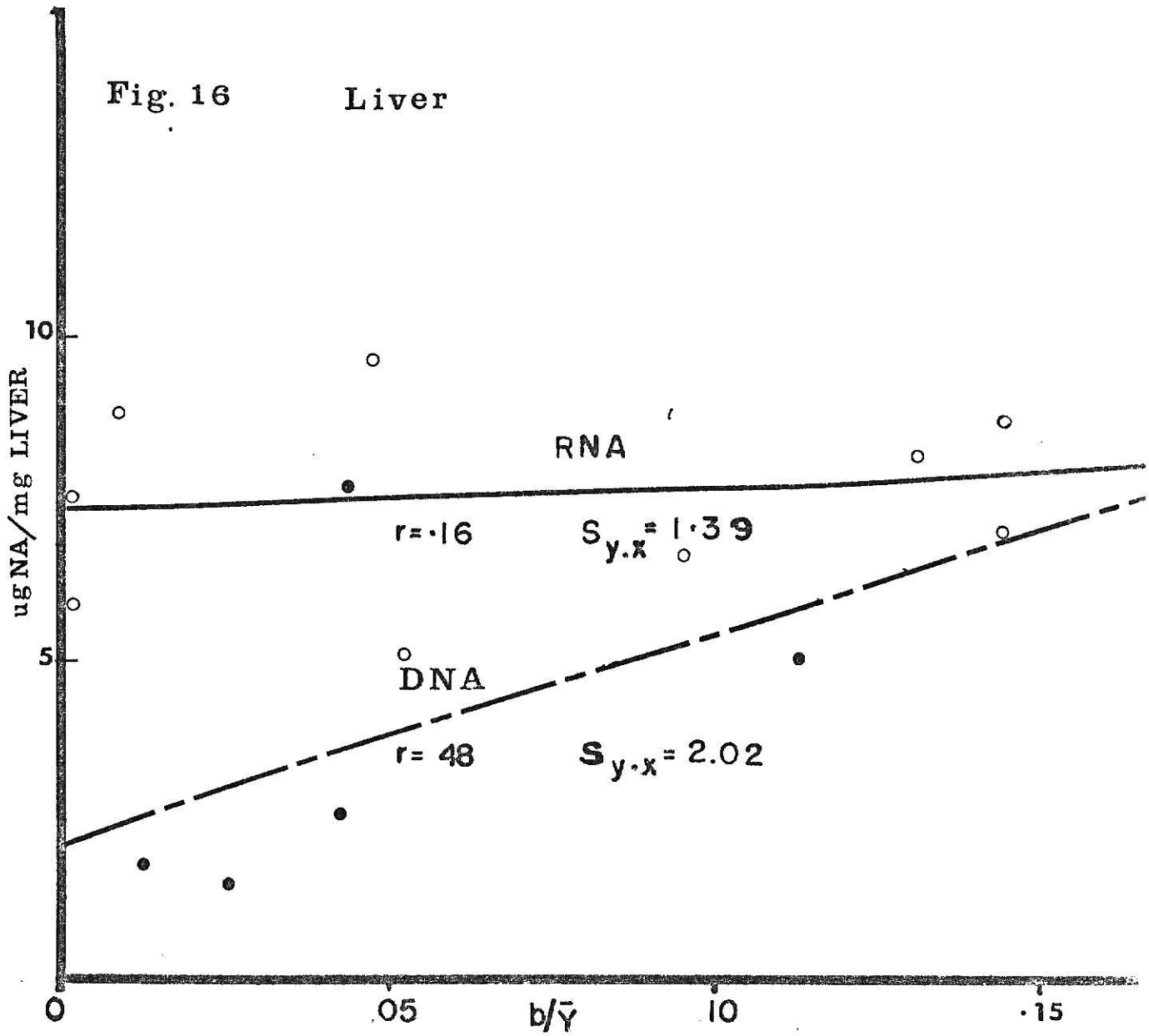

ugNA/mg LIVER
10
5
0
RNA
r=·16
$S_{y.x}$= 1·39
DNA
r= 48
$S_{y.x}$= 2.02
.05
b/$\bar{Y}$
10
·15

Figure 17. Concentrations of µg DNA and RNA per mg kidney of Microtus ochrogaster against the relative growth rate of the kidney.

