

SEX CHROMATIN IN VERTEBRATES
WITH EMPHASIS ON FISHES

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ABSTRACT

Nineteen species of vertebrate, representing fishes, amphibians, reptiles, and birds were investigated to determine if nuclear sexual dimorphism occurs as has been described for mammals.

Feulgen positive masses were present in cell nuclei of most tissues. Some were similar in size, shape, and location to sex chromatin found in human buccal smears of the female; but, it was impossible to detect microscopically a nuclear sex difference in any of the species examined, except the garter snake. In this snake, although intranuclear chromatin bodies were present in tissues of both sexes, the chromatin bodies were larger and were on the nuclear border more frequently in tissues of females than of males. These differences were highly significant.

The difference between the incidences of intranuclear chromatin masses in tissues of females and males was also highly significant in timber rattlers, pigeons and rainbow trout. In timber rattlers and pigeons the females had a higher incidence of chromatin masses, while in rainbow trout the males had the higher incidence.

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TABLE OF CONTENTS

	Page
Introduction	1
Literature Review	2
Characteristics of sex chromatin	2
Discovery	3
Application and significance	4
Distribution	5
Origin and suggested function	7
Materials and Methods	14
Obtaining tissues	14
Preparation of tissues for study	15
Examination of tissues	15
Observations	17
Petromyzones	17
Teleostomi	20
Amphibia	26
Reptilia	30
Aves	36
Discussion	41
Summary	49
Literature Cited	50
Appendices	56

LIST OF TABLES

TABLE	PAGE
1. F values for incidence of chromatin masses by sex and tissue and per cent chromatin masses on nuclear membrane.....	22
2. Comparison of mean size of sex chromatin between male and female garter snake.....	31

LIST OF ILLUSTRATIONS

FIGURE		PAGE
1.	Lamprey. Prosencephalon, female.....	19
2.	Lamprey. Spinal cord, female.....	19
3.	Lamprey. Epidermis, female.....	19
4.	Lamprey. Intestinal epithelium, female.....	19
5.	Lamprey. Kidney, female.....	19
6.	Lamprey. Liver, female.....	19
7.	Rainbow trout. Cerebral hemisphere, female...	23
8.	Rainbow trout. Cerebral hemisphere, male.....	23
9.	Rainbow trout. Cerebellum, female.....	23
10.	Rainbow trout. Cerebellum, male.....	23
11.	Rainbow trout. Medulla, female.....	23
12.	Rainbow trout. Medulla, male.....	23
13.	Rainbow trout. Kidney, female.....	24
14.	Rainbow trout. Kidney, male.....	24
15.	Rainbow trout. Liver, female.....	24
16.	Rainbow trout. Liver, male.....	24
17.	Rainbow trout. Intestinal epithelium, female.	24
18.	Rainbow trout. Intestinal epithelium, male...	24
19.	Spot tail shiner. Optic lobe, female.....	25
20.	Mottled sculpin. Cerebral hemisphere, male...	25
21.	Mottled sculpin. Cerebellum, female.....	25
22.	Stickleback. Cerebellum, female.....	25
23.	Spot tail shiner. Liver, female.....	25
24.	Spot tail shiner. Kidney, male.....	25

FIGURE		PAGE
25.	Manitoba toad. Cerebral hemisphere, female...	28
26.	Leopard frog. Cerebral hemisphere, male.....	28
27.	Manitoba toad. Optic lobe, female.....	28
28.	Tiger salamander. Cerebral hemisphere, male..	28
29.	Leopard frog. Spinal cord, female.....	28
30.	Tiger salamander. Medulla, female.....	28
31.	Tiger salamander. Liver, female.....	29
32.	Leopard frog. Liver, male.....	29
33.	Leopard frog. Smooth muscle, female.....	29
34.	Tiger salamander. Kidney, male.....	29
35.	Tiger salamander. Intestinal epithelium, male	29
36.	Tiger salamander. Epidermis, male.....	29
37.	Garter snake. Optic lobe, female.....	32
38.	Garter snake. Optic lobe, male.....	32
39.	Garter snake. Medulla, female.....	32
40.	Garter snake. Medulla, male.....	32
41.	Garter snake. Spinal cord, female.....	32
42.	Garter snake. Spinal cord, male.....	32
43.	Garter snake. Epidermis, female.....	33
44.	Garter snake. Epidermis, male.....	33
45.	Garter snake. Kidney, female.....	33
46.	Garter snake. Kidney, male.....	33
47.	Garter snake. Liver, female.....	33
48.	Garter snake. Liver, male.....	33

