

SPECTROPHOTOMETRIC DETERMINATION  
OF NUCLEAR DESOXYRIBONUCLEIC ACID  
IN SELECTED TISSUES OF  
TARICHA (TRITURUS) GRANULOSA GRANULOSA SKILTON

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INTRODUCTION

Desoxyribose nucleic acid (DNA) has been known for some time to be an important constituent of cell nuclei. Since Miescher's isolation of a nucleic acid from the nuclear material of pus cells in 1869, the structure and chemistry of DNA has been the object of many investigations, for which a good review has been provided by Davidson (9). Studies of its localization and distribution in the cell showed it to be almost exclusively confined to the nucleus, specifically to the chromosomes. For such studies the Feulgen nuclear reaction, recently reviewed by Lessler (16), has been considered the most reliable. The reaction was first described by Feulgen and Rossenbeck in 1924 (12), and since that time it has been the most widely used reaction specific for DNA.

The first quantitative studies on DNA were based on biochemical analyses of nuclear suspensions. This method was first used by Boivin, Vendrely and Vendrely (3) on beef tissues. The DNA content per nucleus was found to be approximately constant in different tissues, but the amount per sperm nucleus was nearly half of that found in the somatic cells. Similar results were found in other mammal

and bird tissues by Vendrely and Vendrely (31, 32, 33) and in fishes, amphibians, reptiles and birds by Mirsky and Ris (18). These findings led the authors to suggest that the DNA content per nucleus is constant in the nuclei of any one species and that DNA might be viewed as a possible gene component. However, the DNA content per nucleus determined with similar methods by Mirsky and Ris (18) for beef liver, thymus, lymph node and kidney were more than twice the sperm nucleus amount. Mirsky and Ris suggested the presence of some special "duplication of gene material" in mammals to explain the discrepancy found. Their determinations, however, represented averages of a great number of nuclei, and differences between the various types of nuclei of any one organ, as well as individual variations in nuclei of the same tissue type, were obviously lost.

Photometric measurements of individual nuclei presented a better picture of the variations in the DNA content between nuclei of the same and different tissue types. The first cytophotometric method, introduced by Caspersson (6), used the natural ultraviolet absorption at  $2600 \overset{\circ}{\text{A}}$  by the purine and pyrimidine rings of nucleic acids. A method measuring the absorption in visible light of Feulgen-stained nuclei was later developed by Pollister and Ris (23) and by Pollister and Moses (22). Since then,

these cytospectrophotometric methods have been widely used in studies concerned with the quantitative behavior of DNA in nuclear populations. Considerable attention has been given not only to the stable aspect of DNA content in interphasic nuclei but also to its variations during the mitotic cycle, to the degree and frequency of polyploidy in nuclear populations, and to its variations accompanying physiological changes.

The photometric measurements of polyploidy in interphasic nuclei are particularly valuable, since chromosome counts are generally difficult to carry out and polyploidy studies based on nuclear sizes are not reliable. Increased size in nuclei does not always correspond to an increased number of chromosomes or an increased amount of DNA. Polyploid classes and their frequencies have been reported in various normal and abnormal mammalian tissues by Pasteels and Lison (20), Swift (27), Alfert (1) and others. A few rare polyploid nuclei in adult frog tissues have been reported by Swift (27). Adult salamander tissues have been little explored except for an unpublished study on the effect of starvation in liver nuclei of adult Triturus by Kleinfeld, alluded to by Swift (28).

The present study was undertaken to extend this still limited body of information by providing data on the nuclear distribution of DNA in various adult tissues of

the salamander Taricha (Triturus) granulosa granulosa  
Skilton, a form common in the Corvallis area.



## MATERIALS AND METHODS

Fixation and Staining

After preliminary testing of the Feulgen technique on the tissues of several salamanders, two healthy adult males were selected for detailed study. These were taken from a collection made in the MacDonald Forest near Corvallis, Oregon, on March 19, 1954. The salamanders were killed by decapitation and pieces of liver, kidney, pancreas and skin were rapidly removed and fixed in 30 per cent neutral formalin for 8 hours. The fragments were kept small, the largest dimension not exceeding 5 mm. After washing, the tissues were imbedded in paraffin, and sections were cut at  $18\mu$  and  $22\mu$  for the liver,  $18\mu$  for both the pancreas and kidney, and  $30\mu$  for the skin (where only the granular glands were intended for study). Staining with the Feulgen reagent followed the procedure described by Stowell (26). The time of hydrolysis in 1N HCl at  $60^{\circ}\text{C}$  was 20 minutes, though a difference of 5 minutes did not produce an appreciable change in the maximal intensity of the color produced by the subsequent treatment with the sulphurous fuchsin reagent for one hour. Unhydrolysed sections were also stained for controls. Sections of liver tissue from the same block were placed on all the slides studied as a standard of comparison. All the slides measured were stained with the same stock

Feulgen reagent kept completely colorless by storage in tightly closed containers at 5°C.

#### Photometric Apparatus

The photometric apparatus (plate I) consisted essentially of a microscope and a Farrand photomultiplier unit. The light source was a 6-volt tungsten coiled filament in a Griffith Base-Lite Illuminator connected through a voltage stabilizer and transformer to the 110-volt line source. The magnified image of the microscope field was projected onto the reflex ground glass screen of a Leitz Aristophot camera, where the nuclear size could be directly measured in millimeters. By swinging the mirror of the reflex housing out of position, the image was sent up to an RCA 1P21 phototube mounted on a modified plate carrier. An iris diaphragm placed directly under the phototube limited the projection on the photosensitive area to the desired fraction of the nucleus to be measured. The screen bore a mark and was adjusted in such a way that when the nucleus was centered on the mark it was also centered at the diaphragm level. The latter point could be checked by looking down on the plane of the diaphragm through a magnifying lens slid over the diaphragm.

All absorption measurements were taken at the 550 m $\mu$  line isolated from the light source with a Bausch and Lomb

550 m $\mu$  interference filter which was placed over the condenser of the Base-Lite Illuminator. A Bausch and Lomb 4 mm achromatic objective, N.A. 0.65, was used, together with a 6x or 8x Leitz periplan ocular, depending on the magnification required. The objective was mounted on a centerable nosepiece. A Leitz aplanatic, achromatic centerable condenser, N.A. 1.40, completed the optical set-up. Photocurrents were read on a Rubicon spot-light galvanometer of 0.0023  $\mu$ a/mm sensitivity and 108,000 ohms critical damping resistance.

#### Method of Measurement

The following data were recorded (see sample data sheet, plate II):

- 1) magnification at phototube (or focusing screen) level
- 2) nuclear diameter or average of the major and minor diameters for ellipsoid nuclei
- 3) 60 per cent of nuclear diameter (or of average diameter) for setting of diaphragm, which determined size of nuclear plug
- 4) galvanometer deflection when light passed through the nucleus (Tt)
- 5) galvanometer reading through the cytoplasm near the nucleus (Tb)

- 6) per cent transmission (%T) given by  $T_t/T_b$
- 7) extinction  $E = \log_{10} T_t/T_b$
- 8) relative amount of the absorbing substance, or DNA, in the whole nucleus (A). This was calculated from the formula used by Alfert (1), viz.,

$$A = \frac{E x r^2}{0.49} x M^2$$

where A is the amount of DNA in arbitrary units, E is the extinction, r is the plug radius at the phototube level, and M is a magnification factor ( $M = \mu/\text{mm}$  at the phototube level). Derivation of this formula is given in the appendix.

Care was taken to measure only entire nuclei as determined by critical focusing beyond their upper and lower surfaces. Nuclei which were completely out of focus when other objects remained sharply in focus were assumed to be whole. Errors involved in the measurements of incomplete nuclei were thus avoided. Nuclei departing markedly from spherical or ellipsoidal shapes were avoided.

Magnification at the level of the phototube was adjusted in such a way that the 60 per cent nuclear plug diameter would not exceed the range of 3.5 mm to 6 mm. Six millimeters was the upper limit of uniform sensitivity of the phototube. Though 2 mm was the lower limit, measurements below 3.5 mm at 600x magnification were not

possible because of low intensity of light. Nuclear sizes were measured to the nearest quarter of a millimeter at magnifications of 400x or above. Diaphragm settings were made to the closest 0.1 mm. Transmission percentages could be determined within an error of 2 per cent; higher accuracy was not necessary since nuclear volumes could not be determined with greater exactitude.