Fig. 17 Kidney

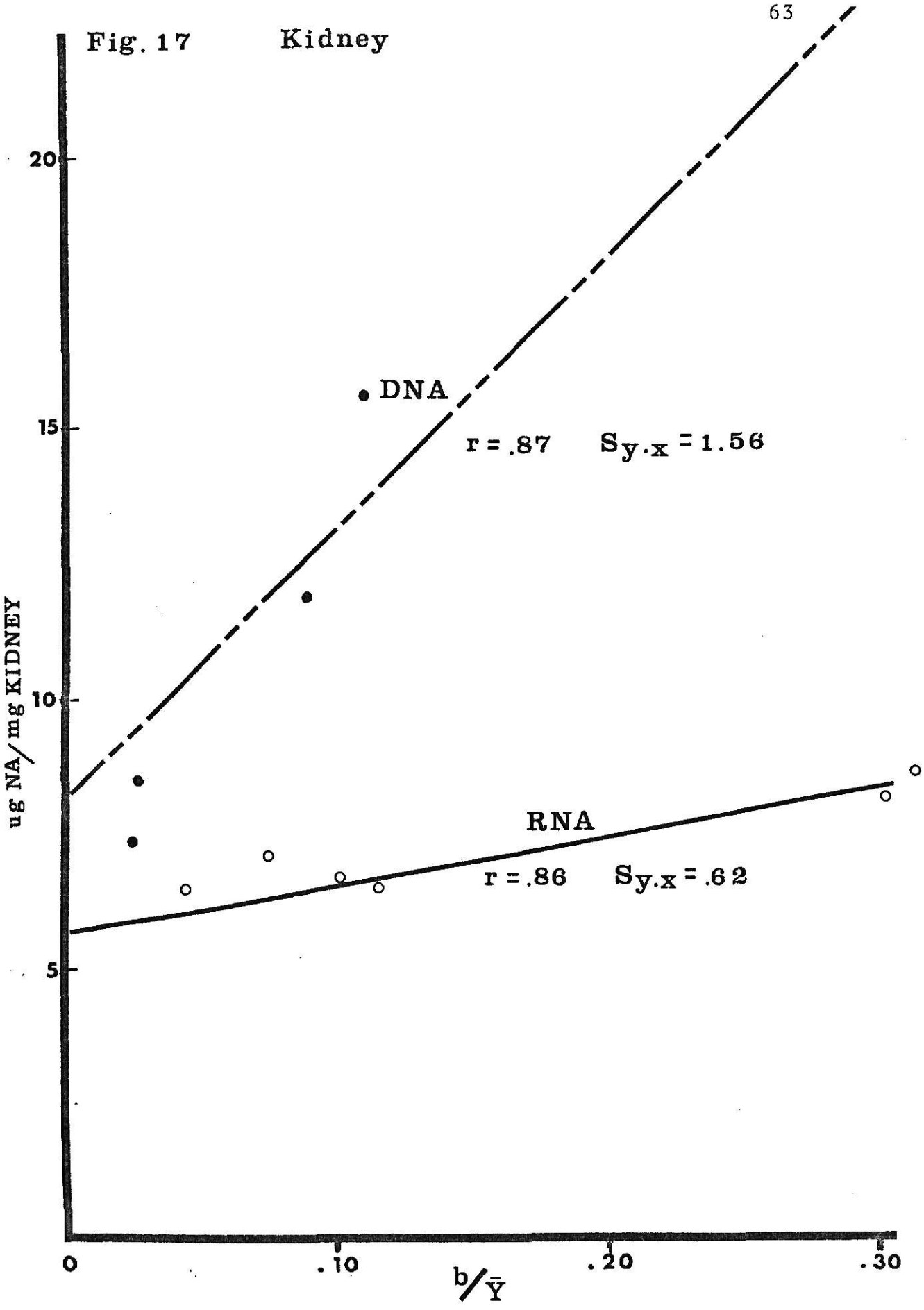


Figure 18. Concentrations of μg DNA and RNA per mg heart of Microtus ochrogaster against the relative growth rate of the heart.

