NUCLEAR VOLUME AND CHROMOSOME NUMBER IN RELATION TO PLOIDY DISTRIBUTION IN THE LIVER OF THE MOUSE

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURES PAGE

CHAPTER I

INTRODUCTION

There has been considerable interest in the occurrence, in the mammalian liver, of parenchymal nuclei which fall into various size classes. Evidence has been presented that nuclear volume in the liver parenchymal nuclei follows a discontinuous pattern such that the modes of nuclear volume make a geometric series 1:2:4:8.

Various investigators have attempted to relate the geometric progression in nuclear volume with polyploidy. The ploidy of a cell refers to the number of haploid sets of chromosomes present in the nucleus. It is considered that most somatic cells have a 2N ploidy known as the diploid condition. The liver is reported to have many cells of 4N and some of 8N ploidy known as tetraploid and octoploid cells. From our knowledge of mitosis and endomitosis it is a logical assumption that increase or decrease in chromosome complement would follow a geometric pattern. Because of this common factor (i.e. increasing by geometric progression) many investigators have assumed that nuclear volume is indicative of chromosome number or ploidy.

Preliminary studies of mouse liver nuclei at the Drake University cancer research laboratory have shown intermediate nuclear volume values which might indicate the presence of odd ploidy groups. Since

odd ploidy groups do not fit into the assumption that chromosome number increases geometrically it was suggested that a more intensive study of the ploidy distribution of the mouse liver, as ascertained by both chromosome counts and nuclear volumes, would be of value.

The present study is to determine if a relationship exists between the frequency distribution of nuclear volume and the frequency distribution of chromosome number in the liver of the mouse.

CHAPTER II

REVIEW OF THE LITERATURE

Many methods have been used to determine the chromosome number in a resting nucleus. Since the liver is mitotically inert some method other than direct chromosome counts is needed in the study of its ploidy distribution under varying experimental conditions.

Jacobj, as cited by Sulkin,[1] was the first investigator to note a discontinuous pattern in the modes of nuclear volume in the liver of mice and rats. By plotting the nuclear volumes against their frequency, he obtained a curve which had definite modes. He concluded that there were various sizes of nuclei, their volumes being in the ratio of 1:2:4:8. He interpreted the differences in nuclear volume as being related to differences in the amount of chromosomal material. Voss, as cited by Wilson and Leduc,[2] studied nuclear size on isolated nuclei to avoid errors due to sectioning. He corroborated Jacobj's conclusions for the adult and newborn mouse.

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[1]Norman M. Sulkin, "A Study of the Nucleus in the Normal and Hyperplastic Liver of the Rat," American Journal of Anatomy, LXXIII (1943), 107.

[2]J. Walter Wilson and Elizabeth H. Leduc, "Nuclear Phenomena in Mouse Liver," American Journal of Anatomy, LXXXII (1948), 359.

Beams and King[1] measured the nuclei of the normal and the three day old restoring liver. They obtained a bimodal curve in both the normal and restoring liver of the rat, with an indication of a third mode for the latter. They found that the nuclei at the peak of the second mode had just double the volume of the nuclei of the first mode and interpret this to indicate the existence of a geometric type of polyploidy. Daoust, as cited by Brauer,[2] noted three basic modes of liver nuclei in the rat. The first having a mean volume of 193 cubic micra he considered to be diploid. The second having a mean volume of 385 cubic micra he considered to be tetraploid. The third having a mean volume of 728 cubic micra he considered to be octoploid. These modes follow a geometric progression.

Sachs[3] found that, in the deciduoma of the rat, nuclear size could be used as an index of the existence of polyploidy.

Heidenhain and others, according to Schreiber and Angeletti,[4]

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[1]H. W. Beams and R. L. King, "The Origin of Binucleate and Large Mononucleate Cells in the Liver of the Rat," Anatomical Record, LXXXIII (1942), 293.

[2]Ralph W. Brauer, Liver Function (Washington, D.C.: American Institute of Biological Sciences, 1958), p. 5.

[3]Leo Sachs and M. C. Shelesnyak, "The Development and Suppressed Deciduoma in the Rat," Journal of Endocrinology, XII (1955), 149.

[4]B. Schreiber and S. Angeletti, "Rhythmic Increase and Decrease of Nuclear Volume of the Hepatic Cell of the Carp. Cyprinus carpio var. specularis," Anatomical Record, LXXVI (1940), 473.

have observed that in spermatogenesis the nuclear volume ratio of the spermatogonium, first spermatocyte, and second spermatocyte is 4:2:1. This would indicate that nuclear volume is decreasing as the chromosomal number decreases during spermatogenesis. Fankhauser and Humphrey,[1] working with the epidermis cells of the axolotl larvae, found a definite relationship between nuclear size and chromosome number. Bradley, as cited by Huskins,[2] worked with chromosome and chromatid number in pit cells of Nicotiana tomentosa. She found that in this material nuclear volume is related directly to chromatid and not to chromosome number. In her estimation it makes little or no difference whether 8N chromatids are present as the 4N number of ordinary two-chromatid chromosomes or as 2N chromosomes each having four chromatids.

Many investigators believe that nuclear volume is not always related to chromosome number. Biesele,[3] working with rat liver, noted that there was an inconsistency between his findings and those of Sulkin and Beams and King. He considered that some of the increase in liver

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[1]G. Fankhauser and R. R. Humphrey, "The Relationship Between Number of Nucleoli and Number of Chromosome Sets in Animal Cells," National Academy of Science Proceedings, XXIX (1943), 347.

[2]C. L. Huskins, "Nuclear Reproduction," International Review of Cytology, I (1952), 15.

[3]J. J. Biesele, "Chromosome Complexity in Regenerating Rat Liver," Cancer Research, IV (1944), 234.

nuclear volume noted by these authors was a result of increased fat content and not necessarily of increased nucleoprotein content. Biesele,[1] in a second paper, considered that a doubling in nuclear volume is related to a doubling in chromosome number if there is also a doubling of the number of plasmosomes. According to Biesele, "In normal rat tissues it cannot be said that an enlarged resting nucleus necessarily contains a greater number of chromosomes unless its plasmosomes are radically increased in number."

Attention has been called to internal factors, such as fat content, which may have an effect on nuclear volume. Other factors, such as the properties of the cytoplasm, may also have an effect on nuclear volume.

Anderson[2] considers that the isolated rat liver nucleus swells and shrinks in response to various conditions in the environmental medium, such as, changes in ph, ionic strength, and ionic composition. These changes parallel the volume changes of DNA in solution. Anderson[3] considers that the capacity of the isolated rat liver nucleus

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[1]J. J. Biesele, "Chromosome Size in Normal Rat Organs in Relation to B Vitamins, Ribonucleic Acid, and Nuclear Volume," Cancer Research, IV (1944), 533.

[2]Norman G. Anderson and Carolyn B. Norris, "The Effects of Amines on the Structure of Isolated Nuclei," Exptl. Cell Research, XIX (1960), 605.

[3]Norman G. Anderson and K. M. Wilbur, "Studies on Isolated Cell Components. Iv. The effect of various solutions on the isolated rat liver nucleus," Journal of General Physiology, XXXV (1952), 791.

to show volume changes depends upon polymerized DNA. He feels that this is supported by the inhibitory action of DNAase in preventing both swelling and shrinkage resulting from changes in the ionic composition of the medium. Swanson[1] considers that nuclear size may be correlated with some property exhibited by the proteins in the cytoplasmic environment of the nucleus. He noted that when he incubated in vitro nuclei from tissues where the nuclear volume is large in proteins from tissues of smaller nuclear volume that a shrinkage of the larger nuclei occurred. He found that the converse was also true. He postulated that the cytoplasmic proteins participate in the determination of nuclear size. He considers that there may also be an effect on the metabolic activity of the nucleus.

Photometric determinations of mean DNA per nucleus are of interest in the study of polyploidy. Results obtained by investigators indicate that most mammalian somatic cells fall into a basic mean DNA per nucleus class, and that most of the remaining cells fall into classes which are even multiples of this mean. These classes evidently represent ploidy classes, the basic class being the diploid one. There has also been shown by many investigators a definite relationship between nuclear volume and DNA content of the nucleus.

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[1]Harold D. Swanson, "A Study of the Relation of Cytoplasmic Proteins to Nuclear Size and Its Implications for Embryonic Differentiation," Doctorate Thesis, The University of Tennessee, August 1960, p. 45.

Huskins[1] determined photometrically the relative DNA content of mouse liver nuclei. He noted that the step from one class to the next higher is a very accurate doubling of the DNA content and is accompanied by a doubling of nuclear size.

Heizer,[2] by plotting the frequency distributions of volumes (segregated by their DNA content) of rat liver nuclei, has found a definite relationship between nuclear volume and DNA content.

Swift[3] has shown, by absorption spectrophotometry, that the nuclei of ten different somatic tissues of young and adult mice all show approximately the same amount of DNA except for some of those in the liver, pancreas, thymus, blood lymphocytes, and Sertoli cells which contained two or four times the common amount. He has found that mouse spermatid nuclei had half the DNA of the common somatic nuclei. Primary spermatocytes had four times and secondary spermatocytes twice the spermatic value. Swift[4] found in mouse tissue that all measurements of liver nuclei fall into three clearly defined classes with means

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[1]C. Leonard Huskins, "Nuclear Reproduction," International Review of Cytology, I (1952), 20.

[2]Pauline Heizer, "Desoxyribose Nucleic Acid (DNA) Content and Size of Rat Liver Nuclei," Chromosoma, VII (1955), 301.

[3]H. H. Swift, "The Desoxyribose Nuclei Acid Content of Animal Nuclei," Physiol. Zool., XXIII (1950), 196.

[4]H. H. Swift, "Nucleoproteins in the Mitotic Cycle," Tex. Reb. Biol. Med., XI (1952), 352.

in a 1:2:4 ratio. He noted that while this method is not sensitive enough to reflect slight degrees of aneuploidy (i.e. slight degrees of variation from the normal haploid progression of DNA content) results obtained by its use disagree with the reports of extensive wide-range aneuploidy and point to a somatic ploidy condition in mammals which is predominantly a diploid one.

In the preceding paragraphs attention has been focused on the occurrence of nuclei whose volumes are related in a geometric manner and whose DNA content is also related in a geometric manner. Many investigators have noted exceptions to this geometric relationship. Salvatore, as cited by Swanson,[1] believed that he found an accumulation of nuclear volumes at 1.5 times the initial volume, and suggested that this was due to doubling of one parental set of chromosomes before the other. Beams and King[2] obtained a bimodal curve for both the normal and restoring liver of the rat with an indication of a third mode for the latter. Heizer,[3] working with regenerating rat liver after thioacetamide injury, found intermediate DNA values and attributed them to DNA synthesis in preparation for mitosis. Swift[4] found intermediate DNA values

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[1]Swanson, op. cit., p. 32.

[2]Beams and King, loc. cit.

[3]Heizer, loc. cit.

[4]Swift (1950), loc. cit.

in the liver, pancreas, thymus, blood lymphocytes and Sertoli cells. He presumed that these values were associated with mitosis.

Actual chromosome counts are of interest in the study of polyploidy. Biesele,[1] using slides prepared by the squash method and stained by acetocarmine, determined the chromosome complement in both regenerating and control rat liver. He found three classes of cells (2N, 4N, and 8N) in both the regenerating and control liver. According to the hypothesis that chromosome number increases geometrically he rounded off the intermediate counts. Hence his upper limits of the diploid, tetraploid, and octoploid ranges were taken as 63, 126, and 252 chromosomes respectively. In both the regenerating and control livers the frequency was highest in the diploid class. Tanaka, as cited by Hungerford,[2] studied the regenerating rat liver. He noted three classes of cells (2N, 3N, and 4N) with the greatest number in the diploid class. Kinosita, as cited by Brauer,[3] determined the chromosome complement of cells in the embryonic and regenerating rat liver. He found complements consisting of 42 or exactly 2N and complements with near 2N, 4N and near 4N in the

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[1]Biesele (1944), op. cit., p. 234.

[2]David A. Hungerford, "Chromosome Numbers of Ten-Day Fetal Mouse Cells," Journal of Morphology, LXXXXVII (1955), 498.