## RESULTS

The microscopic appearances of nuclei in the Feulgen-stained sections of liver, kidney, pancreas and granular glands are shown in plates III and IV. In each case the nuclei of parenchymal cells are easily distinguished by their large and usually round shape, as well as their disposition, from the nuclei of stromal and vascular elements. Only parenchymal cells were considered in this study.

Pancreatic nuclei, though fixed like all the other material in 30 per cent formalin, showed somewhat more discreet aggregation of chromatin than nuclei of liver, kidney or granular glands. As will be explained in the discussion, this difference in the distribution of light-absorbing particles may account for the slightly lower peak DNA values for nuclear classes in pancreatic cells when compared with those from liver and kidney.

In order to obtain a reasonably representative number of determinations for the nuclei of the four tissues, all of the whole nuclei included in any one section were measured, provided their shape fitted the requirements of the formula.

Liver Nuclei

Interphasic nuclei of liver parenchyma were measured in two animals. The distributions of the relative amounts

of DNA are graphically shown in plates V and VI. In one animal 644 nuclei were measured, in the other 910 nuclei. Within the ranges 12 to 20 and 11.5 to 20, respectively, the great majority of DNA values formed in both cases a high, rather sharply delimited and fairly symmetrical unimodal curve. A few DNA values, 2.2 per cent of the total number of determinations in the first animal and 2.6 per cent in the second, were scattered from 22 to 33 and from 24 to 29.5, respectively. The DNA values of liver nuclei were thus grouped into two classes. The peak DNA value for class I nuclei was 14.5 in both animals. Class II nuclei included values that doubled this amount.

#### Kidney Nuclei

The number of kidney tubule nuclei measured was 136 in the first animal, 125 in the second, and the results are shown on the upper and lower graphs of plate VII. Unimodal curves similar to those of liver were obtained. In both animals all DNA values ranging from 11 to 18 and from 11.5 to 19.5, with a peak value of 13.5 in both cases, could be placed into class I. Two DNA values fell at 21, but no values twice the peak amount of class I were found. Careful scanning of several sections did not show any large and darkly stained nuclei which might give class II values. The average DNA content of 13.5 for 10 nuclei of the

standard liver section which was mounted together with the kidney sections for comparison, seemed to indicate a slightly lower intensity of stain in the kidney slides. The comparison, however, also showed that class I nuclei of kidney cells were in close agreement with class I nuclei of liver cells with respect to DNA content.

#### Pancreatic Nuclei

The distribution of the DNA values determined for 675 nuclei in the first animal and 944 nuclei in the second are shown on plates VIII and IX. The results obtained were essentially similar to those found in liver nuclei. Within the ranges 11 to 19 and 11.5 to 19, respectively, the DNA values formed high unimodal curves (class I nuclei). Only a few values, 5.2 per cent and 1.6 per cent, respectively, of the totals were found in the ranges 20.5 to 25.5 and 20 to 24. The class I peak values occurred at 12.5 in the first animal and at 13.5 in the second. DNA content in the standard liver nuclei averaged 14.7 and 15.0 respectively. The slightly lower peak values of pancreatic nuclei can be accounted for by their less homogeneous chromatin distribution, as previously mentioned.



### Granular Gland Nuclei

Skin sections were taken from the dorsal tail region in one animal and from the back region in the other. In the first case, most of the granular glands were at earlier developmental stages than in the second case. In the younger glands cell walls were sharp, the cytoplasm was evenly filled with secretion granules, small nuclei were common, and most of the larger nuclei occupied a central position in the cell. A larger number of mature glands were found in the second location. In these the cell boundaries were lost (apocrine secretion), the cytoplasm was vacuolated, and peripherally located giant nuclei (fig. 3, plate IV) were numerous. These very large nuclei were usually flattened and could not be measured with the present photometric method. Because of this limitation only a small number of measurements were possible, 127 in the first animal and 73 in the second. Therefore the results, graphically shown on plate X, include only a partial representation of the granular gland nuclei. In the first animal (dorsal tail region), in which the smaller nuclei were observed to be the most numerous, DNA values ranging from 14 to 26 were found to be the most common. The peak value in this class (class I) was between 15 and 18. Within the range 30 to 51 a somewhat smaller number of nuclei formed another curve with peak value around 32.

One DNA value at 57 may fall into a third class. In the second animal (back region) the smaller nuclei were less frequently encountered than those of class II. The DNA values of class I nuclei, ranging from 14 to 25, formed a low curve, not well defined. Class II values formed a broad curve with a range of 29 to 50 and a peak around 31. A few DNA values were scattered between 60 and 75 and can be grouped into a third class with double the value of class II. The average DNA amounts of the standard liver nuclei were 14.3 and 14.2, respectively, comparable to class I of the granular gland nuclei.

## DISCUSSION AND CONCLUSIONS

Specificity of the Feulgen Stain

Since the Feulgen nucleal reaction has been often used in cytophotometric studies, the validity of a great part of the photometric data for DNA in cell nuclei depends on the qualitative and quantitative specificity of this reaction. The exact mechanism of the Feulgen nucleal reaction for DNA is not fully known. According to Overend and Stacey (19) a large percentage of desoxyribose and other carbohydrates exists naturally in a non-cyclic form which gives aldehyde tests, due to the terminal  $-CH=O$  group. Mild acid hydrolysis removes some of the purine groups of DNA as shown by Li and Stacey (17), liberating aldehyde groups. According to Wieland and Scheuing (35) these couple with the colorless fuchsin-sulfurous acid of the Feulgen reagent to form a violet-colored complex. Ribonucleic acid (RNA) under such conditions does not give a positive reaction. The mild acid hydrolysis causes very little depolymerization of the DNA and does not split DNA from its nucleoproteins in any appreciable amount, as shown by Ely and Ross (11). The latter point explains the insolubility character of the reaction at the site of the DNA-protein complex in the nucleus.

The qualitative specificity of the Feulgen nucleal reaction for DNA in situ has been demonstrated time and again by various workers. Brachet (4, 5) and Catcheside and Holmes (7) showed that tissue sections did not give a positive reaction after the removal of DNA with desoxyribonuclease. The Feulgen nucleal reaction for DNA can be effectively prevented by the blocking of the aldehyde groups with various aldehyde-coupling reagents, as demonstrated by Lessler (15). Other proofs of the qualitative specificity of the reaction are the staining of isolated chromatin threads in work by Claude and Potter (8) and Barber and Callan (2) and the agreement in the localization of DNA by the Feulgen reaction with its localization by ultraviolet spectrophotometry following treatment with ribonuclease, as discussed by Pollister and Ris (23).

Ris and Mirsky (25) have demonstrated that the cytochemical Feulgen nucleal reaction under properly controlled conditions is quantitatively specific for DNA. This was done by comparing the results obtained on cytological preparations with the DNA values biochemically determined in the same material by Boivin, Vendrely and Vendrely (3). Swift (27) and Hoover and Thomas (13) showed that the Feulgen color intensity of cytological preparations varies directly with the thickness of the absorbing layer, obeying the requirements of the Beer-Lambert law. The constancy in the DNA values of Feulgen-stained nuclei, as well as the

1:2:4 polyploid ratios reported in numerous studies, is a good indication of the quantitative specificity of the Feulgen reaction. Conditions that are known to modify the reaction intensity, such as the pH and temperature of the hydrolytic solution, time of hydrolysis, and time of staining in the Feulgen reagent are to be carefully controlled if reproducible results are to be obtained. In the course of this study particular care was given to such standardization of the Feulgen procedure. Liver sections from the same block, mounted on all the slides, also provided a standard for comparison in the case of slides stained at different times.

#### Sources of Error in Cytospectrophotometry

The various sources of error in this method have been discussed and estimated by many authors among them Swift (27) and Patau (21).