FIGURE		PAGE
49.	Garter snake. Intestinal epithelium, female..	34
50.	Garter snake. Intestinal epithelium, male....	34
51.	Garter snake. Pancreas, female.....	34
52.	Garter snake. Pancreas, male.....	34
53.	Garter snake. Smooth muscle, female.....	34
54.	Garter snake. Smooth muscle, male.....	34
55.	Timber rattler. Optic lobe, female.....	36
56.	Timber rattler. Optic lobe, male.....	36
57.	Timber rattler. Liver, female.....	36
58.	Timber rattler. Liver, male.....	36
59.	Timber rattler. Kidney, female.....	36
60.	Timber rattler. Kidney, male.....	36
61.	Pigeon. Medulla, female.....	38
62.	Pigeon. Medulla, male.....	38
63.	Pigeon. Cerebellum, female.....	38
64.	Pigeon. Cerebellum, male.....	38
65.	Pigeon. Spinal cord, female.....	38
66.	Pigeon. Spinal cord, male.....	38
67.	Pigeon. Kidney, female.....	39
68.	Pigeon. Kidney, male.....	39
69.	Pigeon. Liver, female.....	39
70.	Pigeon. Liver, male.....	39
71.	Pigeon. Intestinal epithelium, female.....	39
72.	Pigeon. Intestinal epithelium, male.....	39

FIGURE		PAGE
73.	Pigeon. Pancreas, female.....	40
74.	Pigeon. Pancreas, male.....	40
75.	Pigeon. Smooth muscle, female.....	40
76.	Pigeon. Smooth muscle, male.....	40
77.	Pigeon. Epidermis, female.....	40
78.	Pigeon. Epidermis, male.....	40

INTRODUCTION

Nuclear sexual dimorphism of interphase somatic cells is a fairly widespread phenomenon in mammals. The dimorphism is based on the presence or absence of sex chromatin. 'Sex chromatin' is the name given to an intranuclear chromocenter which indicates the sex of the organism from which the cell originates. It stains intensely with nuclear dyes and is present in the nucleus during interphase.

The purpose of this study is to gain further knowledge of nuclear sexual dimorphism in vertebrates and if present to determine: (1) the incidence of sex chromatin in the vertebrates with emphasis on fishes; and (2) the correlation between presence of sex chromatin and known chromosomal mechanisms of sex determination.

Previous investigations indicate that nuclear sexual dimorphism of neurons is a general phenomenon in primates, carnivores, ungulates, insectivores and chiropterans. There is no clear indication of sex chromatin in rodents, lagomorphs, edentates, marsupials and birds. Sex chromatin has not been recorded for reptiles, amphibians and fishes.

From karotype studies, sex chromosomes have been identified in mammals, birds, some reptiles, some amphibians; but, not clearly indicated in fishes. However, genetic studies on fishes indicate some form of cytological sex determination. In mammals, the female is homogametic and the male heterogametic. The reverse is true for birds, reptiles and amphibians. Fishes illustrate both mechanisms.

LITERATURE REVIEW

Characteristics of Sex Chromatin

Sex chromatin has been identified in living and fixed cells. Criteria as to size, shape, and location in nuclei, have been established for identification of sex chromatin (Klinger, 1966). The size is about 1μ in diameter, with an average of $0.7 \times 1.2\mu$ in nuclei of buccal mucosa and in sections of several human tissues (Moore and Barr 1953, 1954). Other staining masses may be present in nuclei, but they are generally smaller and regarded as non-specific chromocenters (Mittwoch, 1964). Sex chromatin is most commonly situated at the periphery of the nucleus as a plano-convex or wedge-shaped mass and less frequently found free in the cytoplasm or lying against a nucleolus (Berenbaum, 1960; Klinger, 1957). It is present in a large proportion of nuclei of female origin and almost absent in male nuclei (Moore, 1966b).

Sex chromatin was introduced into human genetics in 1953, when Moore et al. described sex chromatin in skin biopsies (Mittwoch, 1967). In two surveys on sexual dimorphism of nuclei in human skin, sex chromatin incidence was reported for females and males as 52-85% and 1-14% (Emery and McMillan, 1954); and, 51-77% and 2-6% (Marberger, 1954). Other techniques were developed, such as the buccal smear (Moore, 1966c) and the "drumstick" in the polymorphonuclear leucocytes (Mittwoch, 1967). These also illustrate

that the incidence of sex chromatin is higher in nuclei of female origin than in nuclei of males.

Discovery

A female-specific chromocenter was described in insects prior to the identification of sex chromatin in vertebrates. Geitler in 1937, in his studies on bugs (Hemiptera) and flies (Diptera) found that females had two chromatin bodies in each somatic nucleus while males had one (Mittwoch, 1964). Smith in 1944-45, in his work on Archips fumiferana (spruce budworm), described a chromocenter in interphase nuclei of the female (Smith, 1945). He used it for diagnosis of sex of the larvae of Archips. Other arthropods have been studied with similar results (Mosbacher and Traut, 1968; Traut and Mosbacher, 1968). It is known that the male is homogametic (XX) and the female heterogametic (XY or XO) in these species.