[3]Brauer, op. cit., p. 17.

embryonic liver. An almost identical distribution was found in the regenerating liver. In this case, however, 3N and near-3N complements were noted. He noted that in both the embryonic and regenerating liver the greatest number of cells were in the diploid class. Marquardt[1] has noted up to ten ploidy classes in the regenerating liver of the rat. He also notes that when a larger portion of the liver is removed a greater number of polyploid cells are observed. He believes that this indicates that the strength of the stimulus (i.e. the amount of liver removed) determines the number of the higher polyploid cells that will be in division. In rats, which have had two-thirds of their liver removed, he finds cells with 1N, 2N, 3N, 4N, 5N, 6N, 7N, 8N, 9N, and 10N chromosome complements. These observations indicate that an arithmetic progression of ploidy is present in the liver.

Many investigators have studied the chromosome complement of liver cells. Since the liver is mitotically inert, as cited by McKellar,[2] some method of stimulating and storing mitotic activity must be used. According to Hoffman et al,[3] carbon tetrachloride has three major

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[1]Hans Marquardt und Eberhard Glass, "Die Chromosomenzahlenin den Leberzellen Von Ratten Verschiedenen Alters," Chromosoma, VIII (1957), 619.

[2]M. McKeller, "The Postnatal Growth and Mitotic Activity of the Liver of the Albino Rat," American Journal of Anatomy, LXXXV (1949), 267.

[3]Joseph Hoffman et al, "Responses of Liver to Injury: Effects of Acute Carbon Tetrachloride Poisoning," A. M. A. Arch. Path., LIX (1955), 429.

advantages in stimulating mitotic activity: (1) the simplicity of the experimental design; (2) its reproducibility; (3) the rapid course of recovery. Stowell and Lee[1] noted that the liver had the greatest number of mitotic figures three days after hepatic injury by carbon tetrachloride. They also noted that oral feeding of carbon tetrachloride gave the greatest degree of reproducibility.[2] Brues and Jackson[3] noted that in the mitoses of liver cells in rats subjected to partial hepatectomy and subsequently treated with colchicine, the chromosomes were scattered throughout the cell.

It is of interest to note what effect carbon tetrachloride and colchicine have on the liver other than the production and storage of metaphase figures. Hoffman and Himes[4] noted four major stages in the response of the liver to a single feeding of carbon tetrachloride: (1) necrosis 24 hours after feeding; (2) regeneration of the tissue was at its peak 48 hours after feeding; (3) restoration was nearly complete

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[1]C. E. Stowell and C. S. Lee, "Histochemical Studies of Mouse Liver After a Single Feeding of Carbon Tetrachloride," Archives of Pathology, L (1950), 522.

[2]Ibid.

[3]Austin M. Brues and Elizabeth B. Jackson, "Nuclear Abnormalities Resulting from Inhibition of Mitosis by Colchicine and Other Substances," The American Journal of Cancer, XXX (1937), 510.

[4]Joseph Hoffman and H. Himes, "Origin of Polyploid Nuclei in Rat Livers During Regeneration Following Carbon Tetrachloride Poisoning," Jour. Mount Sinai Hospital, XXIV (1957), 936.

after 72 hours; (4) the liver was completely restored by 120 hours after feeding. Stowell and Lee[1] noted that by the third day after carbon tetrachloride injury the mean nuclear volume of viable hepatic cells had increased 66% and the cytoplasmic 58%, or a total increase of mean cell volume of 59%. With continued mitotic activity these values returned to normal by the twelfth day after injury. They also noted that the weight of the liver and the amount of lipid present reached a maximum on the third day after injury. Wachter, as cited by Matko,[2] measuring the size of hepatic cells after the administration of carbon tetrachloride, found the enlargement and diminution, respectively, of the nuclei to be frequent and notable in acute hepatic lesions. Matko[3] noted, in the liver of the rat, that changes occurring in nuclear size both 30 minutes and 72 hours after treatment with colchicine were significant in comparison with the normal values.

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[1]Stowell and Lee, loc. cit.

[2]L. Matko, L. Holozinger and S. Keresztury, "Effect of Colchicine, Podophyllin, and Nitrogen Mustard on the Resting Cells of the Organs," Acta Morphologica, VI (1955), 309.

[3]Ibid., 309.

CHAPTER III

METHODS AND MATERIALS

This study was carried out in two series. The animals used in this study were white mice of the A/JAX strain obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine.

Seven 6-week-old male mice used in the first series were given .1cc of a 40% solution of carbon tetrachloride in olive oil orally under mild ether anesthesia. Three days after administration of carbon tetrachloride the mice were given .1cc of a colchicine solution (i.e. .2mg per 100 gms. body weight) by subcutaneous gluteal injection.[1] Eight hours after administration of colchicine the mice were sacrificed by applying a sharp blow to the base of the skull. The medial lobe of the liver was removed and cut into 2 cubic millimeter portions. These portions were fixed in a 1:3 solution of acetic acid and ethyl alcohol and stained according to the Feulgen method.[2] After staining the pieces of tissue were placed between a slide and coverslip and squashed by gentle pressure with a blunt needle and by thumb pressure exerted on the

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[1] M. McKellar, op. cit., p. 268.

[2] C. D. Darlington and L. F. LaCour, The Handling of Chromosomes, (New York: The Macmillan Company, 1960), p. 156.

coverslip. The slides were then made permanent by the dry ice method.[1]

Drawings were made, with the aid of the camera lucida, of parenchymal nuclei with an oil immersion objective. They were measured, at their longest and shortest diameters, to the nearest 0.5 mm. The nuclei to be drawn were selected at random. The magnification of the camera lucida was 1750X.

Assuming that the nucleus is an oblate spheroid, the radius of the length or long axis and radius of the width or short axis were substituted in the formula for determining the volume of an oblate spheroid. This formula is: $4/3\pi a^2 b$, where (a) is the radius of the short axis and (b) is the radius of the long axis. At the same time, the numbers were converted to microns and correction was made for the increased magnification. The completed formula is: $4/3\pi a^2 b \left(\frac{1}{1750}\right)^3$ equals volume in cubic microns. A total of 200 nuclei were measured for each mouse.

With the aid of colchicine, which acts as an inhibitor of spindle formation, well spread "C" mitoses or exploded metaphases were obtained.[1] Chromosome counts were made on six of the mice in the first series. The seventh mouse did not show countable mitotic figures.

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[1]O. J. Eigsti and Pierre Dustin, Jr., Colchicine (Ames, Iowa: The Iowa State College Press, 1955), p. 45.

Since drawings were made on squash preparations there was a possibility of distortion of the nuclei. It would, therefore, be of value to determine the degree of nuclear distortion, if any, that had occurred through the process of squashing. Two 1-year-old mice were used in the second series, one male and one pregnant female. Squash preparations and camera lucida drawings were utilized in the same manner as described for series one. In addition nuclei in whole cells were measured in a hanging drop suspension. There are four major advantages of the hanging drop suspension: (1) unfixed nuclei may be drawn; (2) there is no pressure exerted on the nuclei; (3) nuclei may be drawn under more normal environmental conditions; (4) a more random sample can be obtained. Drawings were made on nuclei stained with methyl green and on nuclei which had been fixed and stained by the Feulgen method. With this data available a comparison could be made between the nuclear volume of fresh nuclei, of fixed and stained nuclei, and of fixed, stained, and squashed nuclei. The hanging drop suspension was prepared as follows: (1) the entire liver, with exception of that used for the squash preparation, was placed in a test tube containing a solution of (.0094m KH_2PO_4, .0125m K_2HPO_4, .0015m $NaHCO_3$, .145m Sucrose, in 1 liter of water);[1] (2) several glass beads were added and the test tube was agitated for ten

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[1]Norman G. Anderson and K. M. Wilbur, op. cit., p. 788.

minutes; (3) a drop of this suspension was placed on a coverslip and suspended into a depression slide.

Chromosome counts were made on the male mouse but no mitoses were observed in the pregnant female mouse.

The chi square test and a method described by Fisher[1] for making a cumulative probability of all the individual probabilities was used in analysing the variability of ploidy distribution between mice. These methods were used in analysing the variability of ploidy distribution, as determined by nuclear volume and chromosome counts and in comparing the degree of relationship between these ploidy frequencies.

The chi square test is a test of the degree of fit of the data to a particular hypothesis (i.e. the expected value). It is inferred from a high chi square probability that the degree of variation found in the data is most probably due to chance factors. It is inferred from a low chi square probability that there is a real difference between the data analysed.

Since the sample size for nuclear volumes and chromosome counts differed, it was necessary to find some method for equating them. The data from each mouse was composed of two hundred nuclear volume values and fifty chromosome number per metaphase values. The

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[1]Sir Ronald A. Fisher, Statistical Methods for Research Workers, (London: Oliver and Boyd, 1954), p. 99.

expected values, therefore, were determined by adding the observed number of nuclei to the observed number of metaphases and taking 1/5 of this sum to be the expected number of metaphases and 4/5 of this sum to be the expected number of nuclei.

CHAPTER IV

DATA

This study deals with two aspects of the mouse liver. The frequency distribution of parenchyma cell nuclei, according to their volume, and the frequency distribution of chromosome numbers seen in metaphase figures. Four questions were considered in this investigation: (1) what type of numerical relationship is indicated between the volume at which frequency peaks of parenchyma nuclei occur; (2) what type of numerical relationship is indicated between the numbers of chromosomes at which modes of frequency distribution occur; (3) is the type of relationship in each of these the same; and (4) how well can chromosome complements of parenchymal nuclei be correlated with nuclear volumes?

In Figure 1 the upper and lower limits of the classes at which peaks occur are 180-219, 420-459, 480-519, 900-939, and 1200-1239 cubic microns.

In Figure 2 the upper and lower limits of the classes at which peaks occur are 180-219, 260-299, 380-418, 460-499, 580-619, 660-699, 780-819, and 860-899 cubic microns.

In Figure 3 the upper and lower limits of the classes at which peaks occur are 180-219, 340-379, 420-459, 500-539, 580-619, 660-699, 860-899, and 980-1019 cubic microns.

In Figure 4 the upper and lower limits of the classes at which peaks occur are 140-179, 220-259, 340-379, 460-499, 540-579, 620-659, 700-739, 780-819, 860-899, 940-979, and 1060-1099 cubic microns.

In Figure 5 the upper and lower limits of the classes at which peaks occur are 140-179, 220-259, 340-379, 540-579, 620-659, 740-779, 820-859, 940-979, and 1220-1259 cubic microns.

In Figure 6 the upper and lower limits of the classes at which peaks occur are 180-219, 380-419, 500-539, 620-659, 700-739, 780-819, 860-899, 1020-1059, and 1220-1239 cubic microns.

In Figure 7 the upper and lower limits of the classes at which peaks occur are 180-219, 260-299, 340-379, 460-499, 580-619, 660-699, 740-779, 900-939, 1060-1099, 1220-1239, and 1360-1399 cubic microns.

In Figure 8 the upper and lower limits of the classes at which peaks occur are 180-219, 300-339, 380-419, 500-539, 580-619, 700-739, 820-859, 900-939, 1020-1059, 1180-1219, and 1320-1359 cubic microns.

Figures 10-16 represent the number of metaphases having a particular chromosome count, plus or minus one chromosome, for each individual mouse in the first series and the 1-year-old male mouse of series two. Figure 9 represents the total number of metaphases with a particular chromosome number, plus or minus one chromosome, in all of the mice in series one.