The non-homogeneity of the Feulgen-stained nuclear material is a major source of error. It can, however, be reduced by the use of a fixative which produces a minimum clumping of chromatin. In the course of this study 30 per cent formalin was found to be most effective, in agreement with the experiences of other investigators. Inaccuracies resulting from the residual non-homogeneity can be reduced by measuring an adequate nuclear area of constant fractional

size for all nuclei. The plug area at the center of the nucleus should be small enough to eliminate errors due to the curved surfaces of the nucleus, but large enough to present an adequate sampling of the non-homogeneous chromatin. A plug size representing 60 per cent of the average nuclear diameter was found suitable by Alfert (1) and was used in the present study. Swift and Kleinfeld (29), studying mitotic and meiotic prophase nuclei in the grasshopper testis, found the distributional error in their material to be less than 15 per cent. It is certainly no higher in the present study, since the interphasic nuclei are more homogeneous.

Incomplete nuclei or irregularly shaped nuclei may be another source of error. The method for checking wholeness of nuclei has already been explained and it is unlikely that any incomplete nuclei were measured. Nuclei of irregular outline were also avoided, though a slightly flattened shape or flattening caused by cover-glass pressure could conceivably escape detection. In the main, however, this problem could not have been serious, since sections were cut at a thickness several micra greater than the diameter of the largest nuclei, as determined by previous measurement.

Non-specific light loss can occur, due to the scattering of light out of the measured beam by colloidal particles

and by larger bodies of different refractive index from the surrounding medium. The degree of this light loss can be determined by measuring undyed tissue otherwise identical to the test object. Since Swift (27) reported that such blanks give negligible absorption in material comparable to that used in this study, correction for non-specific light loss was deemed unnecessary.

Scattering of light into the measured beam by surrounding tissues (stray light) was reduced by setting the substage diaphragm at the smallest aperture giving good extinction readings. By employing a filter slightly off the Feulgen absorption peak errors arising from stray light, particularly at high extinction values, were further minimized.

Linearity of phototube response was carefully checked before experimental measurements were made. As pointed out earlier, linearity was obtained at focal plane (level of phototube) diameters of 2 to 6 millimeters. Magnifications were adjusted for measurements within these limits.

### Analysis of Results

In the data presented, the measurements formed curves essentially similar to those usually obtained in Feulgen photometric studies on nuclei. The spreads within nuclear classes were, however, somewhat wider, but this is to be

expected, variations being greater in a large series of measurements. In the DNA distribution curves for the liver, kidney and pancreas nuclei, these variations might be accounted for by the over-all error involved in the cytophotometric method. However, the question whether some of the variations might not indicate real differences in the nuclear DNA content should not be ignored. Some of the DNA values intermediate between the classes seem beyond the margin of error, as in the particular case of the granular glands. The spread of the nuclear class II in these glands is approximately twice that in the other tissues, even though a considerably smaller number of determinations were made. There is indication of high polyploid frequency in the granular glands, and part of the spread can be interpreted as endomitotic activity with interphasic synthesis of DNA. The noticeable brevity of the spread below the class I peak would seem to support this view. Interphasic synthesis of DNA (endomitotic activity) might also account to some extent for intermediate values between nuclear classes in liver and pancreas. Aneuploidy in such nuclei is also a possibility, since its occurrence in the somatic tissues of mammals has been reported by Therman and Timonen (30) and by Hsu and Pomerat (14). A valuable critique of these findings has recently been published by Walker and Boothroyd (34).



Unlike mammalian tissues, where polyploidy has been found to be common in beef liver and even reach 50 per cent in old mouse liver, as reported by Swift (27), salamander hepatic and pancreatic nuclei show very low polyploid frequency. Only about 2.5 per cent tetraploidy (class II nuclei) was found in the liver, 5.2 per cent in the pancreas, and no octoploid amounts in either. Intermediate values between the DNA nuclear classes were scarce, indicating that these tissues are not mitotically or endomitotically very active. The polyploid frequency, however, might vary with the age of the animal, as is known to happen in mammals. Seasonal variation might also occur. Further studies in these directions are needed.

In the granular glands, since a great part of the nuclear population could not be measured because of various shape irregularities, the picture of the DNA distribution is not complete. There is, however, sufficient evidence that high polyploid frequency exists and indication that it increases in degree as well as frequency during the development and growth of the glands. In the younger glands, taken from the tail region, the class I (diploid) DNA values were more common than the class II (tetraploid) values, and the class III (octoploid) values were rare. In the more mature glands of the skin sections, taken from the back region, diploid DNA values were less

common than the tetraploid values. Only a few class III determinations were possible, but large, darkly stained and irregular or flattened nuclei, which might give octoploid or higher values, were frequently encountered. Thus it was not possible to determine the full extent of polyploidy in the mature glands. Giant nuclei which stained lightly were also observed, but their irregular size likewise precluded photometric determination of their DNA content.

The conspicuous nuclear changes in size, position and shape accompanying the various phases of secretory activity in the granular glands have been fully described by Dawson (10) for the salamander Triturus viridescens. He expressed the opinion that, though the evidence was not conclusive, these changes might suggest nuclear participation in the glandular activity. In the mature glands he noted nuclei of gigantic size, alveolar patterns of chromatin distribution, vacuolizations, and indications of possible passage of nuclear material into the cytoplasm. It is clearly of interest to establish the DNA relations of these very large nuclei. The "two wave-length method" of Patau (21) may lend itself to such a study, though the more conventional one used here does not. An extension of the present investigation in this direction would be desirable.

## SUMMARY

1) Relative DNA amounts were determined photometrically on individual Feulgen-stained parenchymal nuclei of the liver, kidney, pancreas and granular glands of the salamander Taricha (Triturus) granulosa granulosa Skilton. Graphs of these values indicated the presence of nuclear classes falling into polyploid ratios.

2) A total of 644 liver parenchyma nuclei were measured in one animal and 910 in another. A vast majority of the DNA values fell into the first class, representing the diploid condition. Only 2.2 per cent and 2.6 per cent, respectively, of the total number of nuclei constituted class II, which included values roughly double those of class I. No haploid or octoploid values were found.

3) In the kidney tubules, 136 and 125 nuclei were measured. All could be included in class I. No tetraploid or octoploid values were found.

4) In the pancreas 675 nuclei were measured in one animal, 944 in another. Only 5.2 per cent and 1.5 per cent, respectively, of the total number of nuclei fell into the range of tetraploid values. No octoploid nuclei were found.

5) The 127 DNA values determined in the young granular glands of the tail region fell into classes I

and II; only one nucleus approached the octoploid value. A total of 73 determinations from older granular glands of the back region fell into three classes: diploid, tetraploid and octoploid. However, their frequency in the total nuclear population could not be assessed because of the small number of determinations obtained and the additional presence of very large and irregular nuclei not measurable by the method used. There was clear indication, however, of a much higher frequency and degree of polyploidy than characterized the other tissues examined.

6) Sources of error in the measurements were analysed, and various interpretations were offered for real variations in values, including the role of endomitotic DNA synthesis, aneuploidy, etc. It was pointed out that further investigations would be needed to study any possible variation in the degree and frequency of polyploidy incident to age and seasonal changes in the organism. More complete data would also be needed before conclusive statements could be made on the relationship between changes in DNA amounts and cellular activity in the granular glands.

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APPENDIX

## APPENDIX

$$\text{Derivation of Photometric Formula } A = \frac{E \times r^2}{0.49} M^2$$

According to the Beer-Lambert law the extinction (E) for substances in solution is proportional to the amount of absorbing molecules, i.e., proportional to the concentration (c) and the thickness (d) of the absorbing layer:

$$E = kcd \quad (1)$$

where k is a constant characteristic of the substance measured. Pollister and Swift (24) and Ris and Mirsky (25) have shown that Feulgen-stained DNA in nuclei follows approximately the Beer-Lambert law and that relation (1) could be used in the calculation of relative amounts of DNA in individual nuclei. From equation (1)

$$\text{concentration } c = \frac{E}{kd}. \quad (2)$$

The amount of absorbing substance (A) is given by concentration x volume. If the volume is that of a cylinder,

$$A = c\pi r^2 d = \frac{E}{kd} \cdot \pi r^2 d. \quad (3)$$

The thickness (d) cancels out in equation (3) and the constant (k) can be neglected for relative measurements of the same absorbing substance. The amount of absorbing substance is then proportional to the extinction times the cross-sectional area of the cylinder measured, or

$$A = E \times \pi r^2. \quad (4)$$

In the case of spherical nuclei, the amount of Feulgen dye can be determined in a cylinder delimited by a plug at the center of the nucleus ( $E \times$  area of plug). The total amount of Feulgen-stained DNA in arbitrary units in the whole nucleus is given by the relation

$$A \text{ (in arbitrary units)} = \frac{E \times \pi r^2}{F} \quad (5)$$

where  $F$  is the per cent volume of the sphere represented by the central cylinder. Ellipsoid nuclei can be considered as spherical by taking the average of the major and the minor diameters, the error involved being small.