The discovery of sex chromatin in mammals is attributed to M. L. Barr and E. G. Bertram (Mittwoch, 1964). While examining chromatolytic neurons of the cat, they identified a female-specific chromocenter in the nuclei of the neurons (Barr and Bertram, 1949). Investigations of other tissues and other mammals, adults and embryos, established a means of diagnosis of sex by nuclear dimorphism. (Moore and Barr 1953, 1954). In mammals, the male is heterogametic (XY) and the female homogametic (XX).

Application and Significance

When methods for studying human chromosomes became available, a correlation was revealed between sex chromatin and the number of X-chromosomes. Presence of sex chromatin was associated with sixteen chromosomes in group 6-12, that is two X-chromosomes. While fifteen chromosomes in group 6-12, one X-chromosome, was associated with absence of sex chromatin. Harnden proposed a formula to express the sex chromatin number in terms of the number of sex chromosomes and ploidy of the cell (Harnden, 1961).

$$S.C. = X - P/2$$

In man p (ploidy) is $2n$; therefore, the sex chromatin number is one less than the number of X-chromosomes. However two things should be considered; first not all nuclei contain sex chromatin, and second, the maximum number of sex chromatin is not always expressed (Miller and Warburton, 1968; Mittwoch, 1964; Schwarzacher, 1963). This may be the result of technique, position of sex chromatin, or perhaps DNA duplication. However it could be that sex chromatin is not formed in all cells. Nevertheless sex chromatin does reflect the number of X-chromosomes.

In man, there appears to be a phenotypic response to the ratio of X-chromosomes and sex chromatin number, particularly in the expression of sex. Therefore, sex chromatin number can distinguish discrepancies between genetic and phenotypic sex; the XO type Turner's syndrome,

XXY-type Klinefelters syndrome and multiple X-chromosomes may be detected (De Mars, 1963; Hamerton, 1962; Klinger *et al.*, 1965; Maclean *et al.*, 1968; Moore, 1966a).

Therefore, a sex chromatin test such as the buccal smear, provides a simple aid in diagnosis of such patients and makes possible to screen large populations, and thus obtain estimates regarding the incidence of these abnormalities.

Although sex chromatin is a useful tool in human genetics, its main significance lies in providing an opportunity to study genes in two different states: heterochromatic versus euchromatic; and to study the effect of heterochromatization on gene function (Brown, 1966). Hence a survey of presence and incidence of sex chromatin in animals other than man would extend the experimental field.

Distribution

The extent of nuclear sexual dimorphism in vertebrates has been investigated in all classes (Beckert, 1962; see Barr, 1966 and Moore, 1966b for review).

Of the eutherian mammals, the most comprehensive coverage has been on primates. Apes and monkeys exhibit a sex chromatin pattern which in size, frequency and shape is similar to that in man (Hamerton *et al.*, 1963; Prince, Graham and Barr, 1955). Sex chromatin is present also in members of the carnivores, artiodactyls and perissodactyls. In rodents and rabbits, nuclear sexual dimorphism does not

always follow a similar pattern. In many tissues, nuclei contain coarse chromatin masses from which it is difficult to distinguish the sex chromatin. Sex chromatin has been found in metamyelocytes of mice (Berenbaum, 1960) and in epithelial cells of the buccal cavity and vagina of the rat (Baig and Telford, 1967). In the creeping vole, Microtus oregoni, the nuclei of male origin contain two chromocenters while those of the female contain only one. Both sexes are heterogametic, the male XY and the female XO (Ohno and Stenuis, 1963). Sex chromatin has been reported in blastocysts of the rabbit as early as ninety-six hours after mating of the parents (Issa, Blank and Atherton, 1969). But, the authors did not show any direct proof of the sex of the experimental material; hence the "sex chromatin" may be a non-specific chromocenter.

Some marsupials that have been studied illustrate a unique type of sexual dimorphism. In opossums, nuclei of both sexes contain a chromatin mass, but that in the nuclei of female origin is larger (Graham and Barr, 1959; Perondini and Perondini, 1966; Steyer and Paulete-Vanrell, 1969). In the bandicoot, Perameles nasuta, a sex chromatin-like body was present in corneal epithelial cells of both males and females (Walton, 1969). Other Australian marsupials were examined, but many chromatin masses in individual nuclei made identification of sex chromatin impossible. The chromosomal mechanism of sex determination appears to differ

from that in eutherian mammals. Multiple sex chromosomes have been described in marsupials (Martin and Hayman, 1966).