In Figures 10-16 the greatest number of metaphases are observed to have 40 chromosomes. Metaphases having 60, 80, 100, 120, and 140

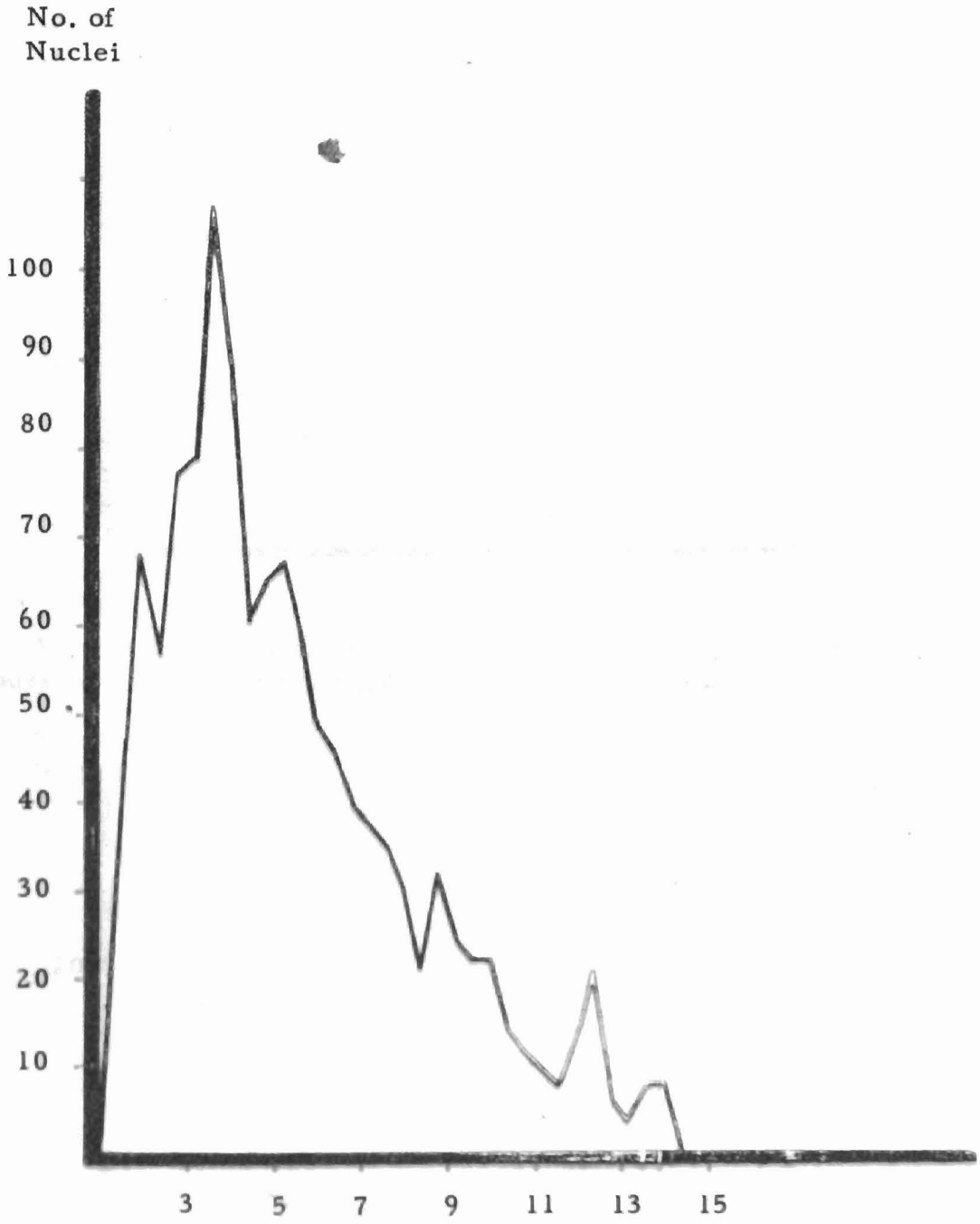

Figure 1. The distribution of nuclei, according to their volume, in all of the mice of series one. Units are in hundreds of cubic microns.

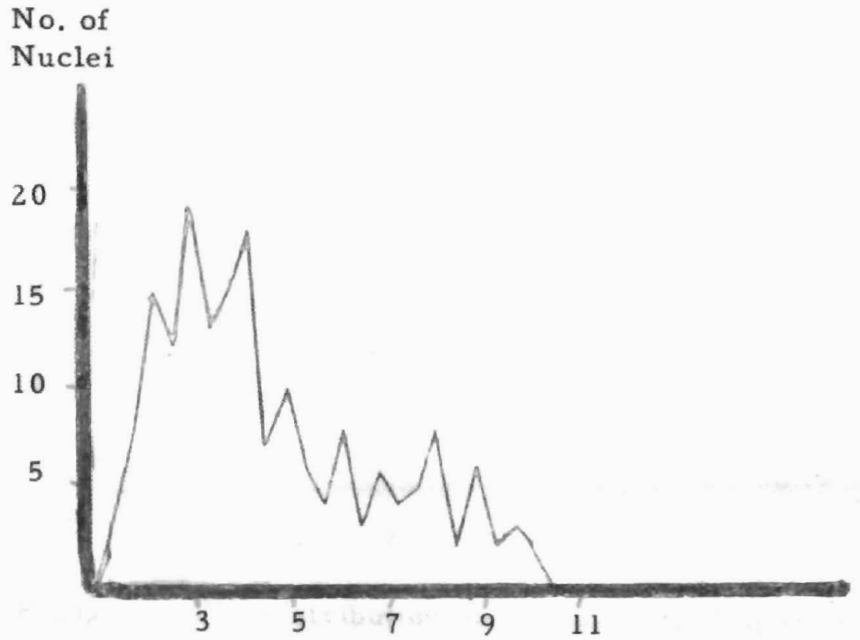

Figure 2. The distribution of nuclei, according to their volume, in mouse one. Units are in hundreds of cubic microns.

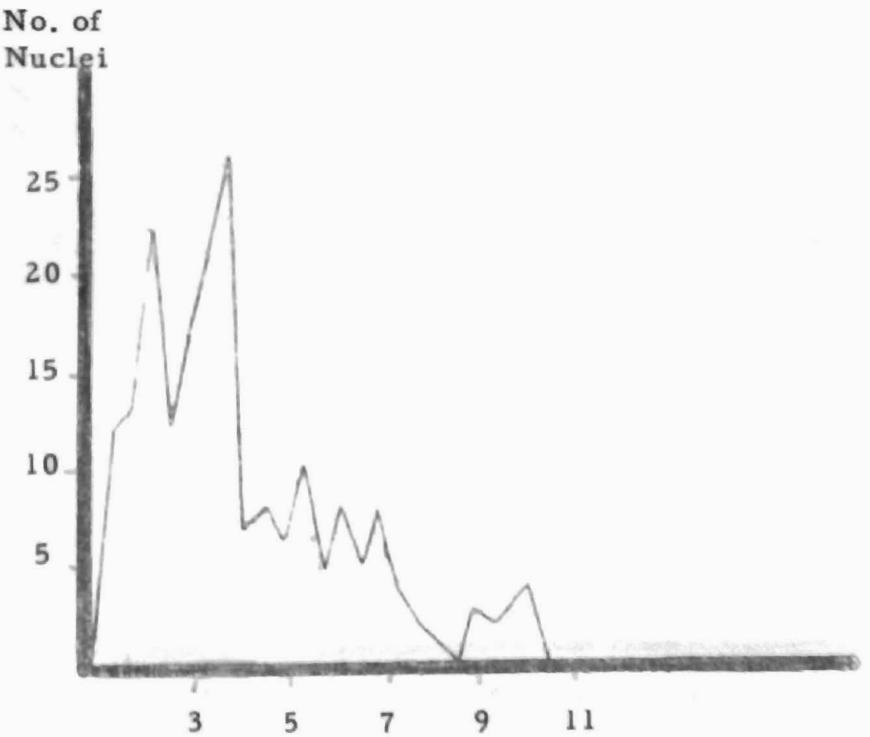

Figure 3. The distribution of nuclei, according to their volume, in mouse two. Units in hundreds of cubic microns.

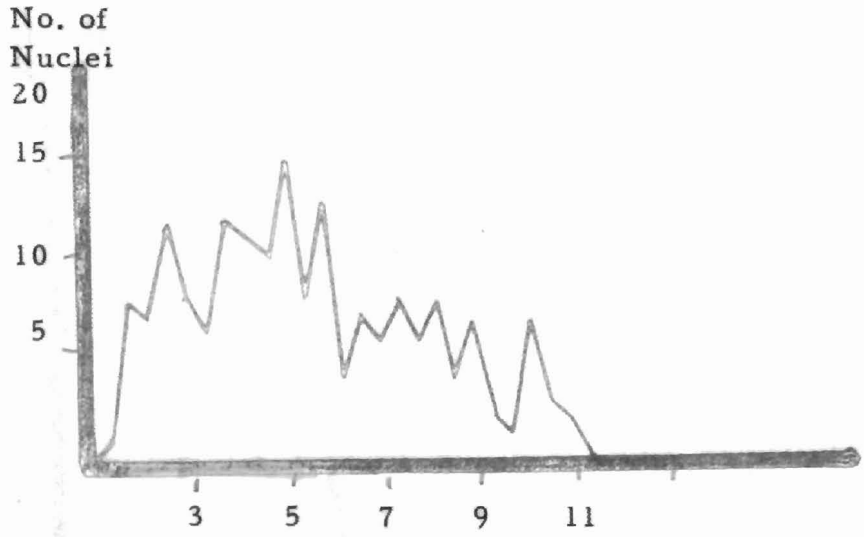

Figure 4. The distribution of nuclei, according to their volume, in mouse three. Units are in hundreds of cubic microns.

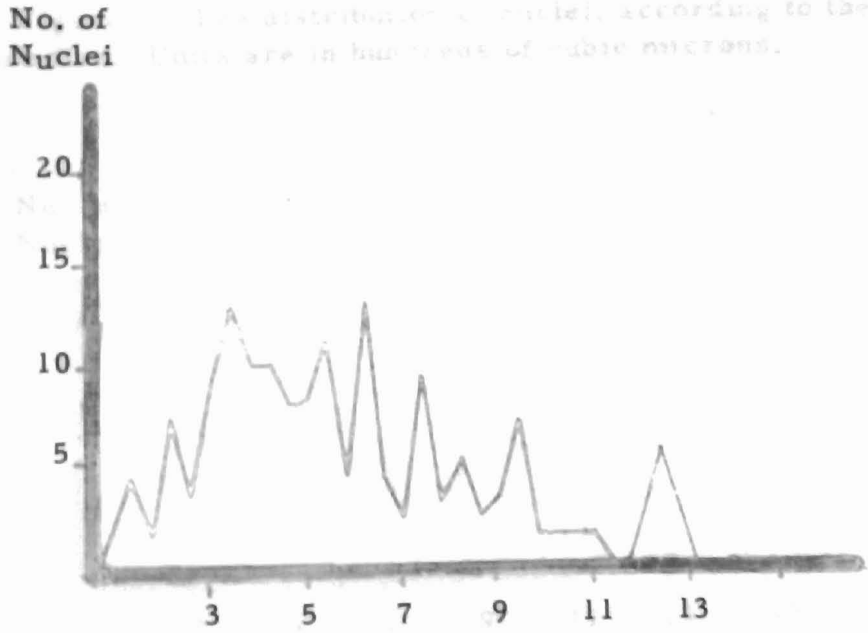

Figure 5. The distribution of nuclei, according to their volume, in mouse four. Units are in hundreds of cubic microns.

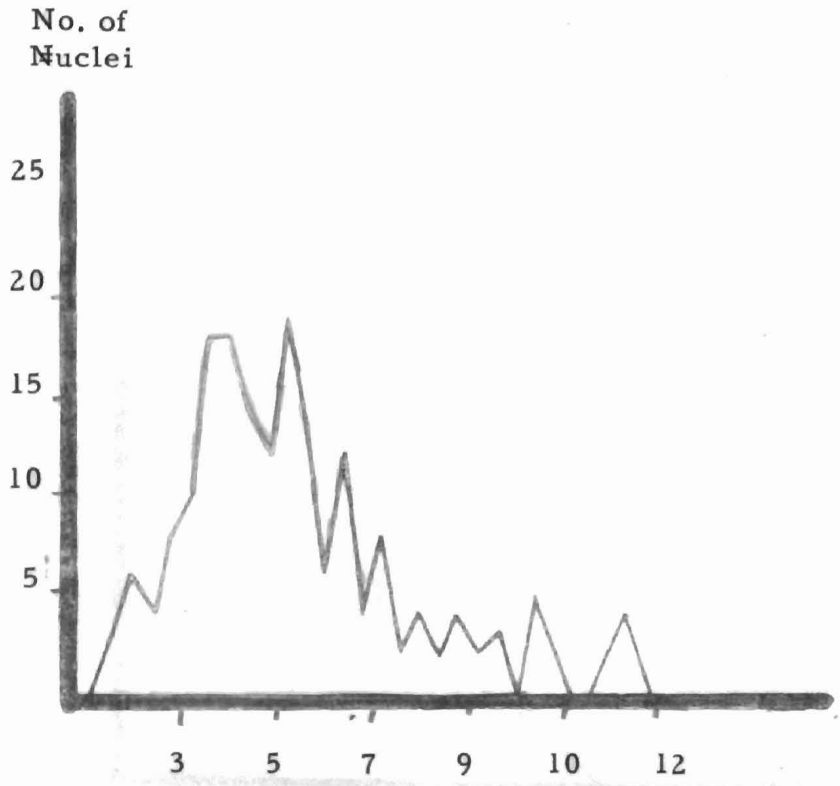

Figure 6. The distribution of nuclei, according to their volume, in mouse five. Units are in hundreds of cubic microns.