As Alfert (1) pointed out, if the plug radius ( $r$ ) is constant for nuclei of different sizes, the percentage of nuclear volume measured would vary considerably and sizable errors due to the non-homogeneous distribution of chromatin in the nuclei could result. To reduce these errors a constant fraction ( $F$ ) can be measured for each nucleus.  $F$  can be computed from

$$F = \frac{R^3 - (R^2 - r^2)^{3/2}}{R^3} \quad (6)$$

$R$  being the radius of the spherical nucleus and  $r$  the radius of the plug. When the plug diameter is set equal to 60 per cent of each nuclear diameter,  $F$  is uniformly equivalent to 49 per cent of the nuclear volume. For convenience of computation the area ( $\pi r^2$ ) of the plug may be replaced simply by its proportional square radius. Then

$$A \text{ (in arbitrary units)} = \frac{E \times r^2}{0.49}. \quad (7)$$

A final modification to permit comparison of measurements made at different magnifications introduces a factor  $M^2$ , where  $M = \mu/\text{mm}$  at the level of the phototube. Thus,

$$A \text{ (in arbitrary units)} = \frac{E \times r^2}{0.49} M^2. \quad (8)$$

## EXPLANATION OF PLATES

## PLATE I

Cytospectrophotometric apparatus. Description given in text.

## PLATE II

Sample data sheet, showing recordings for the computation of relative DNA values in individual nuclei.

## PLATES III and IV

Photomicrographs of Feulgen-stained nuclei in tissue sections of the salamander Taricha granulosa granulosa. Thickness 18 $\mu$  in figs. 1-3; 30 $\mu$  in figs. 4 and 5. All magnifications 750x.

Fig. 1. Liver.

Fig. 2. Kidney.

Fig. 3. Pancreas.

Fig. 4. Granular gland.

Fig. 5. Granular gland (giant nucleus).

## PLATE V

Distribution of relative amounts of DNA (in arbitrary units) in liver nuclei of first animal.

## PLATE VI

Distribution of relative amounts of DNA (in arbitrary units) in liver nuclei of second animal.

## PLATE VII

Distribution of relative amounts of DNA (in arbitrary units) in kidney nuclei of two animals (upper and lower graphs, respectively).

## PLATE VIII

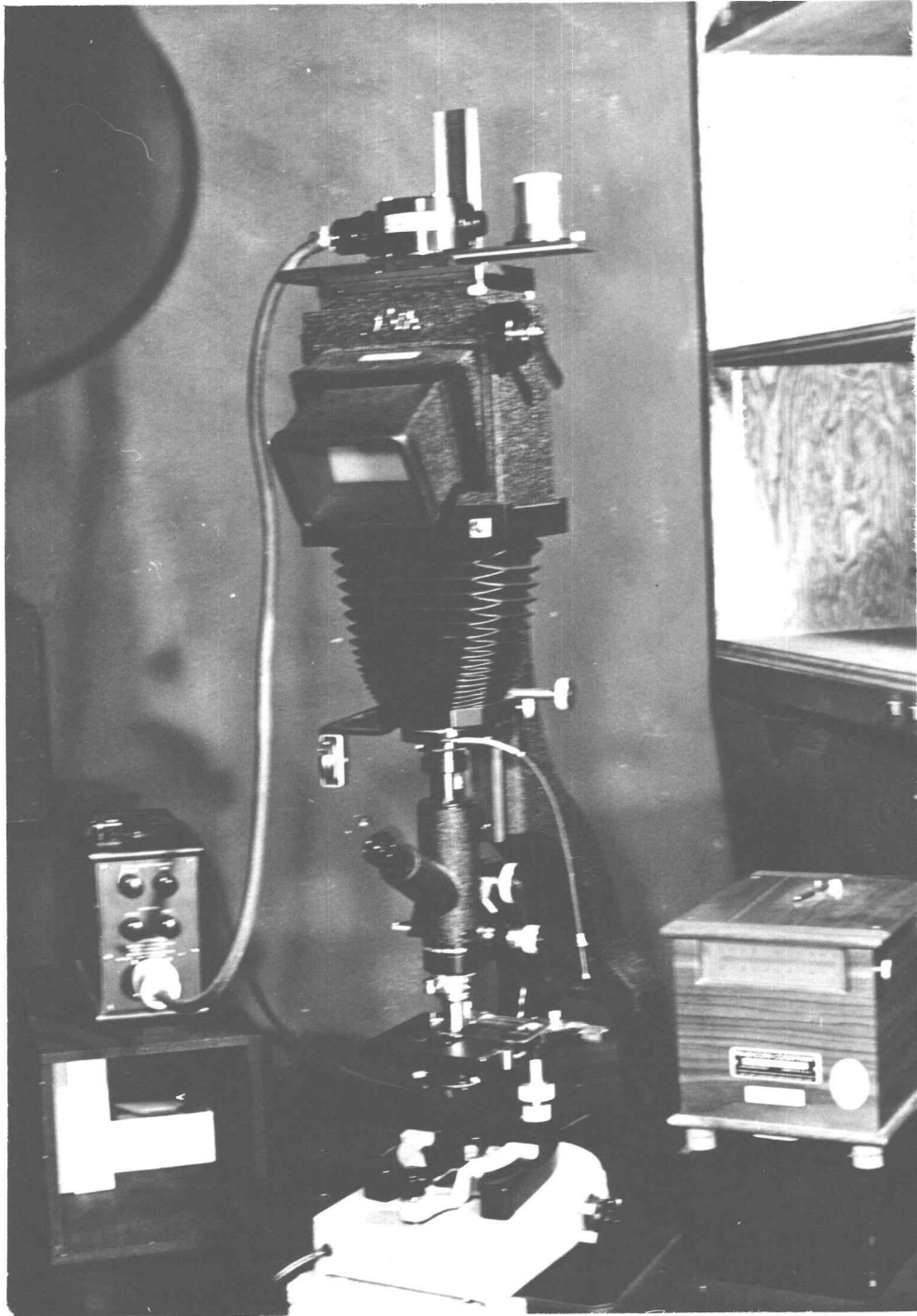
Distribution of relative amounts of DNA (in arbitrary units) in pancreatic nuclei of first animal.

## PLATE IX

Distribution of relative amounts of DNA (in arbitrary units) in pancreatic nuclei of second animal.

## PLATE X

Distribution of relative amounts of DNA (in arbitrary units) in nuclei of granular glands. Upper graph represents younger glands from tail region. Lower graph represents older glands from back region.