Fig. 18 Heart

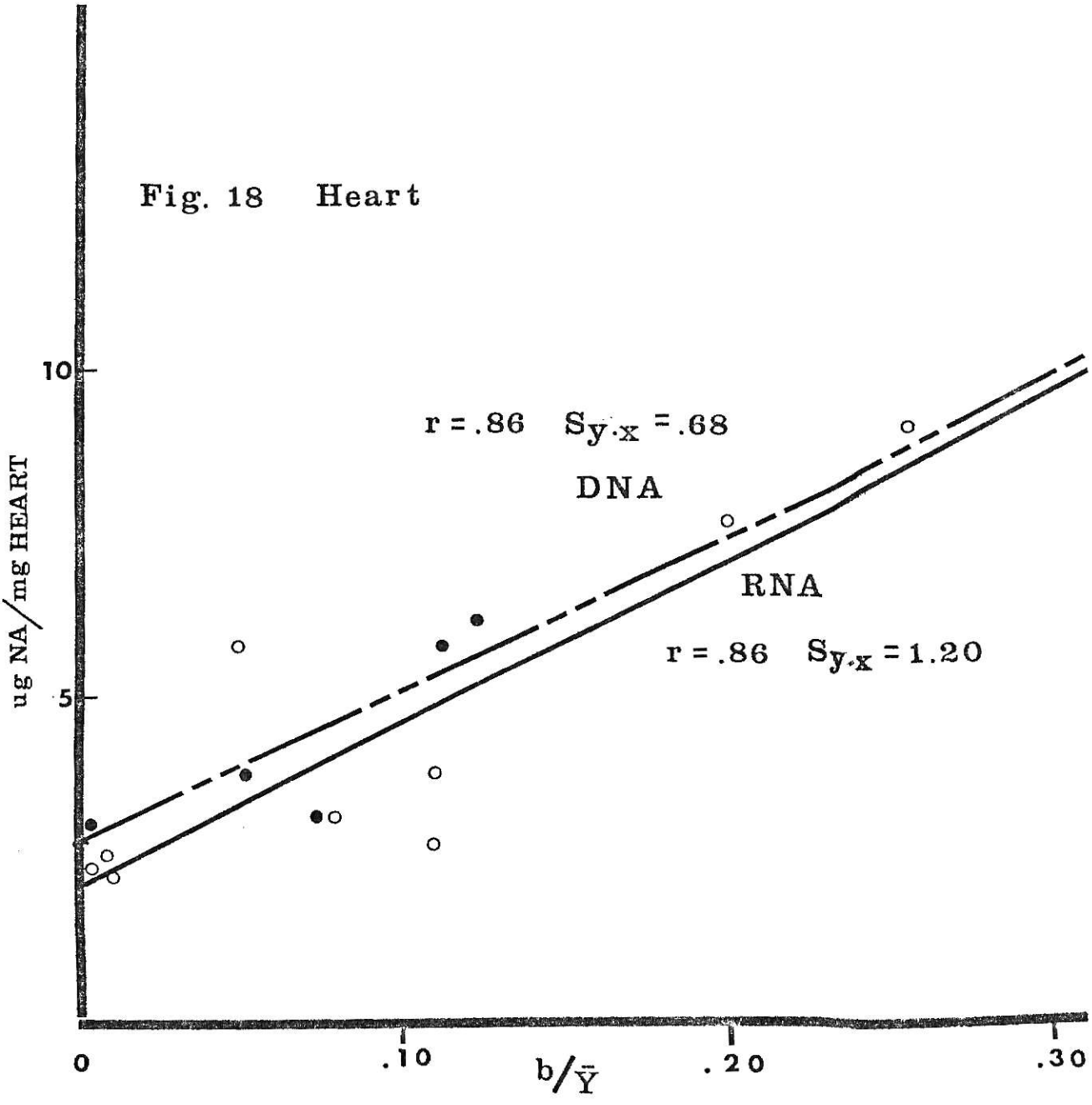

10
5
ug NA/mg HEART
r = .86 $S_{y \cdot x}$ = .68
DNA
RNA
r = .86 $S_{y \cdot x}$ = 1.20
0
.10
.20
.30
$b/\bar{Y}$

Figure 19. Concentrations of μg DNA and RNA per mg brain of Microtus ochrogaster against the relative growth rate of the brain.