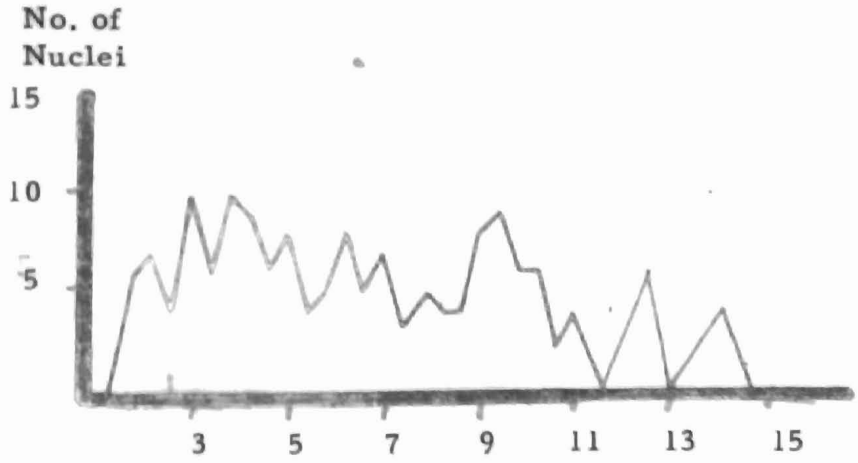

Figure 7. The distribution of nuclei, according to their volume, in mouse six. Units are in hundreds of cubic microns.

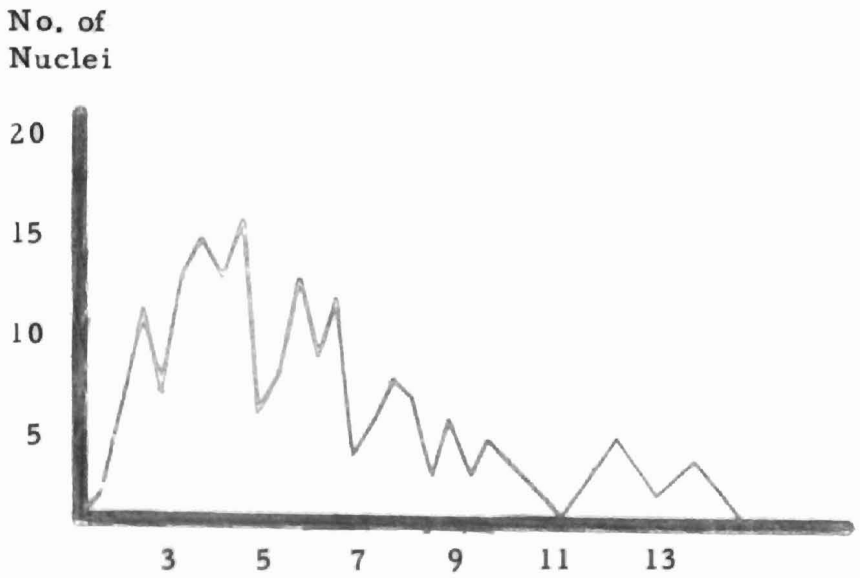

Figure 8. The distribution of nuclei, according to their volume, in mouse seven. Units are in hundreds of cubic microns.

chromosomes have also been observed. The diploid cell in the mouse is considered to have 40 chromosomes. Metaphases having multiples of 20 chromosomes are considered to be of a particular ploidy. Those cells having 20, 40, 60, 80, 100, 120, 140, etc., chromosomes are considered haploid (1N), diploid (2N), triploid (3N), tetraploid (4N), pentaploid (5N), hexaploid (6N), heptaploid (7N), etc., respectively.

Table I represents the data obtained by actual chromosome counts. In Table I the greatest number of metaphases are found in the diploid class. Metaphases containing the haploid, triploid, tetraploid, pentaploid, hexaploid, and heptaploid chromosome complement are also noted. No clustering of metaphases around the tetraploid class was observed and the octoploid class was not observed. Seven ploidy classes were noted in mouse 3, 4, and 5. Six ploidy classes were noted in mouse 2 and 6. Four ploidy classes were noted in mouse 1. The hypodiploid, diploid, triploid, and tetraploid classes were observed in all of the mice.

Table II gives the average nuclear volume in cubic microns in the mice of series one and the average chromosome number per metaphase with the average nuclear volume in all of the mice and the average chromosome number per metaphase in six of the mice. The seventh mouse in series one did not show mitotic figures.

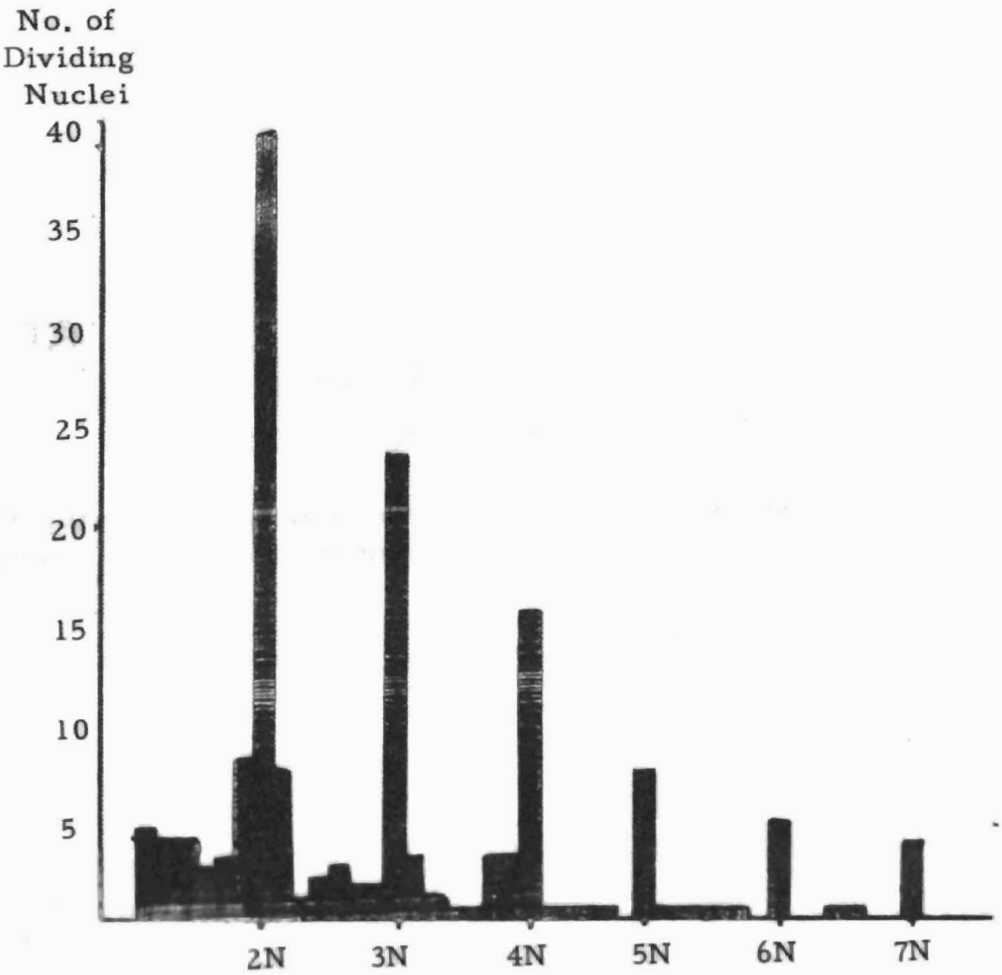

Figure 9. The distribution of dividing nuclei, according to their chromosome complement, in all of the mice of series one.

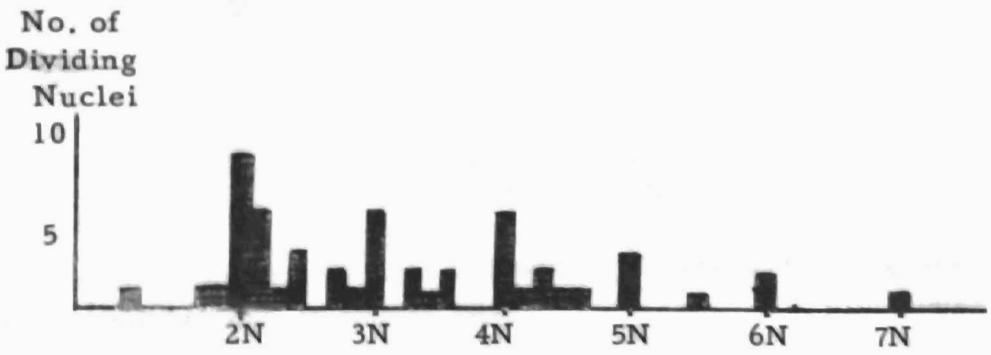

Figure 10. The distribution of dividing nuclei, according to their chromosome complement, in the 1-year-old male mouse of series two.

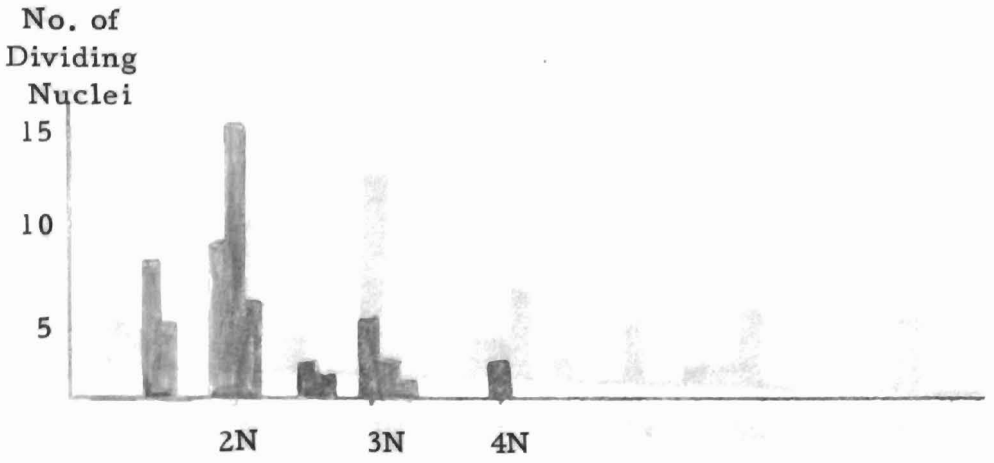

Figure 11. The distribution of dividing nuclei, according to their chromosome complement, in mouse one.

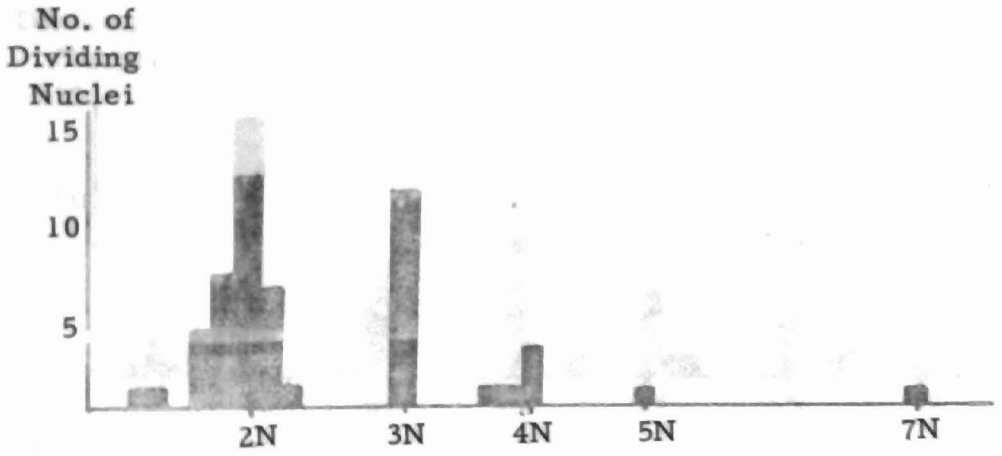

Figure 12. The distribution of dividing nuclei, according to their chromosome complement, in mouse two.