Cytospectrophotometric Apparatus

Tissue: *Liver parenchyma*      Animal: #2      Slide: #3      22μ      Date:

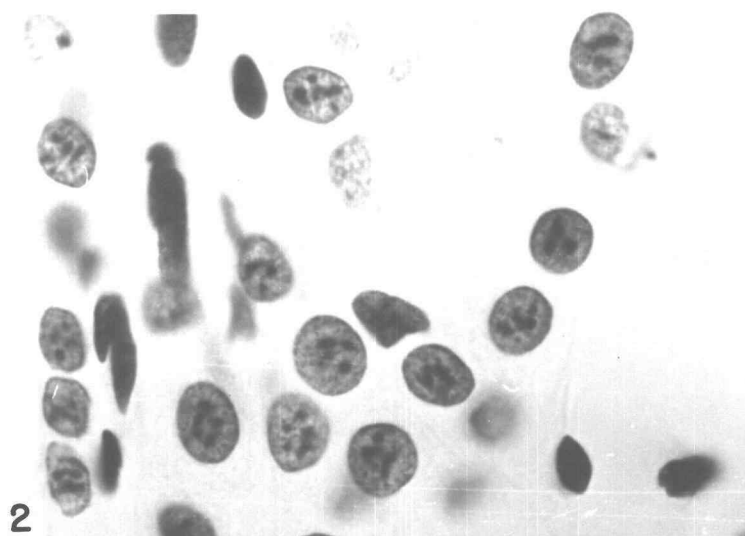
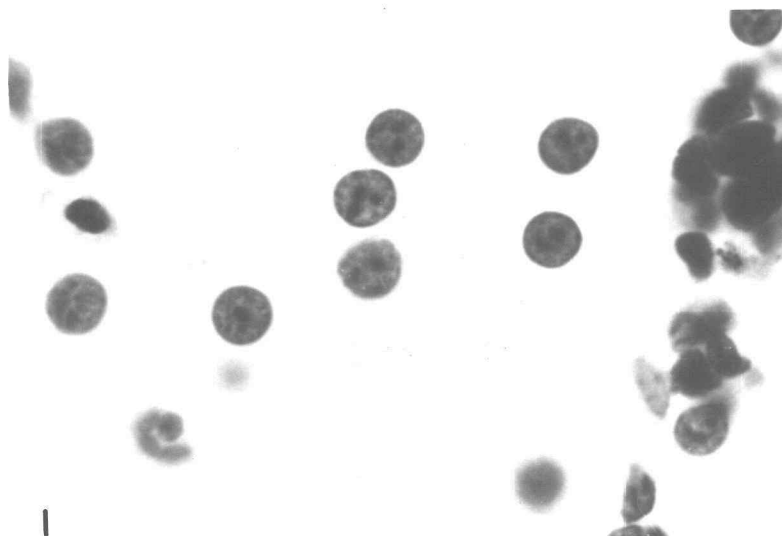
Nucleus	Mag	Diam 1 mm	Diam 2 mm	Av Diam mm	60% Av Diam (Plug Diam)	r <sup>2</sup> mm (Plug rad <sup>2</sup> )	T <sub>t</sub>	T <sub>b</sub>	%T	E	M (u/imm)	M <sup>2</sup>	A = $\frac{E \times r^2}{0.49} M^2$
row section (#1, 3)	600X	7.2	6.2	6.7	4	4	18	73	24.7	0.607	1.666	2.76	13.7
"	"	7	6.5	6.75	4	4	17	72	23.6	0.627	"	"	14.1
"	"	7	6.5	6.75	4	4	19	73	26	0.585	"	"	13.2
"	"	7.2	6.2	6.7	4	4	19	73	26	0.585	"	"	13.2
"	"	7.2	6.2	6.7	4	4	16	71	22.5	0.648	"	"	14.6
"	"	7.2	6.2	6.7	4	4	16	71	22.5	0.648	"	"	14.6
"	"	7	6.5	6.75	4	4	17	72	23.6	0.627	"	"	14.1
"	"	7	6.5	6.75	4	4	17	71	23.9	0.622	"	"	14
"	"	7	6.5	6.75	4	4	17	71	23.9	0.622	"	"	14
"	"	7.2	6.2	6.7	4	4	19	73	26	0.585	"	"	13.2
"	"	7.2	6.2	6.7	4	4	16	72	22.2	0.654	"	"	14.7
"	"	7.2	6.2	6.7	4	4	21	73	28.9	0.541	"	"	12.2
"	"	7	6.5	6.75	4	4	17	71	23.9	0.622	"	"	14
"	"	7	6.5	6.75	4	4	16	72	22.2	0.654	"	"	14.7
"	"	7.2	6.2	6.7	4	4	16	72	22.2	0.654	"	"	14.7
"	"	7.2	6.2	6.7	4	4	16	69	23.2	0.634	"	"	14.3
"	"	7.2	6.2	6.7	4	4	16	69	23.2	0.634	"	"	14.3
"	"	7.2	6.2	6.7	4	4	15	70	21.4	0.670	"	"	15.1
"	"	7	6.5	6.75	4	4	15	70	21.4	0.670	"	"	15.1
"	"	7.2	6.2	6.7	4	4	16	70	22.8	0.642	"	"	14.4
"	"	7	6.5	6.75	4	4	16	71	22.5	0.648	"	"	14.6
"	"	7	6.5	6.75	4	4	15	71	21.1	0.676	"	"	15.2
"	"	7.2	6.2	6.7	4	4	13	68	19.1	0.719	"	"	16.2
"	"	7	6.5	6.75	4	4	15	68	22.1	0.656	"	"	14.8
"	"	7.2	6.2	6.7	4	4	16	68	23.5	0.629	"	"	14.2
"	"	7.5	6	6.75	4	4	15	70	21.4	0.670	"	"	15.1
"	"	7.5	6	6.75	4	4	16	72	22.2	0.654	"	"	14.7

PLATE II

Sample Data Sheet

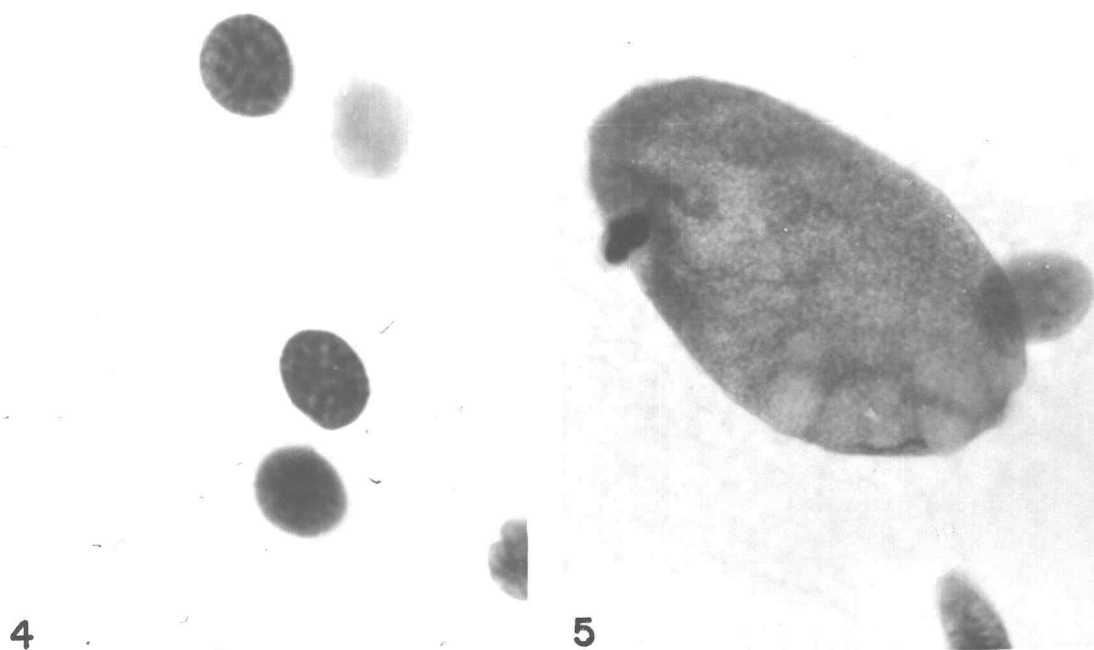
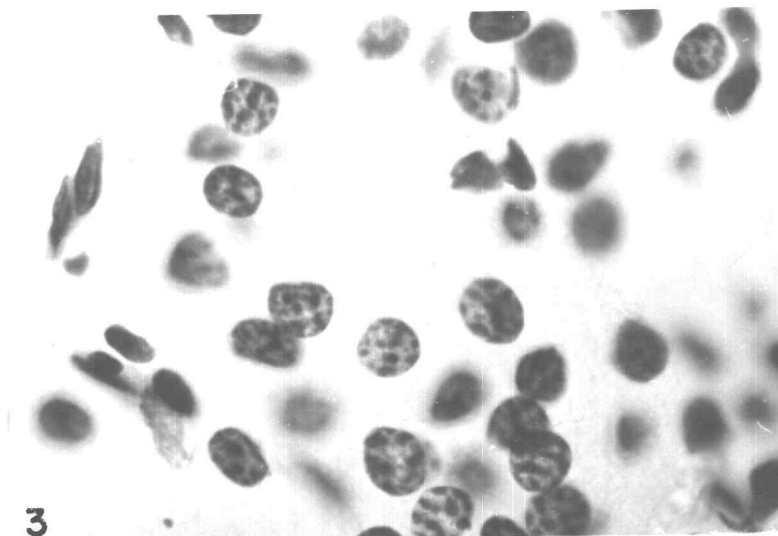