Fig. 19 Brain

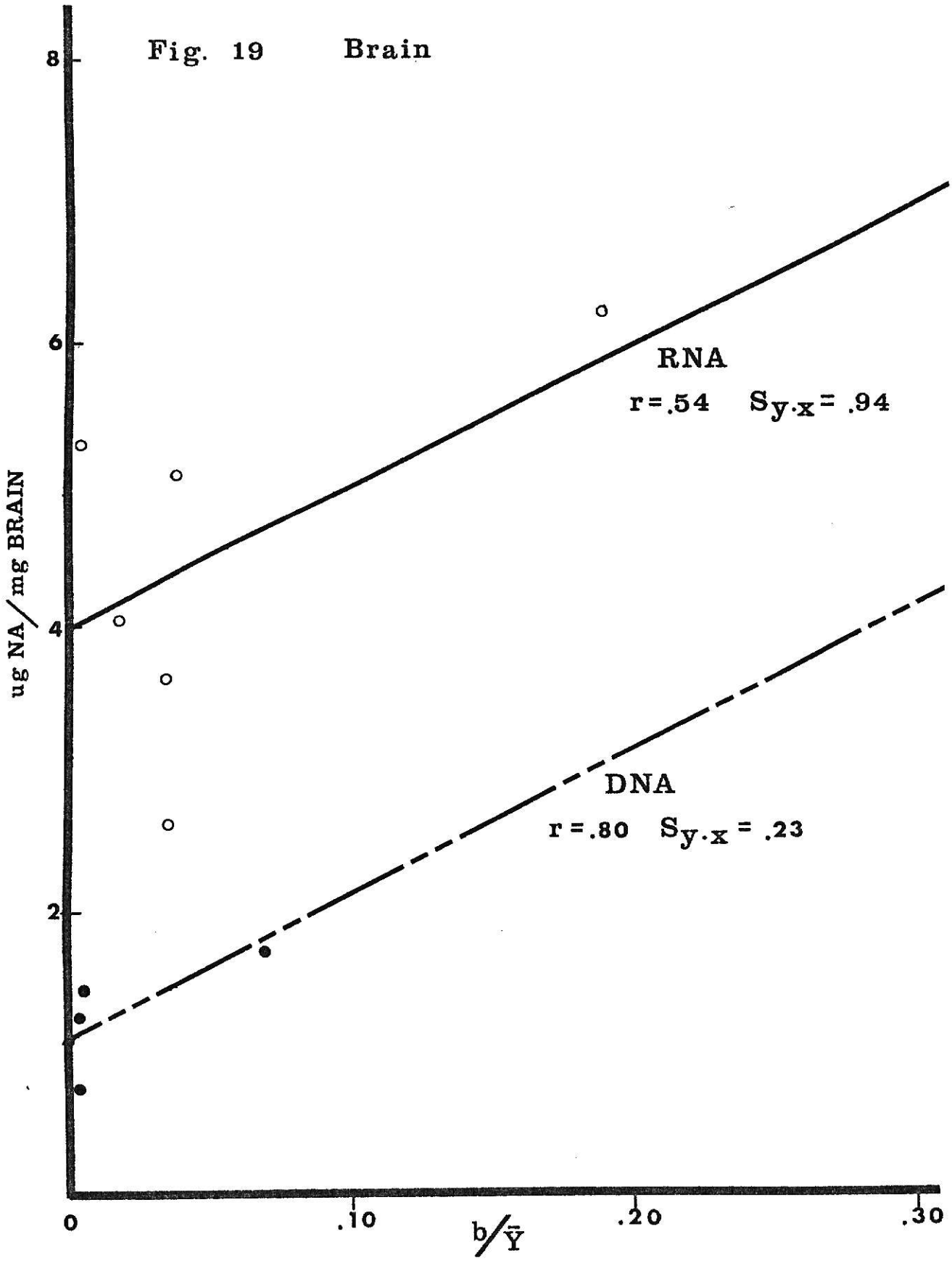
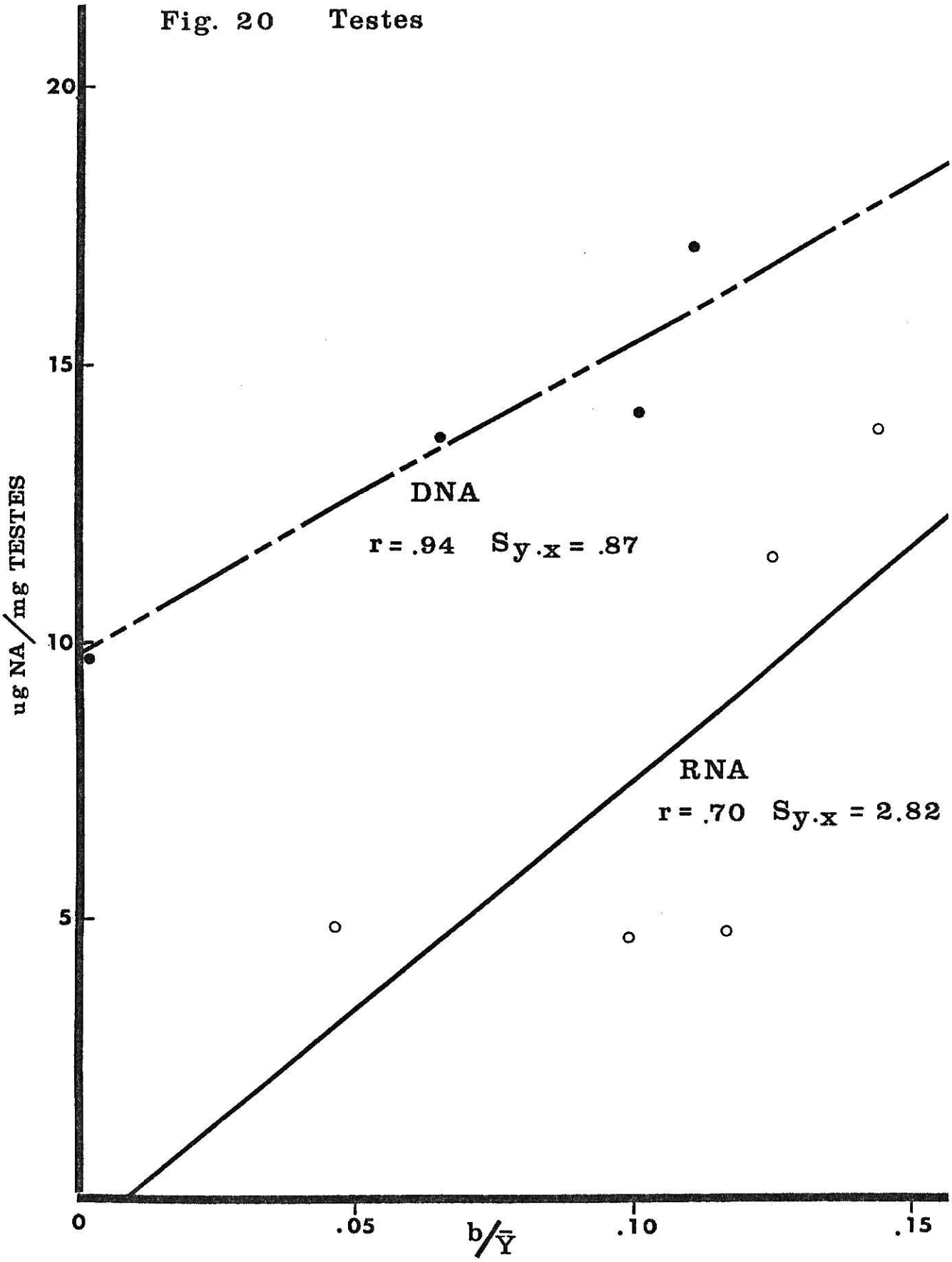