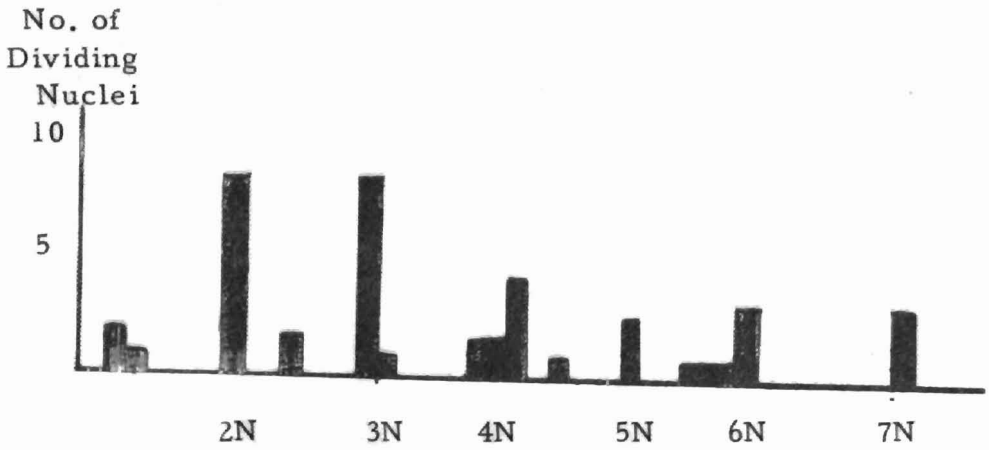

Figure 13. The distribution of dividing nuclei, according to their chromosome complement, in mouse three.

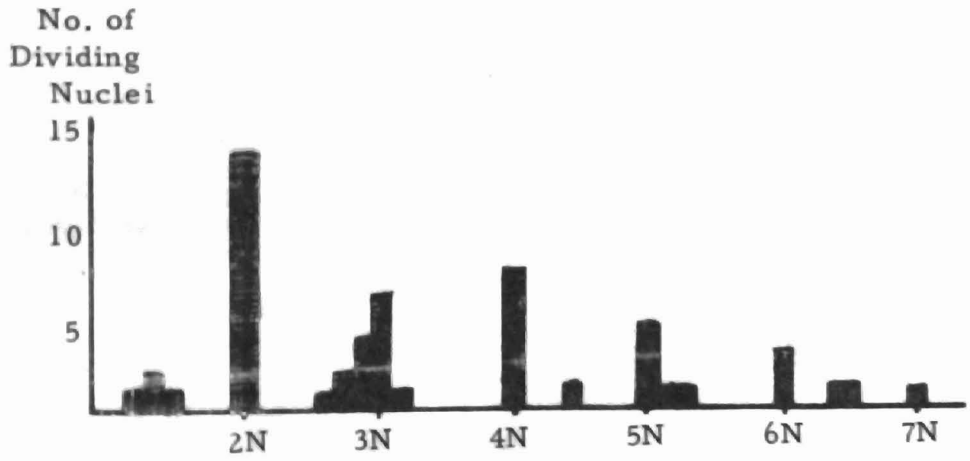

Figure 14. The distribution of dividing nuclei, according to their chromosome complement, in mouse four.

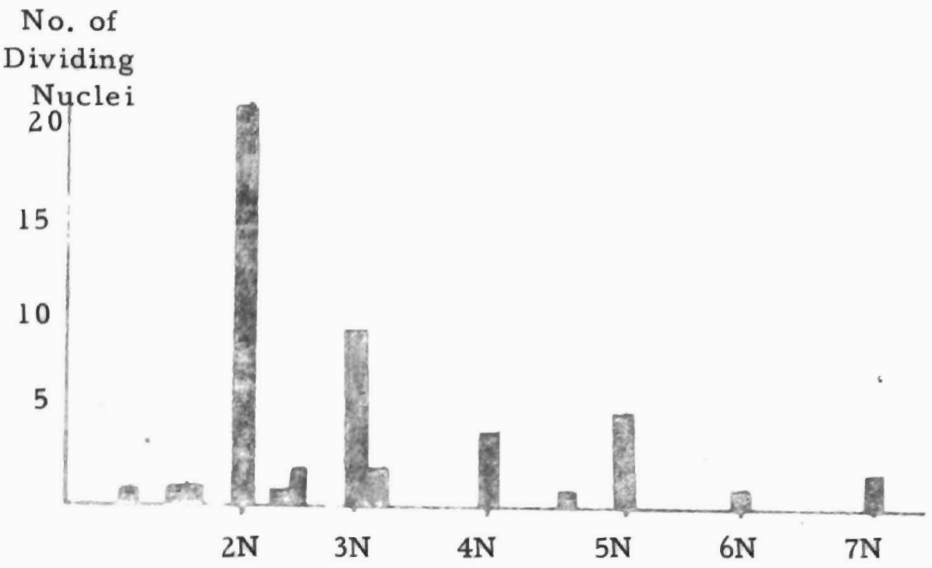

Figure 15. The distribution of dividing nuclei, according to their chromosome complement, in mouse five.

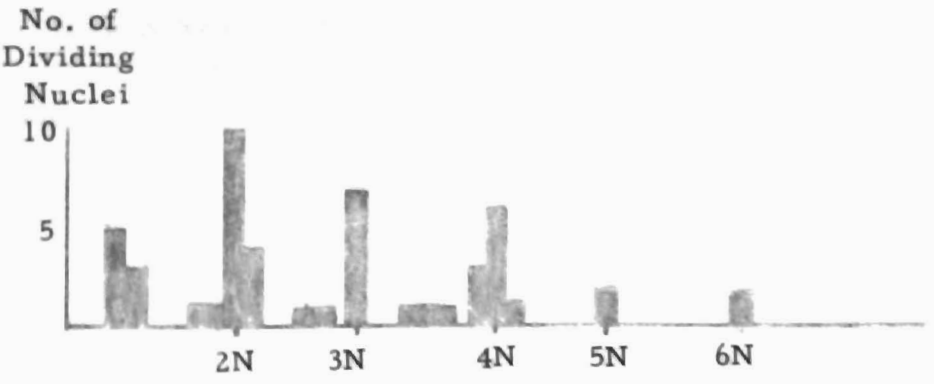

Figure 16. The distribution of dividing nuclei, according to their chromosome complement, in mouse six.

Figures 17-21 show the frequency distribution of nuclei in the 1-year-old male and pregnant female mouse of series two. Figures 17 and 18 show the frequency distribution of nuclei in the male mouse. Two different techniques were used on this mouse in determining nuclear volume: (1) a hanging drop suspension of cells having their nuclei stained with methyl green; (2) a squash preparation of cells having their nuclei stained by the Feulgen method. The second method is the same as used in the first series mice. Figure 17 shows the frequency distribution of nuclei using the hanging drop suspension technique. Figure 18 shows the frequency distribution of nuclei using the squash technique.

TABLE I

THE NUMBER OF DIVIDING NUCLEI OBSERVED IN THE VARIOUS PLOIDY CLASSES WITH THE MEAN NUMBER OF DIVIDING NUCLEI IN EACH PLOIDY CLASS AND THE PROBABILITY THAT EACH PLOIDY CLASS REPRESENTS THE SAME SAMPLE WITH THE COMBINED PROBABILITY THAT EACH OF THE PROBABILITIES IS SIGNIFICANT

Mouse	Hypodiploid	2N	3N	4N	5N	6N	7N
1	11	14	4	2	0	0	0
2	2	12	11	3	1	0	1
3	3	10	10	5	3	4	4
4	4	13	6	7	4	3	1
5	3	20	9	4	5	1	2
6	8	10	7	6	2	2	0
Mean	5.1	13.2	7.1	4.5	2.6	1.7	1.3
Probability	.02	.50	.50	.70	.30	.20	.20
Combined Probability		.30					

Figures 19-21 show the frequency distribution of nuclei in the pregnant female mouse. Three different techniques were used to determine nuclear volume in this mouse. The first two were the same as those used with the male mouse. The third consisted of fixing and staining the nuclei as described in Chapter III for the first series of mice, and then suspending the cells. Figures 19-21 represent the methyl green, Feulgen squash, and Feulgen hanging drop techniques respectively.

TABLE II

THE AVERAGE NUCLEAR VOLUME AND CHROMOSOME NUMBER PER METAPHASE IN SERIES ONE MICE WITH THE AVERAGE NUCLEAR VOLUME IN ALL OF THE MICE AND THE AVERAGE CHROMOSOME NUMBER PER METAPHASE IN SIX OF THE MICE

Mouse	1	2	3	4	5	6	7
Average Nuclear Volume in Cubic Microns	508	397	645	755	614	831	639
Average Chromosome Number per Metaphase	41.86	49.46	73.16	67.02	59.38	55.12	- -
Average Nuclear Volume in Cubic Microns in All Mice		627					
Average Chromosome Number per Metaphase in Six Mice		57.66					

In Figure 17 the upper and lower limits of the classes at which peaks occur are 179-211, 344-376, 509-541, 575-640, 773-805, 872-904, 1004-1036, 1070-1135, 1169-1200, 1268-1300, 1367-1399, 1433-1465, 1598-1630, 1697-1729, and 1895-1927 cubic microns.

In Figure 18 the upper and lower limits of the classes at which peaks occur are 380-419, 500-579, 740-779, 940-979, 1020-1059, 1140-1179, 1220-1239, 1320-1359, 1440-1479, and 1560-1599 cubic microns.

In Figure 19 the upper and lower limits of the classes at which peaks occur are 500-600, 650-700, 750-800, 900-950, 1100-1150, 1250-1300, 1400-1500, 1600-1650, 1750-1800, and 2100-2150 cubic microns.

In Figure 20 the upper and lower limits of the classes at which peaks occur are 350-450, 550-600, 650-700, 750-800, 900-950, 1050-1100, 1150-1250, 1450-1500, 1550-1600, and 1800-1850 cubic microns.

In Figure 21 the upper and lower limits of the classes at which peaks occur are 113-145, 344-376, 443-475, 509-541, 575-607, 674-706, 773-805, 872-937, 971-1003, 1103-1135, and 1235-1267 cubic microns.

In Table III the average nuclear volume and chromosome number per metaphase in each mouse and in each technique is given. It should be noted that no mitotic figures were observed in the pregnant female. Table III represents the data from figures 17-21.

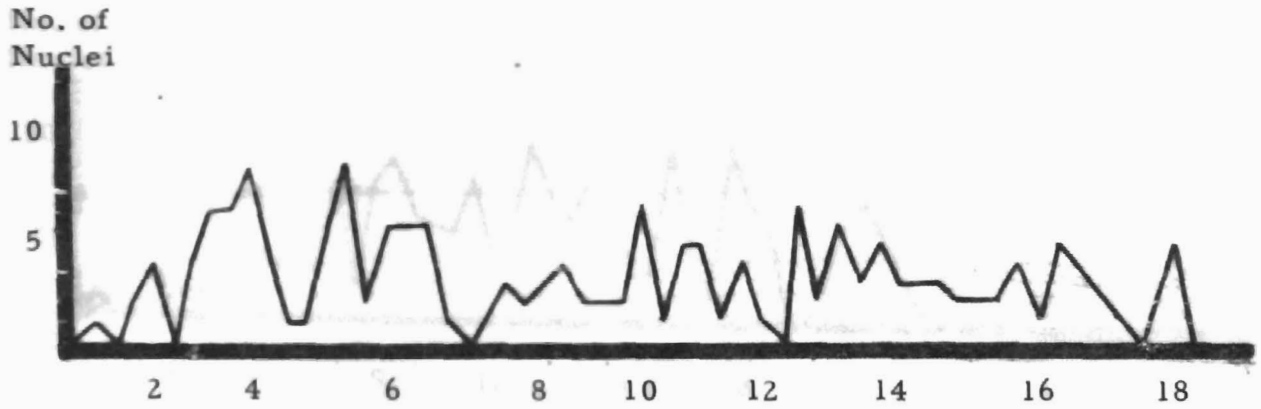

Figure 17. The distribution of nuclei, according to their volume, in the 1-year-old male mouse of series two. The tissue was prepared by the hanging drop technique. Units are in hundreds of cubic microns.