PLATE III

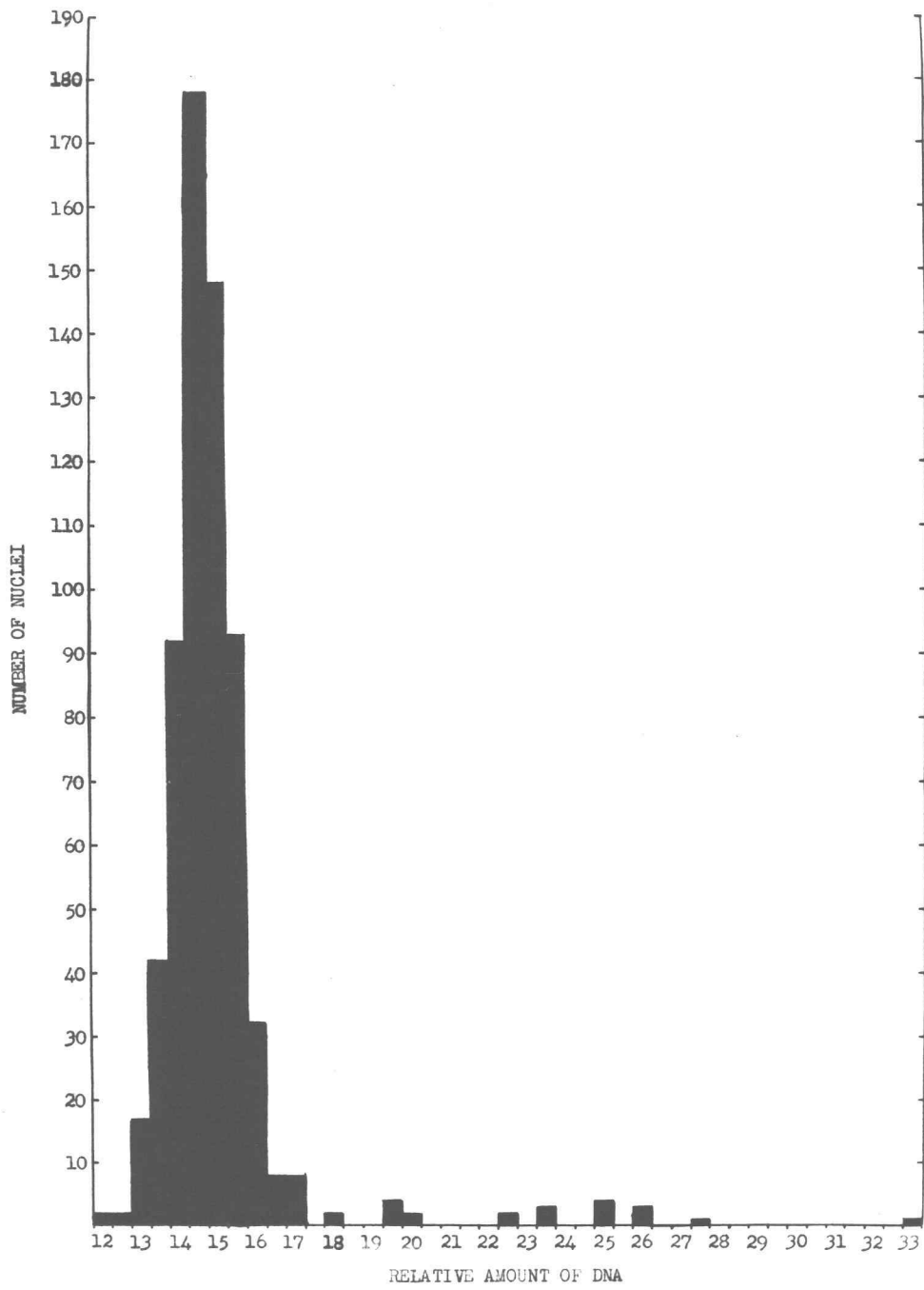


Liver (1) and Kidney (2) Nuclei

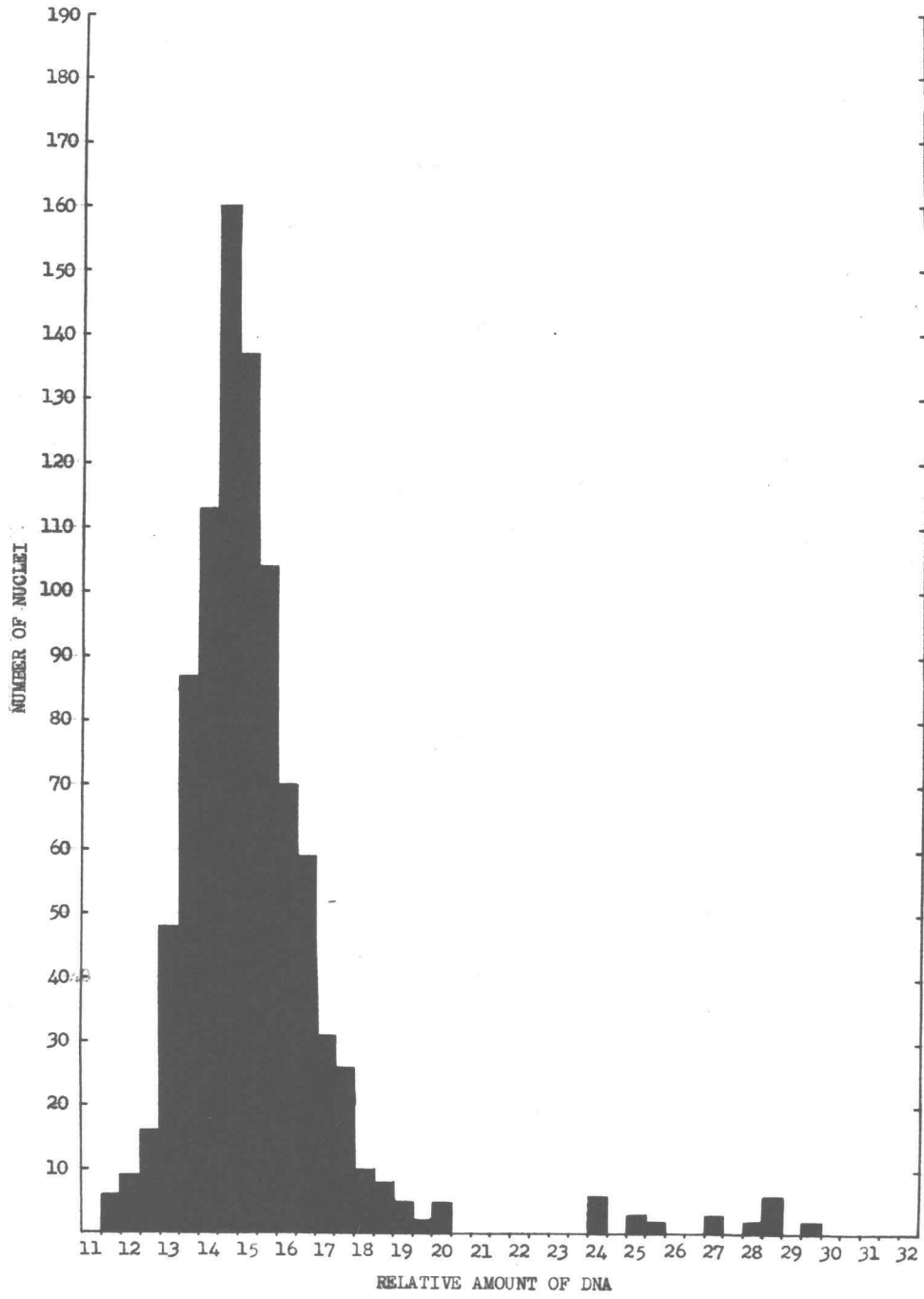
## PLATE IV



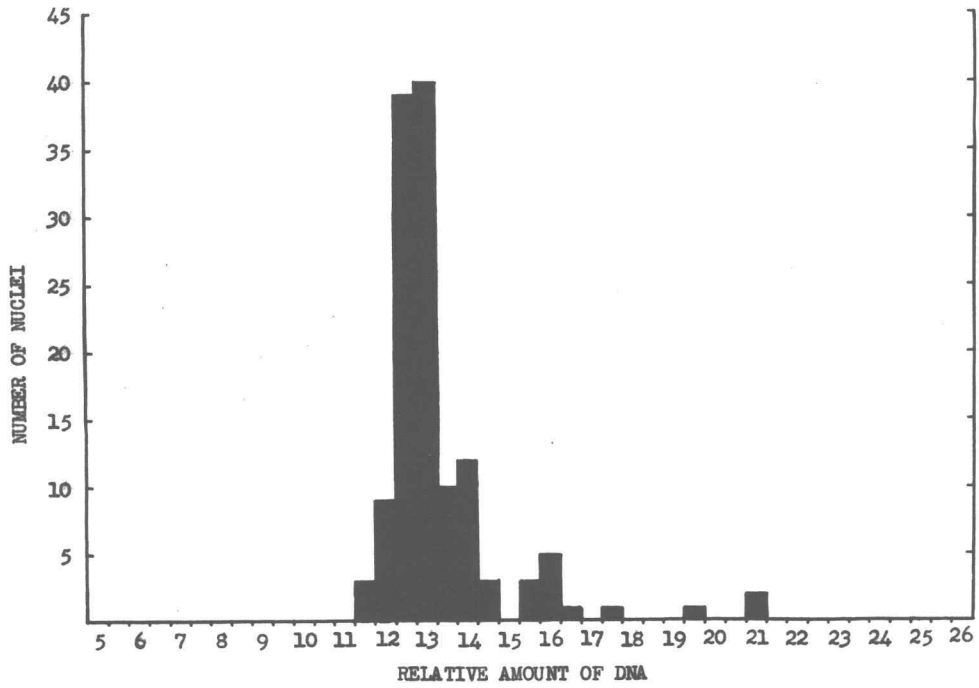