Figure 20. Concentrations of µg DNA and RNA per mg testes of Microtus ochrogaster against the relative growth rate of the testes.

Fig. 20 Testes



TABLES

TABLE 1. Average weights and length of Microtus ochrogaster at various stages during growth.

Age (days)	no.	Total Weight (g)	Total Length (mm)	Tail Length (mm)	Hind Foot (mm)
0	5	3.01 ± 0.10*	44.80 ± 0.97*	6.32 ± 0.42*	6.90 ± 0.25*
5	4	6.40 ± 0.89	59.75 ± 5.69	11.27 ± 0.65	10.72 ± 0.82
12	4	11.69 ± 1.15	84.50 ± 2.40	17.95 ± 0.58	14.55 ± 0.19
18	4	17.39 ± 0.83	97.75 ± 3.20	19.87 ± 1.97	16.75 ± 0.57
23	2	22.49 ± 0.69	108.00 ± 2.00	22.70 ± 0.40	16.70 ± 0.70
28	4	29.92 ± 1.83	125.50 ± 1.32	25.90 ± 1.03	18.20 ± 0.30
34	5	32.65 ± 1.51	126.00 ± 2.35	25.70 ± 1.60	17.54 ± 0.14
39	4	36.65 ± 2.40	134.25 ± 2.02	29.97 ± 1.30	19.15 ± 0.39
45	2	39.45 ± 7.55	126.50 ± 5.50	28.45 ± 5.65	17.75 ± 0.05
50	4	36.20 ± 1.94	136.25 ± 2.63	28.10 ± 1.71	19.32 ± 0.05

*Standard error of the mean.