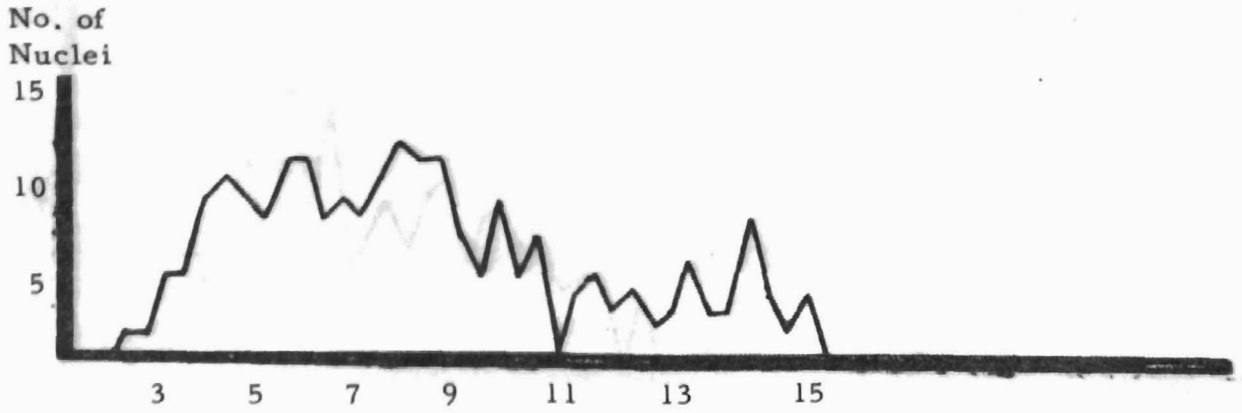

Figure 18. The distribution of nuclei, according to their volume, in the 1-year-old male mouse of series two. The tissue was prepared by the squash technique. Units are in hundreds of cubic microns.

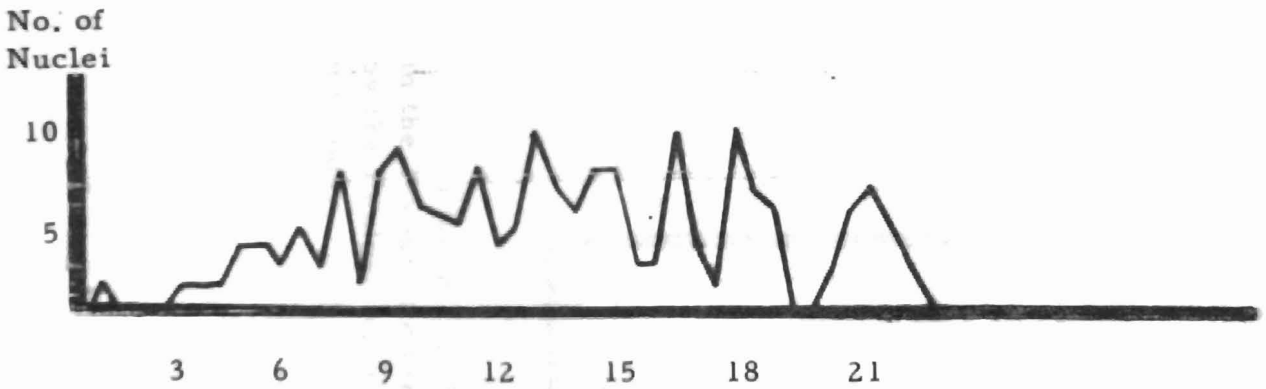

Figure 19. The distribution of nuclei, according to their volume, in the 1-year-old female mouse of series two. The tissue was prepared by the methyl green hanging drop technique. Units are in hundreds of cubic microns.

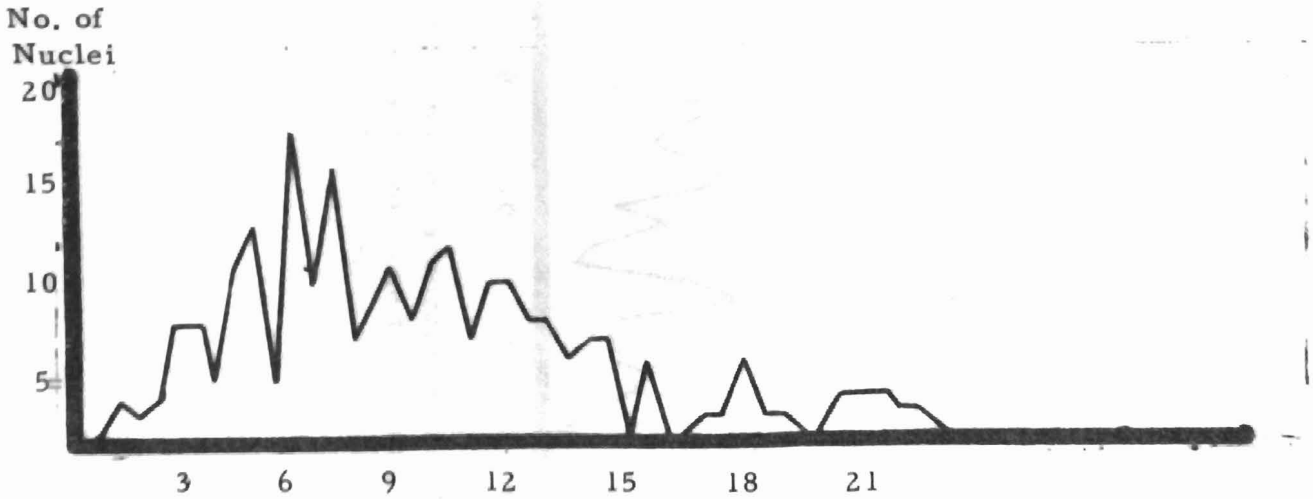

Figure 20. The distribution of nuclei, according to their volume, in the 1-year-old female mouse of series two. The tissue was prepared by the Feulgen squash technique. Units are in hundreds of cubic microns.

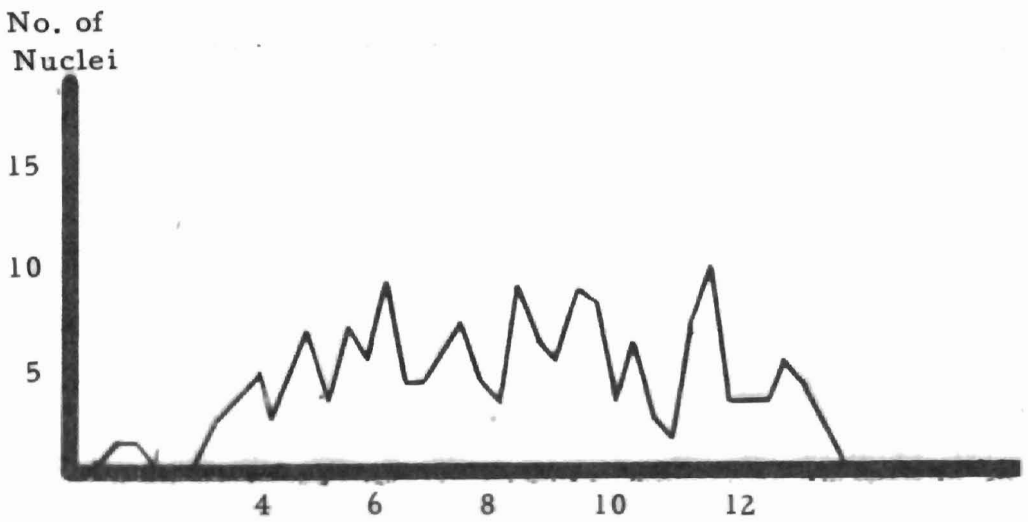

Figure 21. The distribution of nuclei, according to their volume, in the 1-year-old female mouse of series two. The tissue was prepared by the Feulgen hanging drop technique. Units are in hundreds of cubic microns.

TABLE III

THE AVERAGE NUCLEAR VOLUME, ACCORDING TO THE TECHNIQUE USED, IN THE SERIES TWO MICE AND THE AVERAGE CHROMOSOME NUMBER PER METAPHASE IN THE MALE MOUSE

Technique	IMG	IFS	IFH	IIMG	IIFS
Average Nuclear Volume in Cubic Microns	1,346	964	978	1,045	877
Average Chromosome Number Per Metaphase	- -	- -	- -	- -	61.14
Average Nuclear Volume in the Male Mouse		961			
Average Nuclear Volume in the Female Mouse		1,096			

Note: IMG equals pregnant female, methyl green stain, hanging drop technique

IFS equals pregnant female, Feulgen stain, squash technique

IFH equals pregnant female, Feulgen stain, hanging drop technique

IIMG equals male mouse, methyl green stain, hanging drop technique

IIFS equals male mouse, Feulgen stain, squash technique

CHAPTER V

DISCUSSION

Prior to the plotting of the data it was necessary to determine what numerical relationships were present and what interval size would best exhibit this relationship. The following hypotheses ask the question; what numerical relationship will have the greatest number of nuclei fall into the range of its modes. The hypotheses considered were: (1) a 120-240-360-480 relationship; (2) a 140-280-420-360 relationship; (3) a 160-320-480-640 relationship; (4) a 180-360-540-720 relationship; (5) a 200-400-600-800 relationship; (6) a 220-440-660-880 relationship; (7) and a 240-480-720-960 relationship. The relationships indicate modes at which nuclei, according to their volume, should fall. Using 120 as the base volume frequency modes of parenchymal nuclear volume would be expected at 2, 3, 4, etc., times the base mode. The 120 hypothesis postulates that the volumes of the nuclei must fall within plus or minus 30 cubic microns of each expected mode. The plus or minus range of the modes in these relationships is determined by dividing the base volume by four. The interval was determined by dividing the base number by five. An odd number such as five is used so that the numerical relationships cited would fall at the middle of the mode. It was found that one numerical relationship would fit all of the mice in series one.

This was a 200-400-600-800 relationship. An interval size of forty cubic microns would give the best picture of this relationship. It should be noted that the interval size would lend greatly to the form of the peaks in a frequency distribution and where those peaks would lie.

Figures 2-8 show an arithmetic relationship with peaks generally every 100 cubic microns. Not all of the peaks fit a perfect arithmetic relationship but the peaks usually fall within 60 cubic microns of it. Figure 1 has fewer frequency peaks than would be indicated by the number of frequency peaks in each mouse. The reason for this is that some mice peak at 200, 300, 400 cubic microns while others peak at 260, 360, 460 cubic microns. Although these peaks are at a different position the relationship between them is the same.

Figures 2-8 were interpreted in two different ways. Table IV shows the distribution of nuclei as taken directly from the figures. The data in Table IV was determined by considering the 200 cubic micron peak to be the diploid peak. If this is done the tetraploid peak would be expected at 400 cubic microns and the octoploid peak at 800 cubic microns. Since peaks are found approximately every 100 cubic microns this would suggest that a 1:2:3:4:5:6:7:8 relationship of volume or an arithmetic relationship is characteristic of parenchymal cell nuclei. In this table it is noted that all of the mice with the exception of mouse four and five have the greatest per cent of nuclei in the dip-

loid class. Mouse four and five have the greatest per cent of nuclei in the tetraploid class. A triploid peak is found in four of the seven mice. From the average per cent of nuclei in each ploidy class, it is noted that the greatest per cent of nuclei occur in the tetraploid class.