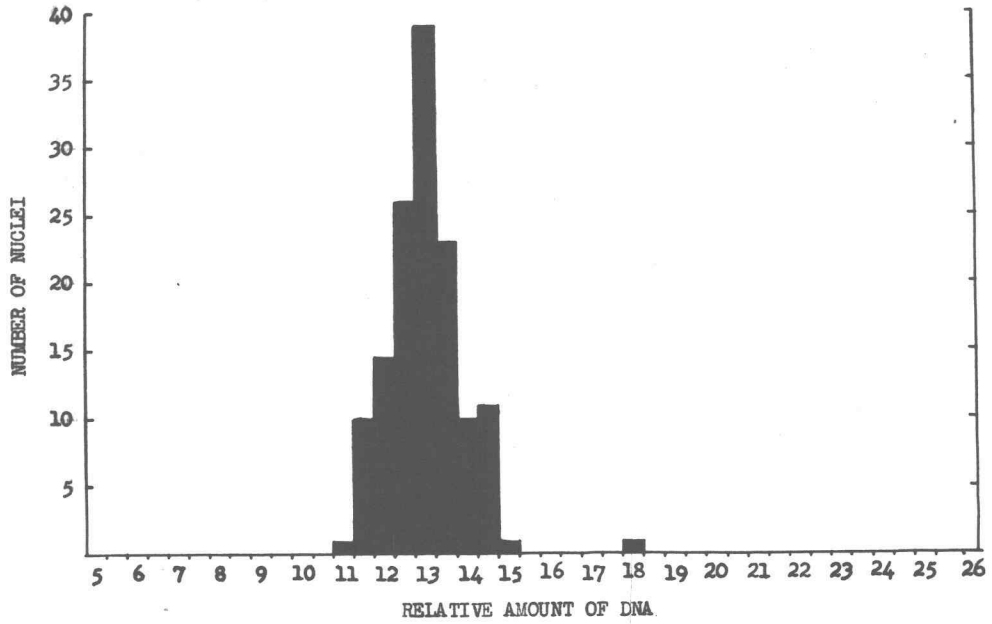
Pancreas (3) and Granular Gland (4,5) Nuclei



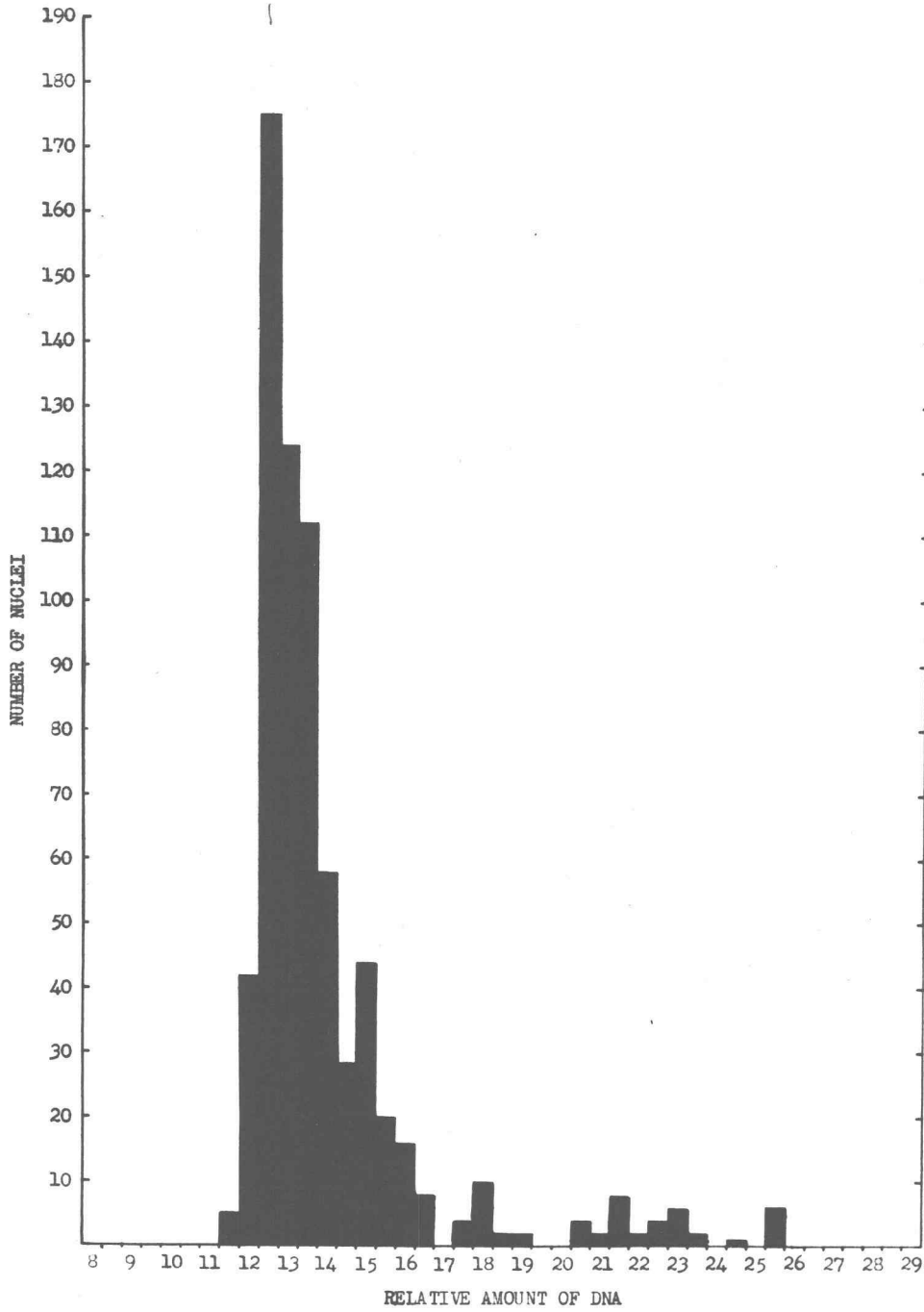
Distribution of DNA in liver nuclei of first animal