TABLE 2. Average weights of organs of Microtus ochrogaster at various stages during growth.

Age (days)	no.	Liver (g)	Kidney (gm)	Heart (mg)
0	5	0.153 ± 0.020*	32.60 ± 1.96*	13.60 ± 0.40*
5	4	0.250 ± 0.034	87.75 + 7.71	38.50 ± 3.30
12	4	0.551 ± 0.069	162.50 ± 19.84	71.50 ± 7.18
18	4	0.945 ± 0.078	279.00 ± 12.52	124.50 ± 15.28
23	2	1.257 ± 0.177	397.50 ± 17.50	152.50 ± 7.50
28	4	1.493 ± 0.169	432.50 ± 42.30	167.50 ± 13.92
34	5	2.434 ± 0.097	435.20 ± 28.37	174.20 ± 12.98
39	4	2.395 ± 0.425	421.20 ± 34.54	161.70 ± 17.85
45	2	2.315 ± 0.475	515.00 ± 65.00	176.00 ± 4.00
50	4	2.200 ± 0.254	417.50 ± 41.31	140.25 ± 21.17

Age (days)	no.	Brain (mg)	no.	Testes (mg)
0	5	144.2 ± 6.26*	2	3.7 ± 1.1*
5	4	340.0 ± 20.41	2	12.0 ± 2.0
12	4	480.0 ± 3.54	2	26.5 ± 6.5
18	4	557.5 ± 10.31	2	50.0 ± 5.0
23	2	580.0 ± 20.00	1	160.0 ± ---
28	4	566.0 ± 22.86	3	168.3 ± 28.92
34	5	561.0 ± 23.58	2	279.0 ± 34.00
39	4	640.0 ± 10.60	2	265.0 ± 45.00
45	2	610.0 ± 10.00	1	510.0 ± ---
50	3	616.0 ± 27.28	1	390.0 ± ---

* Standard error of the mean.

TABLE 3. Average µg RNA/mg liver, kidney, heart, brain and testes during various time intervals, in days.

Days	no.	Liver	no.	Kidney	no.	Heart
0-5	13	9.47 ± 0.67*	14	8.40 ± 0.37*	14	7.90 ± 1.01*
6-10	8	9.22 ± 1.04	8	8.07 ± 0.68	8	6.51 ± 0.53
11-15	14	7.42 ± 0.71	13	6.55 ± 0.57	14	3.64 ± 0.26
16-20	9	8.90 ± 0.84	9	7.01 ± 0.57	9	3.06 ± 0.45
21-25	5	6.00 ± 0.68	5	4.43 ± 0.61	4	2.48 ± 0.13
26-30	11	6.27 ± 0.68	13	5.85 ± 0.80	13	2.64 ± 0.32
31-35	8	5.47 ± 0.46	7	5.41 ± 0.50	9	2.74 ± 0.24
36-40	5	9.06 ± 1.04	5	6.77 ± 0.81	5	2.68 ± 0.40
41-50	4	7.18 ± 0.39	4	6.07 ± 0.49	4	2.66 ± 0.09

Days	no.	Brain	no.	Testes
0-5	8	5.96 ± 1.30*	5	14.95 ± 4.22*
6-10	3	5.25 ± 0.30	4	15.10 ± 3.65
11-15	7	3.71 ± 0.54	7	9.69 ± 0.97
16-20	4	4.73 ± 0.68	4	4.69 ± 0.58
21-25	2	2.63 ± 0.03	3	3.92 ± 1.06
26-30	6	2.57 ± 0.53	8	5.73 ± 0.69
31-35	5	4.35 ± 0.33	4	3.65 ± 0.57
36-40	4	5.67 ± 0.69	2	5.70 ± 0.82
41-50	2	4.62 ± 0.90	2	5.45 ± 0.11

*Standard error of the mean.

TABLE 4. Average µg DNA/mg of liver, kidney, heart, brain and testes during time intervals, in days.