The studies on birds have been conflicting. In the fowl, Gallus domesticus, there have been reports on the presence of sex chromatin in some tissues of female origin (Koshida and Kosin, 1968). Other investigators were not able to establish a sex difference, since cells from both males and females contained many chromatin masses (Ashley and Theiss, 1959; Hammar, 1964). Likewise, a sex difference was not detectable in other birds such as pigeon and duck. In birds the male is homogametic: XX (ZZ) and the female heterogametic: XY (WZ).

Sexual dimorphism of nuclei has not been detected in reptiles, amphibians and fishes. For most reptiles and amphibians, the male is homogametic: XX, and the female heterogametic: XY (Becak, 1968; Witschi, 1959), although sex reversal appears to be possible in at least one species of amphibian, Xenopus laevis (Weiler and Ohno, 1962). In some fishes, based on genetic studies, heterogamety and homogamety of the female may be operative (Gordon, 1957). Male heterogamety, based on chromosomal analysis has been reported for one species of fish (Chen and Ebeling, 1965), and female heterogamety in another species (Chen and Ebeling, 1968).

Origin and Suggested Function

From the earliest descriptions of sex chromatin, it was postulated that sex chromosomes are involved in the

formation of sex chromatin. The first suggestion was that sex chromatin represents condensed portions of both X-chromosomes; however, this was inadequate to explain multiple sex chromatin associated with multiple X-chromosomes. The more acceptable proposal regards sex chromatin to be formed by condensation of one X-chromosome or part of one (Grumbach and Morishima, 1962; Russell, 1964). Based on sex linkage in the mouse, M. Lyon developed the "inactive-X hypothesis" to account for origin of sex chromatin and its genetic function (Mittwoch, 1967; Lyon, 1968). She proposed that in a female mammal one of the two X-chromosomes may be inactive in different cells of the same animal. The original inactivation occurs randomly in early embryonic development, and the descendants of each X-chromosome will remain inactive. This makes the female a natural mosaic, as in one line of cells the paternal X-chromosome functions and in the other, the maternal X-chromosome is active. Sex chromatin represents the inactive X-chromosome. But since the incidence of sex chromatin is never 100 per cent in any tissue, there is a third population of cells in which both X-chromosomes must operate (Klinger et al., 1967). It is interesting to speculate on the relevance of a $X^P/X^M/X^P X^M$ female to the survival of the species. A mosaic such as this would indicate cell populations with alternating gene activity due to the state of sex chromosomes. Since many sex-linked characteristics in the recessive form are deleterious, this

arrangement favors the expression of the dominant normal allele. Thus insuring that the female fulfills her reproductive potential.

Russel (1964) cautions the acceptance of the hypothesis in its simplest form. In sex-linkage experiments using an X-autosome translocation in mice, she did not get results as predicted by the hypothesis. She expected that females heterozygous for a coat color would show mottling. Instead the females were the same color as the dominant hemizygous males. She suggests that complete inactivation of an X-chromosome does not occur.

Used as supporting evidence of incomplete inactivation, is the Xg^a blood group in man. This is sex linked, and women heterozygous for the gene do not have two red-cell populations (Reed et al., 1963).

The irony of the whole proposal is that phenotypic results are being used to describe a cytological feature which is not present in cells of tissues involved. The sex chromatin pattern in the mouse is inconclusive and there is no indication that hair follicles have been investigated for the presence of sex chromatin (Moore, 1966b). Also the use of a substance which occurs on the surface of a mature erythrocyte for describing a nuclear phenomenon is not justified.

The technique of autoradiography indicates more accurately the origin of sex chromatin. By introducing a

radioactive base precursor of DNA, such as tritiated thymidine, at different periods of the cell cycle, it is possible to observe chromosome duplication because of the incorporation of the radioactive substance during DNA replication. Some chromosomes, particularly in their heterochromatic regions, incorporate the tritiated thymidine later than others; they are termed late labelling. It has been shown by several groups of investigators that one X-chromosome in nuclei of somatic cells of the female is heterochromatic and also late labelling (Morishima et al., 1962; Hsu et al., 1964; Takagi and Makino, 1966). A late labelling X-chromosome is not found in the nuclei of the male nor in the nuclei of an XO individual (Atkin et al., 1962; Morishima et al., 1962).

However, the pattern of late replication is not conclusive evidence of presence of sex chromatin. At present what determines heterochromatin also determines late replication of a chromosome, even though late replication does not necessarily lead to heterochromatization in interphase nuclei (Isa et al., 1969; Lima-de-Faria and Jaworska, 1968). However, one X-chromosome can be late in DNA replication with subsequent formation of sex chromatin (Brown, 1966; Comings, 1967a; 1967b). The indications are that sex chromatin in the female mammal represents the heterochromatic part of one X-chromosome and that this region lags in synthesis of DNA.