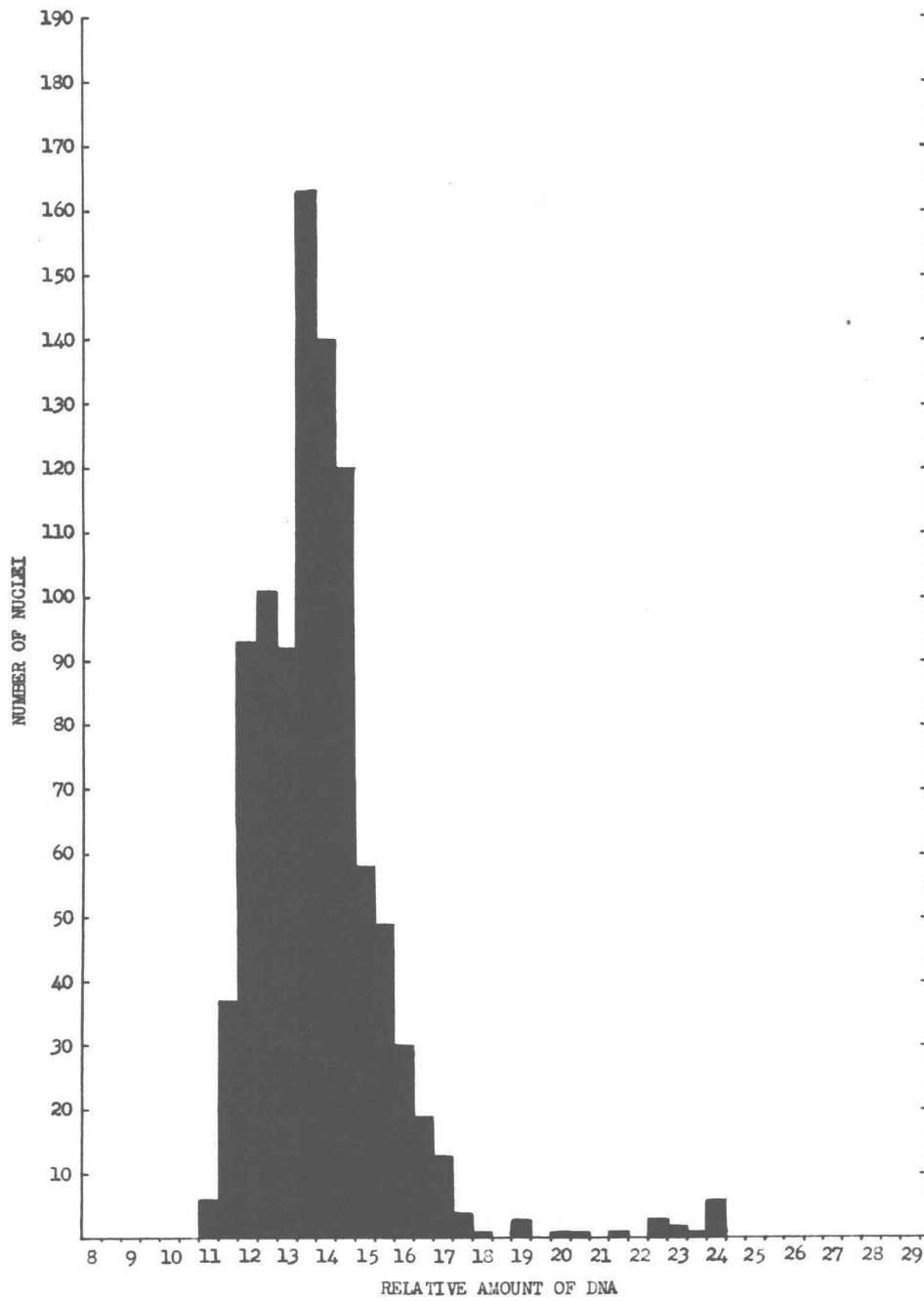
Distribution of DNA in liver nuclei of second animal



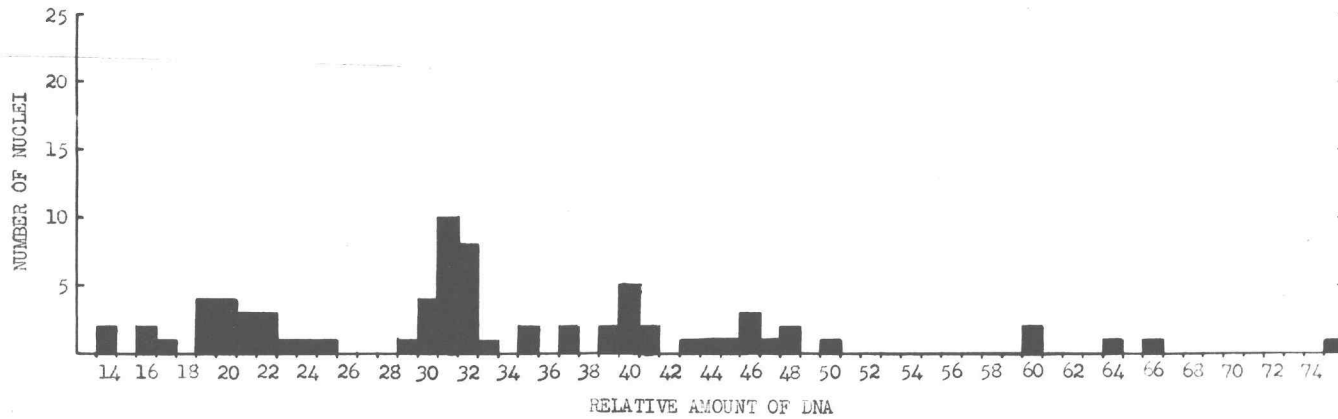
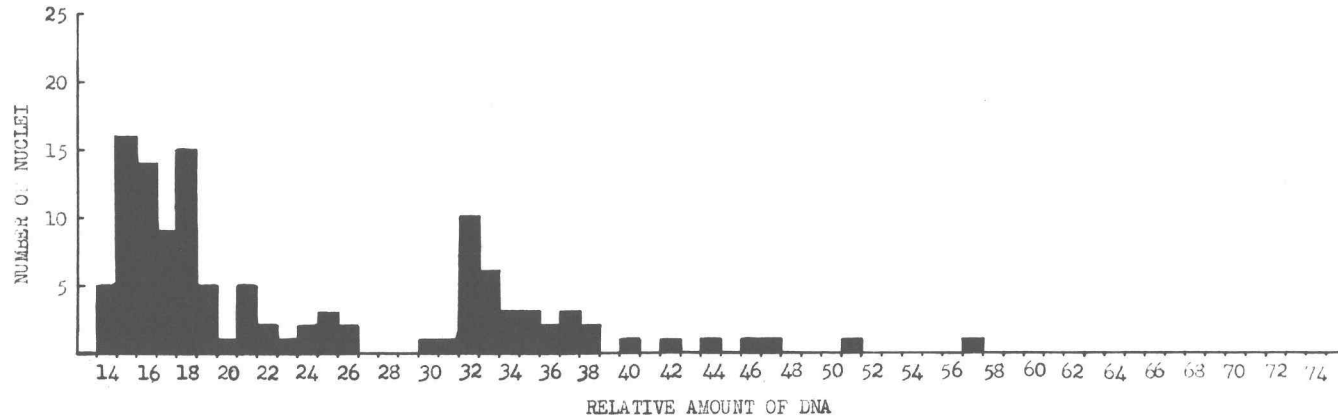
Distribution of DNA in kidney nuclei of two animals



Distribution of DNA in pancreatic nuclei of first animal



Distribution of DNA in pancreatic nuclei of second animal



Distribution of DNA in granular gland nuclei  
 (Younger glands of tail region in upper graph;  
 older glands of back region in lower graph)

PLATE X