Days	no.	Liver	no.	Kidney	no.	Heart
0-5	4	7.96 ± 1.51*	4	15.36 ± 0.81*	4	6.55 ± 0.75*
6-10	2	6.82 ± 0.04	2	16.00 ± 0.54	3	6.23 ± 1.34
11-15	4	5.07 ± 0.73	4	11.30 ± 1.00	4	5.06 ± 0.39
15-20	2	3.99 ± 1.00	2	9.67 ± 0.35	2	4.39 ± 0.00
21-25	2	1.46 ± 0.12	2	8.36 ± 1.04	2	3.30 ± 0.29
26-30	2	2.17 ± 0.55	2	7.47 ± 0.22	2	3.05 ± 0.47
31-35	5	1.60 ± 0.26	4	7.51 ± 0.60	5	3.28 ± 0.20
36-40	3	1.07 ± 0.61	3	7.30 ± 1.07	3	2.42 ± 0.29
41-50	2	0.41 ± 0.20	2	7.75 ± 0.05	2	2.02 ± 0.24

Days	no.	Brain	no.	Testes
0-5	2	2.22 ± 1.61*	2	14.14 ± 4.45*
6-10	2	1.42 ± 0.63	1	24.08 ± ---
11-15	1	1.53 ± ---	3	16.69 ± 1.62
16-20	2	1.71 ± 0.03	0	---
21-25	1	0.95 ± ---	1	10.50 ± ---
26-30	2	1.15 ± 0.12	1	10.50 ± ---
31-35	2	1.37 ± 0.34	3	9.79 ± 2.20
36-40	3	0.80 ± 0.22	1	8.93 ± ---
41-50	2	0.59 ± 0.07	0	---

* Standard error of the mean.

GROWTH RATES OF ORGANS OF MICROTUS OCHROGASTER WITH CHANGES IN NUCLEIC ACID CONCENTRATIONS

by

RANDALL KENT HAHN

B.S., Kansas State University, 1968

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1970

ABSTRACT

Growth curves were established for (1) the total body weights of Microtus ochrogaster from the 12th day of gestation to the 50th postnatal day, (2) crown-rump length of the fetuses, and, (3) total length of postnatal animals. Growth curves also were established from measurements of the length of the hind foot and length of the tail of animals from birth to day 50. Graphs of the weight changes of the liver, kidney, heart, brain and testes were established and the external dimensions of these organs (except liver) given.

Extractions of RNA and DNA from known wet weights of liver, kidney, heart, brain (cerebrum) and testes were performed, the RNA and DNA quantitated colorimetrically from these extractions and the amount of RNA and DNA present per mg tissue was calculated. These concentration levels were graphically plotted against the age of the animal from which the tissue was obtained. With the exception of the brain, definite trends were observed of decreasing concentrations of both the nucleic acids with increasing age. These trend lines ultimately stabilized. In the brain, RNA levels decreased to under 3 μg/mg then increased.

Relative growth rates of liver, kidney, heart, brain and testes were determined by partitioning 6 to 8 points on the growth curve of an organ and obtaining the absolute growth rate (slope, b) of each partition. The slope was then divided by the mean weight of the organs within that partition. The relative growth rate was plotted against the average nucleic acid

concentrations of the organs of the respective partition and linear regressions computed. Concentrations of DNA and RNA in the kidney, heart and testes were correlated with the relative growth rates of those organs. Although no correlations were found between growth rate and nucleic acid concentrations of liver and brain, relative levels of DNA and RNA were determined and plotted.

Functional and structural explanations for the relative concentrations of nucleic acids and the correlations drawn were discussed. Because of the high protein synthetic capacity that is required of liver tissue, liver RNA exceeded DNA concentrations in all ages studied. It was proposed that the nucleic acid constituents of the kidney serve only to maintain the organ, as the RNA/DNA ratio was never greater than 0.70 during all stages of growth studied. Heart tissue had approximately 0.5 μg/mg more DNA than RNA, and brain tissue generally had 3 μg/mg more RNA than DNA during all relative growth-rate intervals; this indicated an increase in the size of the cells in this growth period rather than high mitotic activity from 0 to 50 days of age. A decrease in testicular DNA is explained as a function of the structural changes that occur in that organ; i.e. a change from tightly-packed cord cells in the younger animals to the expansion of the testes with lumen formation in the puberal animals.