The idea incorporated into the Lyon hypothesis, that heterochromatin is genetically inert comes from evidence of dosage compensation in Drosophila. Dosage compensation in Drosophila describes the effect of genes borne on the X-chromosomes. In females two X-chromosomes produce the same amount of product as the one X-chromosome in the male. The effects of dosage is cancelled out by the heterochromatization of the second X-chromosome in the female. Complete adoption of dosage compensation as expressed for Drosophila is not possible in mammalian genetics (Stern, 1960). The inactivation of one X-chromosome by heterochromatization is not expressed in all cells (Reed et al., 1963).

Rather than attribute genetic inertness to heterochromatin, Commoner (1964) suggests that heterochromatin and euchromatin participate in two systems of genetic control. He proposes that Mendelian inheritance due to specific biochemical differences is localized in the euchromatic regions, while genetic regulation of quantitative characteristics such as cell size and rate of mitosis, is localized in the heterochromatic regions. He claims that the sex chromosome mechanism of mammals provides a striking example of this dual control. The single X-chromosome of the male and one X-chromosome of the female are euchromatic; sex-linked genes are expressed by them. The second X-chromosome of the female is heterochromatic and insures normal female development.

According to Hamerton (1968) one X-chromosome in the female and the Y-chromosome of the male act through their heterochromatin as controlling elements for genes affecting sex determination, development and certain quantitative somatic characteristics. The Y-chromosome is needed for development of the testes, the heterochromatic X-chromosome in the female is needed to control the rate of follicular atresia. The euchromatic X-chromosome may carry genes for testicular as well as for ovarian development. A similar idea was expressed by Correns who suggested that sex was determined by Mendelian factors (Mittwoch, 1969).

Mittwoch (1969) has suggested that it would be more fruitful to the understanding of the action of abnormal chromosome constitution to analyse cell kinetics and relative growth instead of relying on a "hypothetical balance of hypothetical genes." She regards sex chromatin as a ballast in that the human female probably contains between 1-2 per cent more DNA per cell than the male. Therefore, DNA synthesis takes longer in the female, hence the female is smaller. Unlike the X-chromosome, the Y-chromosome increases the rate of mitosis and hence males are bigger. This function of the Y-chromosome would explain the early differentiation of testis; and the larger stature of XYY males.

Investigations of the relationship between gene inactivation, late replication of DNA and sex chromatin

formation have been primarily in mammals. Any comprehensive theory on the significance of these phenomena will have to take account findings in animals with female heterogamety.

METHODS AND MATERIALS

Obtaining the tissues

Tissues from twelve species of fish, three species of amphibian, two species of reptile and two species of bird were examined in this study. The major classification taxa are listed for each species in Appendix A. A maximum of three females and three males of each species was obtained. The number, length or approximate age, sources, and date of fixation are recorded in Appendix B for each species.

All specimens were obtained live, instantly sacrificed, and the tissues removed immediately and fixed. The fish and salamanders were decapitated upon removal from water. The rest of the amphibians, the reptiles and birds were given an overdose of ether. The tissues removed were: nervous system: brain, cranial and dorsal root ganglia, nerve cord, sympathetic trunk, non-nervous: skin, gonad, kidney, spleen, pancreas, liver, anterior part of the small intestine. Preliminary work on the fishes indicated that buccal epithelium and respiratory epithelium could not be used. These tissues were also omitted in the other animals studied. Ganglia and the sympathetic trunk were used when they could be successfully removed from the specimen. Although the adrenal cortex has been used in other projects on sex chromatin, the histological nature of the adrenals in the species in this investigation could not be accurately identified. The size of each tissue was restricted to a

maximum of three cubic millimeters. All tissues were fixed in 10 per cent formalin in isotonic saline and remained in the fixative for seven to ten days.

Preparation of tissues for study

The tissues were processed through the Technicon and embedded in paraffin. The blocks per specimen were kept to a minimum by embedding several tissues per block.

Tissues from the nervous system were cut at 10μ ; all other tissues at 6μ . Three slides per block with multiple sections on each slide were prepared. One slide was stained by the Feulgin reaction (Appendix C) and used for counting. The other two slides were spares which could be used for additional staining techniques, such as, cresyl echt violet, hemotoxylin (Harris's) and eosin. These slides were used as checks on identification of tissues (H. and E.) and of sex chromatin (cresyl echt violet). Permount was used as the mounting media.

Examination of tissues

The slides were examined using a Wild binocular microscope with 12.5X wide angle eyepieces and a 100X fluotar objective.