TABLE IV

THE PER CENT OF LIVER NUCLEI FOUND IN EACH PLOIDY CLASS, AS TAKEN DIRECTLY FROM FIGURES 2-8, IN EACH OF THE MICE IN SERIES ONE, WITH THE MEAN PER CENT IN EACH OF THE PLOIDY CLASSES

Mouse	2N	3N	4N	5N	6N	7N	8N	9N	10N	11N
1	26.5	15.5	22.5	10.0	4.0	4.0	5.5	4.0	- -	- -
2	36.5	- -	26.0	20.5	6.0	6.5	1.0	3.5	4.0	- -
3	30.0	- -	17.0	11.0	7.5	8.5	6.0	5.0	5.0	4.5
4	3.0	6.5	26.0	12.5	10.0	8.0	5.0	6.5	- -	- -
5	10.5	- -	53.5	10.5	6.5	5.5	3.0	3.0	2.0	3.5
6	12.5	7.5	11.5	9.0	10.0	5.0	5.5	9.5	- -	- -
7	18.5	13.0	14.5	13.0	7.0	7.5	3.5	4.5	- -	- -
Mean	19.6	6.0	24.4	12.3	4.3	6.4	4.2	5.1		

Beams and King[1] noted a 1:2 relationship in the modes of parenchymal nuclear volume in the normal and regenerating liver of the rat. They also noted that twice as many nuclei were in the second mode. These modes might be comparable to the 2N and 4N modes found in Table IV. Sulkin[2] working with the normal and restoring rat liver, noted

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[1]Beams and King, loc. cit. [2]Sulkin, loc. cit.

four modes. The modes of nuclear volumes formed a 1:2:4:8 numerical relationship which he considered to represent the 2N, 4N, 8N, and 16N ploidy classes. In both the restoring and normal liver the greatest number of nuclei occurred in the tetraploid class. Daoust, as cited by Brauer,[1] noted three modes of parenchymal nuclear volume. The volumes followed a 1:2:4 progression which he considered to represent 2N, 4N, and 8N ploidy class. He noted that the greatest number of nuclei occurred in the tetraploid class.

The aforementioned investigators have noted a geometric progression in parenchymal nuclear volume. They have also noted that the greatest number of nuclei occurred in the 4N or tetraploid class. 2N, 4N, and 8N classes are noted in Table IV with the greatest number of nuclei occurring in the tetraploid class. The major differences between the findings of these investigators and the findings in this study are the intermediate modes observed between the 2N, 4N, and 8N ploidy classes in this study. Although the data tends to corroborate the findings of these investigators, that the 4N parenchymal nuclear volume mode is twice that of the 2N parenchymal nuclear volume mode and the 8N parenchymal nuclear volume mode is four times that of the 2N parenchymal nuclear volume mode, the data as interpreted will not correspond to the data

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[1]Brauer, op. cit., p. 5.

obtained by chromosome counts. The data as interpreted in Table IV does not indicate a hypodiploid group, whereas, the data in Table I, dealing with actual chromosome counts, does show a hypodiploid group. In Table I the greatest number of dividing nuclei occur in the diploid class. In Table IV the greatest average per cent of nuclei occur in the tetraploid class.

If the assumption is made that the dividing nuclei accurately reflect the number of nuclei that are truly diploid, triploid, etc., the data in Figures 2-8 must be interpreted in a different way than they were in Table IV. It was therefore decided to adapt the peaks in Figures 2-8 (i.e. to combine some of the peaks in Figures 2-8) so that they would correspond to the greatest degree with the data from the chromosome counts. In correlating the nuclear volume data with the chromosome count data it was necessary to depart from the arithmetic relationship in the nuclear volume data. This is particularly true in dealing with the diploid class. In order to have approximately the same number of diploid cells, determined by nuclear volumes and from chromosome counts, it is necessary to combine the 300 and 400 cubic micron peaks. The following adaptations were made on Figures 2-8: (1) in Figure 2 the 300 and 400 cubic micron peaks were combined; (2) in Figure 3 no adaptation was necessary; (3) in Figure 4 the 360 and 400 cubic micron peaks, the 660 and 760 cubic micron peaks, and the 800 and 900 cubic micron

peaks were combined; (4) in Figure 5 no adaptation was necessary; (5) in Figure 6 no adaptation was necessary; (6) in Figure 7 the 300 and 400 cubic micron peaks were combined; (7) in Figure 8 the 360 and 400 cubic micron peaks were combined. The following peaks were considered to represent the various ploidy classes: (1) the hypodiploid peak was at 200 cubic microns; (2) the diploid peak was at 360 cubic microns; (3) the triploid peak was at 500 cubic microns; (4) the tetraploid peak was at 600 cubic microns; (5) the pentaploid peak was at 700 cubic microns; (6) the hexaploid peak was at 800 cubic microns; (7) and the heptaploid peak was at 900 cubic microns. The reader is cautioned to evaluate the results of this interpretation in light of the adaptations on the frequency peaks of nuclear volume.

In Table V the data on nuclear volumes after the adaptations were considered is found. Table V shows the per cent of nuclei found in the various ploidy classes in each mouse of series one with the mean per cent of nuclei that are found in each ploidy class. The probability that the same per cent of nuclei occur in each ploidy class with the combined probability that all of the probabilities are significant is also found in Table V.

In Table V the lowest chi square probability is found in the hypodiploid group. The chi square value for the hypodiploid group is 65.77 at six degrees of freedom. This value indicates that in this group there is great variability between mice. Since the hypodiploid group includes all

nuclei smaller than diploid, rather than representing an actual ploidy state, such as diploid or triploid, a much higher variability might be expected. The other ploidy classes, with the exception of the diploid, show a fair relationship between mice with the highest probability in the 5N class. It should be noted that in the hypodiploid group and diploid class, where the sample size is largest, there is great variability between mice. The sample size in the 4N, 5N, 6N, and 7N classes is too small to lend significance to their probability values. Taking these facts into consideration, it seems that the frequency distribution of parenchymal nuclear volume is variable between mice.

TABLE V

THE PER CENT OF LIVER NUCLEI FOUND IN EACH PLOIDY CLASS, FROM THE ADAPTED PEAKS IN FIGURES 2-8, IN EACH OF THE MICE IN SERIES ONE, WITH THE PROBABILITY THAT THE SAME PER CENT OF NUCLEI OCCUR IN EACH PLOIDY CLASS AND THE COMBINED PROBABILITY THAT ALL OF THE PROBABILITIES ARE SIGNIFICANT

Mouse	Hypodiploid	2N	3N	4N	5N	6N	7N
1	26.5	38	10	4	4	5.5	4
2	36.5	26	20.5	6	6.5	1	3.5
3	5.5	24.5	17	11	7.5	8.5	6
4	9.5	26	12.5	10	8	5	6.5
5	10.5	35.5	18	10.5	6.5	3	3
6	12.5	18	9	10	5	5.5	9.5
7	18.5	27.5	13	7	7.5	3.5	4.5
Mean	17.1	27.9	14.2	8.4	6.4	4.6	5.3
Probability	.00	.05	.30	.50	.95	.30	.50
Combined Probability			.50				

Note: The chi square value in the hypodiploid group is 65.77 at 6 degrees of freedom. All probabilities are at 6 degrees of freedom.

In Table I, dealing with the distribution of metaphase figures, the lowest probability is found in the hypodiploid group. The reason for the high degree of variability between mice in this group is the same as that given for its high variability in reference to nuclear volumes, that is, that the hypodiploid group does not represent an actual ploidy class but represents all metaphases less than diploid. The other six ploidy classes show good correlation between mice with the correlation falling off as the higher ploidy classes are encountered. The greatest correlation is found in the tetraploid class. These statistical values should be interpreted cautiously because of the small sample of data on which they are based.

Since much investigation has centered around the ploidy distribution of the liver in rodents of various ages, it would be of interest to compare the ploidy distribution, as determined by actual chromosome counts, that is found in the 1-year-old male mouse of series two with the ploidy distribution of the 6-week-old mice of series one. The hypodiploid group showed the lowest degree of correlation, as it did when compared between the younger mice. The degree of correlation increased as the higher ploidy values were reached with the ploidy classes 4N-7N having a probability of .80. A combined probability of .95 was obtained. It should be noted that although this shows a very high correlation between the metaphase distribution in the young and old mice, this

correlation is based on a small amount of data. Seven ploidy classes were observed in the 1-year-old male mouse with the greatest number of metaphases occurring in the diploid class.

Tanaka,[1] Kinosita,[2] and Marquardt[3] have noted, in regenerating rat liver, that the diploid class is most prevalent. Their work is based on actual chromosome counts. Marquardt[4] notes that the relative number of polyploids, as determined by chromosome counts, is dependent upon the strength of the stimulus. His data indicates that removing larger portions of the liver increases the number of dividing polyploid cells and may induce high polyploid cells which do not normally divide to do so. It may be that the gap between the data obtained by nuclear volume determinations and chromosome number determinations may be bridged by Marquardt's ideas on the frequency of division of polyploid cells.

Tables VI-A to VI-F compare the number of nuclei found in each ploidy class with the number of metaphases found in each ploidy class in each of the mice in series one. The nuclear volume data for this comparison was taken from Table V. The chromosome count data for this comparison was taken from Table I. The chi square probability is given for each mouse for N-1 or 13 degrees of freedom. Tables VI-C to VI-E have a chi

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[1]Tanaka, loc. cit.

[2]Kinosita, loc. cit.

[3]Marquardt, loc. cit.

[4]Ibid.

square probability of .99. This indicates a very high degree of correlation between the number of nuclei, according to their volume, found in each ploidy class and the number of metaphases, according to their chromosome complement, found in the corresponding ploidy class. Tables VI-A and VI-F have a chi square probability of .95. Table VI-B has a chi square probability of .30.

From the average per cent of nuclei in each ploidy class in Table V, it is noted that the greatest average per cent of nuclei occur in the diploid class. Alfert, as cited by Brauer,[1] found that, in the 6-week-old rat, the largest per cent of nuclei occurred in the diploid class. It should be noted that the data in Table V conflicts with the first hypothesis found in Table IV. In Table IV it was noted that the greatest average per cent of nuclei occurred in the tetraploid class. The data in Table IV was taken from the arithmetic relationship found in Figures 2-8. The data in Table V, which is the basis for the data in Tables VI-A to VI-F, does not follow an arithmetic relationship. This means that the volumes of the nuclei in the 2N, 4N, and 8N modes do not form a 1:2:4 numerical relationship (i.e. the volumes of the nuclei in the 4N mode are not twice those in the 2N mode and the volumes in the 8N mode are not four times larger than those in the 2N mode). The fact that the data in Tables VI-A

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[1]Brauer, op. cit., p. 12.

to VI-F was taken from nuclear volume modes that did not follow a 1:2:4 relationship in the 2N, 4N, and 8N ploidy classes must be seriously taken into consideration. Investigators that have noted 2N, 4N, and 8N modes

TABLE VI

A COMPARISON BETWEEN THE NUMBER OF NUCLEI OBSERVED IN EACH PLOIDY CLASS AND THE NUMBER OF METAPHASES OBSERVED IN EACH PLOIDY CLASS IN EACH OF THE MICE IN SERIES ONE WITH THE PROBABILITY THAT THESE TWO METHODS OF DETERMINING PLOIDY ARE RELATED

Table VI-A: Mouse one

Ploidy		Observed	Expected
Hypodiploid	Nuclear Volume	53	51.2
	Chromosome Count	11	12.8
2N	Nuclear Volume	76	72
	Chromosome Count	14	18
3N	Nuclear Volume	20	19.2
	Chromosome Count	4	4.8
4N	Nuclear Volume	8	8
	Chromosome Count	2	2
5N	Nuclear Volume	8	6.4
	Chromosome Count	0	1.6
6N	Nuclear Volume	5.5	4.4
	Chromosome Count	0	1.1
7N	Nuclear Volume	4	3.2
	Chromosome Count	0	.8
Chi square Probability[1]		.95	

[1](n-1), 13 degrees of freedom

Table VI-B: Mouse two

Ploidy		Observed	Expected
Hypodiploid	Nuclear Volume	73	60
	Chromosome Count	2	15
2N	Nuclear Volume	52	51.2
	Chromosome Count	12	12.8
3N	Nuclear Volume	41	41.6
	Chromosome Count	11	10.4
4N	Nuclear Volume	12	12
	Chromosome Count	3	3
5N	Nuclear Volume	13	11.5
	Chromosome Count	1	1.2
6N	Nuclear Volume	2	1.6
	Chromosome Count	0	.4
7N	Nuclear Volume	7	6.4
	Chromosome Count	1	1.6
Chi Square Probability		.30	