In nervous system tissue, the cells selected were those which appeared to be restricted in distribution at specific regions of the cerebral hemispheres, optic tectum, cerebellum, medulla and nerve cord. Cells chosen were those

relatively larger than others at that region and in which the cytoplasm was apparent and the nucleus vesicular. In the non-nervous tissues, the cells chosen were those in which the position of the cell border could be inferred and the nucleus was vesicular. Overlapping or distorted cells were not included in the counts.

For each cell type selected, a hundred nuclei were examined. Absence and presence of sex chromatin-like masses were recorded. In those nuclei in which chromatin masses were present, the number and position (perinucleolar, free in nucleoplasm, on the nuclear membrane) were scored.

Factorial analysis of variance with two factors (sex X tissue) was applied when warranted by results. The analyses were carried out by a computer, IBM model 360/65.

OBSERVATIONS

Petromyzones

Ichthyomyzon castaneus (chestnut lamprey).

Eight tissues were studied, four from regions of the central nervous system, three from viscera, and the epidermis from the skin. In these tissues from females and males, Feulgen positive chromocenters were seen in a proportion of nuclei. The chromocenters were small with no obvious differences as to number or position in either sex (Appendix D, Table 1). Figures 1 to 6 illustrate selected cells from female tissues. Several chromocenters per nucleus were found in all tissues: most frequently in liver, less in intestinal epithelium and epidermis, and least in kidney tubules and nervous system.

Statistical analysis did not reveal any sexual dimorphism (Table 1). The F value for sex was not significant. The significant F value ($P < 0.01$) for tissue verifies the assumption made in choosing the factorial analysis program: the tissues are different to one another. The interaction, sex-tissue, was significant ($P < 0.01$). In tissues from the nervous system and epidermis the means were greater in males than females; but, the reverse was true for the means of kidney and liver which were greater in females. This could account for the significant interaction term.

Enumerating only those nuclei in which the chromatin mass was at the nuclear membrane (Table 1) did not indicate any sexual dimorphism either.

FIGURE

1. Lamprey. Cells from the prosencephalon, female. The chromatin masses are small and irregular in shape. 10μ , Feulgen reaction, X1600.
2. Lamprey. Neuron from spinal cord, female. A very small chromatin mass is free in the nucleoplasm. 10μ , Feulgen reaction, X1600.
3. Lamprey. Epidermis, female. There are small chromatin masses free in the nucleoplasm. 10μ , Feulgen reaction, X1600.
4. Lamprey. Intestinal epithelium, female. The chromatin masses are spherical and appear adjacent to nucleoli. 6μ , Feulgen reaction, X1600.
5. Lamprey. Kidney tubule, female. A chromatin mass is present between the nucleolus and nuclear membrane. 6μ , Feulgen reaction, X1600.
6. Lamprey. Liver, female. Several chromatin masses are present in each nucleus. A few appear adjacent to nucleoli. 6μ , Feulgen reaction, X1600.