Table VI-C: Mouse three

Ploidy		Observed	Expected
Hypodiploid	Nuclear Volume	11	11.2
	Chromosome Count	3	2.8
2N	Nuclear Volume	49	47.2
	Chromosome Count	10	11.8
3N	Nuclear Volume	24	31.2
	Chromosome Count	10	7.8
4N	Nuclear Volume	22	21.6
	Chromosome Count	5	5.4

Table VI-C: Mouse three (continued)

Ploidy		Observed	Expected
5N	Nuclear Volume	15	14.4
	Chromosome Count	3	3.6
6N	Nuclear Volume	17	15.2
	Chromosome Count	4	3.8
7N	Nuclear Volume	12	12.8
	Chromosome Count	4	3.2
Chi Square Probability		.99	

Table VI-D: Mouse four

Ploidy		Observed	Expected
Hypodiploid	Nuclear Volume	19	18.4
	Chromosome Count	4	4.6
2N	Nuclear Volume	52	52
	Chromosome Count	13	13
3N	Nuclear Volume	25	24.6
	Chromosome Count	6	6.2
4N	Nuclear Volume	20	21.6
	Chromosome Count	7	5.4
5N	Nuclear Volume	16	16
	Chromosome Count	4	4
6N	Nuclear Volume	10	10.4
	Chromosome Count	3	2.6
7N	Nuclear Volume	13	11.2
	Chromosome Count	1	2.8
Chi Square Probability		.99	

Table VI-E: Mouse five

Ploidy		Observed	Expected
Hypodiploid	Nuclear Volume	21	19.2
	Chromosome Count	3	4.8
2N	Nuclear Volume	71	72.8
	Chromosome Count	20	18.2
3N	Nuclear Volume	36	36
	Chromosome Count	9	9
4N	Nuclear Volume	21	20
	Chromosome Count	4	5
5N	Nuclear Volume	13	14.4
	Chromosome Count	5	3.6
6N	Nuclear Volume	6	5.6
	Chromosome Count	1	1.4
7N	Nuclear Volume	6	6.4
	Chromosome Count	2	1.6
Chi Square Probability		.99	

Table VI-F: Mouse six

Ploidy		Observed	Expected
Hypodiploid	Nuclear Volume	25	26.4
	Chromosome Count	8	6.6
2N	Nuclear Volume	36	36.8
	Chromosome Count	10	9.2
3N	Nuclear Volume	18	20
	Chromosome Count	7	5
4N	Nuclear Volume	20	20.8
	Chromosome Count	6	5.2

Table VI-F: Mouse six (continued)

Ploidy		Observed	Expected
5N	Nuclear Volume	10	9.6
	Chromosome Count	2	2.4
6N	Nuclear Volume	11	10.4
	Chromosome Count	2	2.6
7N	Nuclear Volume	19	15.2
	Chromosome Count	0	3.8
Chi Square Probability		.95	

have found that the parenchymal nuclear volume in the 4N mode is very close to twice that of the 2N mode and that the parenchymal nuclear volume in the 8N mode is very close to four times that found in the 2N mode.

Another objection, which should be considered, to the data in Tables VI-A to VI-F is concerned with the presence of the hypodiploid group. The hypodiploid group has not been indicated in the work of other investigators who have worked with parenchymal nuclear volumes. Marquardt[1] has noted parenchymal cells which have a 1N complement of chromosomes. His work was also done on squash prepared tissue. Therman and Timonen, as cited by Hungerford,[2] working with human uterine epithelium and human embryonic tissue, found hypodiploidy to be the rule, reporting for uterine epithelium a bimodal distribution having

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[1]Marquardt, loc. cit.

[2]Hungerford, loc. cit.

a major peak at 20-25 chromosomes and a secondary one at 45-50. This work was done using squash preparations. Walker and Boothroyd,[1] working with mouse tissue and human endometrium, found wide deviations from the diploid in the direction of hypodi ploidy when the tissue was prepared by the squash technique. They found that when the tissue was prepared by the "swirling method" only slight deviations from the diploid were noted.

Sachs,[2] using the Feulgen squash technique, has counted chromosomes from 50 cells each of human, white rat, and the short-tailed bank vole, Microtus agrestis. Claiming accuracy within 5% or better, he found in all cases no deviation from the diploid. Furthermore, in figures which were not countable, he found little indication of subdiploid variation.

In Table II it is difficult to see a relationship between the average nuclear volume and the average chromosome number per metaphase. The data indicates that the average cell from all of the mice in series one would have a triploid complement of chromosomes and a volume of 627 cubic microns. The small sample size must be taken into consideration when considering these values. No attempt was made to select the

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[1]B. E. Walker and E. R. Boothroyd, "Chromosome Numbers in Somatic Tissues of Mouse and Man," Genetics, XXXIX (1954), 216.

[2]Leo Sachs, "Polyploid Evolution and Mammalian Chromosomes," Heredity, VI (1952), 362.

metaphases counted in a random manner. If the data is representative of the mice it would indicate that some mouse livers are predominantly diploid while others may be predominantly triploid or tetraploid. It is of interest to note that these are all male mice of the same age.

One of the problems in working with series two mice was the indiscreteness of the peaks of nuclear volume frequency. In Figure 19 and 20 an interval of 50 cubic microns was used. In Figure 17 and 21 an interval of 32 cubic microns was used. It is of interest to note that the numerical relationship in the male mouse, in which countable metaphase figures occurred, was the same as that found in the series one mice (i.e. a 200-400-600-800 relationship). A general numerical relationship which would fit all of the mice in series two was not noted. It was noted, in Figures 17-21, that the peaks are not as sharp and cover a greater range than the peaks in Figures 2-8 for the first series of mice. It is of interest to note that the range in volume, from the smallest to the largest nuclei, was greater in the older mice. It was also noted that a greater number of nuclei with large volumes occurred in the older mice.

The major purpose for doing the second series of mice was to determine if any distortion existed, in reference to nuclear volume, from the squash technique. In order to determine if any distortion occurred, nuclei from the pregnant female were drawn from squashed and suspended liver tissue. The assumption being made was that the suspended nuclei would not be distorted. Figure 20 represents the frequency distribution

of nuclei using the squash technique. Figure 21 represents the frequency distribution of nuclei using the hanging drop technique. The nuclei in Figure 21 were fixed and stained by the same method used in Figure 20. Since the tissues are from the same mouse, any differences between the distribution of the nuclei might be due to the technique used. In Figure 20 more nuclei are found in the lower volume peaks. In Figure 21 the curve is more flattened and the nuclei are more evenly distributed. The average nuclear volume in Figure 20 was 864 cubic microns. The average nuclear volume in Figure 21 was 978 cubic microns. This indicates that the average nucleus is only 14 cubic microns larger in the suspended tissue. The average nuclear volume in the male mouse was 961 cubic microns. The average nuclear volume in the female mouse of series two was 1,096 cubic microns. This indicates that the average nuclear volume in the female mouse is 135 cubic microns larger than the average nuclear volume in the male mouse. The average nuclear volume from the squash technique in the male mouse was 877 cubic microns. The average nuclear volume in the younger mice in series one was 627 cubic microns. This indicates that, although the same technique had been used, the average nuclear volume in the 1-year-old male mouse was 150 cubic microns larger than the average nuclear volume in the 6-week-old male mice. The average number of chromosomes per metaphase was 61.14 in the 1-year-old male mouse of series two. No metaphase figures were noted in the 1-year-old pregnant female. The average number of chromo-

somes per metaphase was 57.66 in the 6-week-old male mice. This would indicate that the average cell in both series of mice has a triploid complement of chromosomes. It should be noted that the value for the average number of chromosomes per metaphase may be an overestimation, as metaphase figures having a large number of chromosomes are more easily observed and since the chromosomes of said metaphases are less frequently clumped.

Two hypotheses were postulated in interpreting Figures 2-8. Figures 2-8 are concerned with the frequency distribution of parenchymal nuclear volume. The first hypothesis is based on the arithmetic progression of nuclear volume modes noted in Figures 2-8. The per cent of nuclei in each ploidy class, according to the first hypothesis, is shown in Table IV. The data in Table IV indicates modes in the 2N, 4N, and 8N ploidy classes. A 1:2:4 progression in the volumes of the nuclei which make up these modes is noted. This type of progression in the volumes of parenchymal nuclei has been noted by other investigators. The data in Table IV does not show a hypodiploid class. This also follows the findings of other investigators. Preliminary studies of mouse liver nuclei at the Drake University cancer research laboratory have shown intermediate nuclear volume values. Although the parenchymal nuclear volume data and the chromosome number data follow an arithmetic progression, these two arithmetic progressions do not show the same relative number of parenchymal nuclei and metaphase figures in each ploidy class.

Marquardt[1] has also noted an arithmetic progression in the number of chromosomes per metaphase in liver cells. He also noted that the strength of the stimulus (i.e. the amount of liver removed) seemed to increase the number of dividing nuclei having a high chromosome complement. He also believes that high polyploid cells that do not normally divide are induced to do so by a stronger stimulus. From this it might be concluded that the differences observed between the relative number of parenchymal nuclei and the relative number of dividing nuclei in each ploidy class might be explained on the basis of Marquardt's[2] findings (i.e. that polyploid nuclei require a stronger stimulus to induce division). Marquardt's[3] observations might be even more exemplified in light of the findings in regard to the 1-year-old male mouse of this study. The parenchymal nuclear volume distribution in this mouse showed a much greater range between the smallest and largest nuclei and a greater number of large nuclei were observed than in the 6-week-old male mice. Although a greater number of large nuclei were observed in the older mouse, no appreciable increase in the number of polyploid cells, as determined by actual chromosome counts, was observed. This might indicate that the higher polyploid cells were not induced to divide.

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[1] Marquardt, loc. cit.

[2] Ibid.

[3] Ibid.

The second hypothesis is based on the assumption that the frequency distribution of parenchymal nuclear volume is the same as the frequency distribution of dividing nuclei. In Figures 2-8 it was assumed that the 300 cubic micron mode and the 400 cubic micron mode represented the diploid class. The 200 cubic micron mode would then represent the hypodiploid class. With these assumptions a high degree of correlation is noted between the frequency distribution of nuclei of a particular volume and the frequency distribution of dividing nuclei of a particular chromosome complement.

This study and others, such as Marquardt's,[1] indicate that further investigation into the mechanics of mitosis are needed.

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[1] Ibid.

CHAPTER VI

SUMMARY

The present study is to determine if a relationship exists between the frequency distribution of nuclear volume and the frequency distribution of chromosome number in the liver of the mouse.

The frequency distribution of parenchymal nuclei, according to their volume, was determined in seven 6-week-old male mice. Modes of parenchymal nuclear volume were noted. The volumes of the nuclei at these modes followed an arithmetic progression. The frequency distribution of dividing nuclei, according to their chromosome number, was determined in six of the 6-week-old mice. Dividing nuclei were not noted in the seventh mouse. The dividing nuclei, according to their chromosome number, also formed modes which followed an arithmetic progression. By adapting the nuclear volume distribution peaks (i.e. by combining some peaks) a high correlation was obtained between the arithmetic progression of the modes of dividing nuclei.

Two 1-year-old mice were used to check the squash technique used on the 6-week-old mice. Drawings were made of nuclei that were suspended. The suspended nuclei were considered to have no distortion force acting on them. Little difference was noted in the average nuclear volume between nuclei that were suspended and nuclei that were squashed. Both the 6-week-old mice and the 1-year-old male mouse had an average

chromosome complement of near 60 chromosomes. The chromosome complement in the older mouse ranges from hypodiploid to 7N. Seven ploidy classes were also noted in three of the younger mice. The first six ploidy classes were noted in two of the seven mice and the first four ploidy classes were noted in one of the mice. From tissues prepared in the same manner, the older mice had a larger average nuclear volume than that found in the younger mice.

Two hypotheses were made in interpreting the data on parenchymal nuclear volume. The first hypothesis was based on the arithmetic progression of the parenchymal nuclear volume modes. The second was based on the assumption that the frequency distribution of parenchymal nuclear volume is the same as the frequency distribution of dividing nuclei. The merits and shortcomings of both hypotheses have been discussed in Chapter V.

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