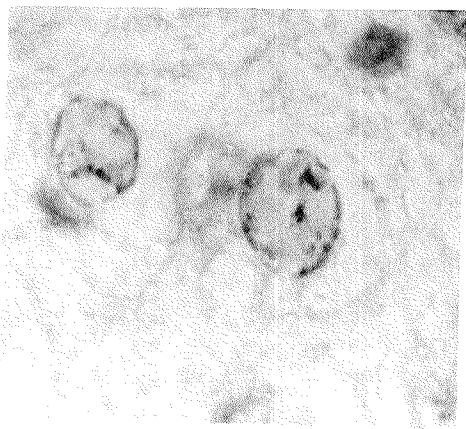


Figure 1

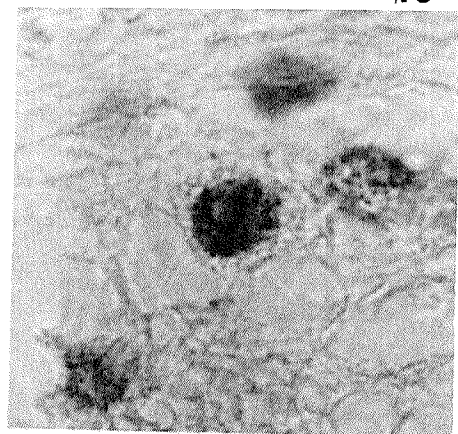


Figure 2

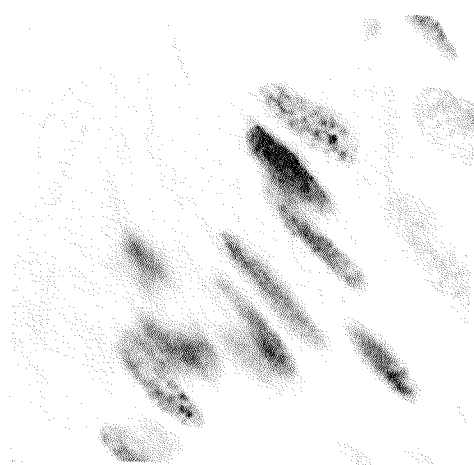


Figure 3

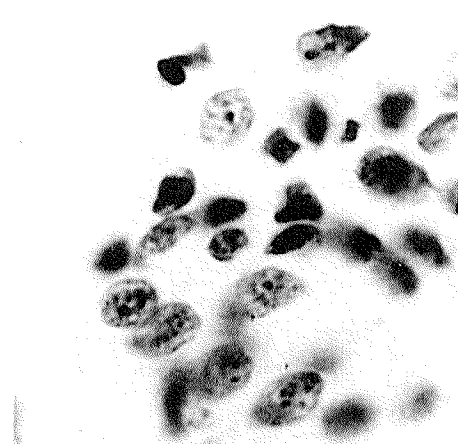


Figure 4

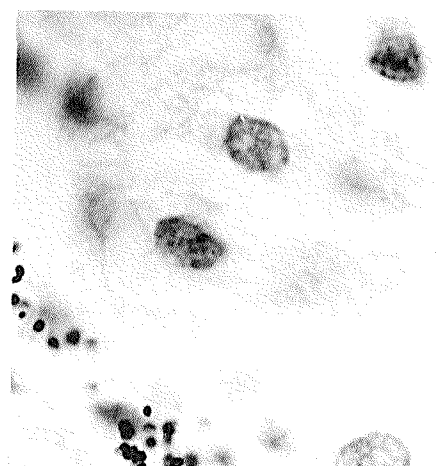


Figure 5

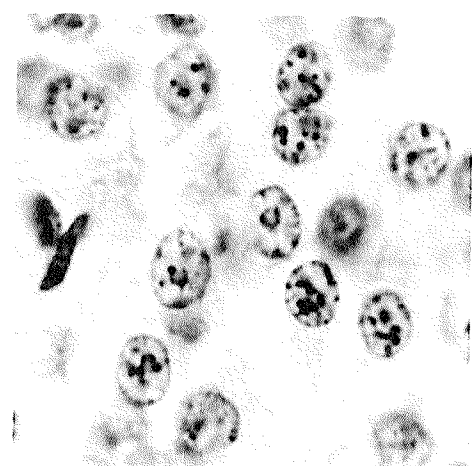


Figure 6

Teleostomi

Salmo gairdneri (rainbow trout), Coregonus clupeaformis (whitefish), Esox lucius (northern pike), Catostomus commersoni (common white sucker), Notropis hudsonius (spottail shiner), Culaea inconstans (brook stickleback), Percopsis omiscomaycus (troutperch), Percina caprodes (logperch), Perca flavescens (yellow perch), Stizostedion vitreum (walleye), Cottus bairdi (mottled sculpin).

Nine tissues were studied, six from the nervous system and three from viscera. Smooth muscle and epidermis were not studied because nuclei were too small for intranuclear detail at the magnification available. Nuclei of the tissues used were also small relative to those in corresponding human tissues, but it was possible to identify and count distinct chromatin masses. Figures 7 to 24 illustrate selected cells of tissues from females and males of several species of fish. Chromatin masses varied in size and in location within the nucleus. Occasionally one similar to sex chromatin in mammalian cells was observed (see Figures 20 and 23).

The total number of nuclei with chromatin masses per hundred cells and analysis of variance table for each species are tabulated in Appendix D, Tables 3 to 26. In all-species of fish studied, except for rainbow trout and mottled sculpin, tissues from viscera had a greater incidence of intranuclear chromatin masses than tissues from the nervous system. Also several chromatin masses per nucleus were more frequent in tissues from viscera. An attempt to rank tissues in accordance to incidence of chromatin masses did not reveal any consistent pattern, just the overall impression that

tissues from the nervous system had a lower incidence than non-nervous tissues.

A sex difference in nuclear morphology could not be determined microscopically in any species of fish studied. This was verified statistically, except that in rainbow trout there was a significant difference ($P < 0.01$); tissues from males had a greater incidence than those from females.

Table 1 lists the F values for sex, tissue, and interaction. The F values for sex were not significant for the fishes except for rainbow trout. As expected the F values for tissue were significant to highly significant for all species of fish except rainbow trout and mottled sculpin. This reinforces the microscopic observations. There was no interaction as the F value was not significant.

The per cent of chromatin masses on the nuclear membrane was low except in mottled sculpin, 20.3 for females and 33.9 for males. The analysis of this difference was not significant.

TABLE 1. F values for incidence of chromatin masses by sex and tissue and per cent chromatin masses on nuclear membrane.

Species (common name)	F values			% on nuclear membrane	
	Sex	Tissue	Interaction	Female	Male
chestnut lamprey	0.60	6.41**	4.72**	2.5	3.8
rainbow trout	40.90**	1.26	0.85	0.7	1.6
whitefish	3.44	69.78**	1.42	1.0	0.4
northern pike	0.59	24.57**	0.30	4.1	3.1
common white sucker	2.58	3.14*	0.83	0.5	0.9
spottail shiner	0.76	17.63**	0.61	3.2	4.6
brook stickleback	0.43	42.78**	2.46	0.8	1.2
troutperch	0.04	19.62**	1.74	4.6	5.0
logperch	1.04	4.56**	0.46	7.9	5.4
yellow perch	2.28	40.12**	2.30	0.6	0.2
walleye	0.05	173.64**	1.07	0.8	0.6
mottled sculpin	2.98	0.28	0.18	20.3	33.9
mottled sculpin (NM)	2.92	0.26	0.37	-	-
Manitoba toad	0.05	19.72**	0.47	23.2	17.4
tiger salamander	0.76	0.16	0.58	7.8	12.7
garter snake	27.98**	25.78**	5.38**	9.3	0.8
garter snake (NM)	49.57**	11.75**	12.50**	-	-
timber rattler	33.17**	5.89**	7.06**	1.7	1.0
pigeon	3.33	172.50**	1.37	21.2	14.0
pigeon (NM)	18.27**	66.88**	4.75**	-	-
mallard duck	-	-	-	17.1	5.8

significance: * P<0.05
** P<0.01

NM - analysis of variance on number of chromatin masses on nuclear membrane.

FIGURE

7. Rainbow trout. Neuron from cerebral hemispheres, female. A small chromatin mass appears free in the nucleoplasm. 10μ , Feulgen reaction, X1600.
8. Rainbow trout. Neurons from cerebral hemisphere, male. Very small, nearly indistinct chromocenters are present in nuclei. 10μ , Feulgen reaction, X1600.
9. Rainbow trout. Neurons from cerebellum, female. Nuclei contain small chromocenters. 10μ , Feulgen reaction, X1600.
10. Rainbow trout. Neurons from cerebellum, male. Several chromatin masses are present in the nucleus, one appears adjacent to a nucleolus. 10μ , Feulgen reaction, X1600.
11. Rainbow trout. Neuron from medulla, female. A small chromatin mass appears free in the nucleoplasm. 10μ , Feulgen reaction, X1600.
12. Rainbow trout. Neuron from medulla, male. The nucleus contains several chromatin masses of varying sizes. 10μ , Feulgen reaction, X1600.

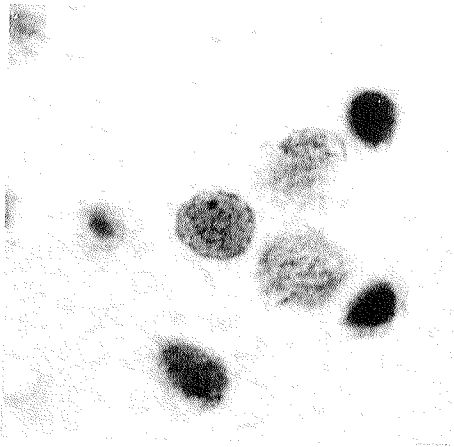


Figure 7

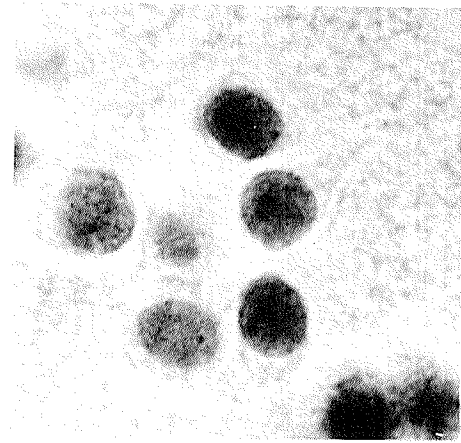


Figure 8

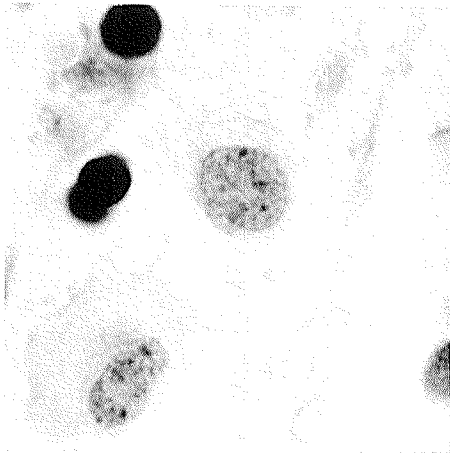


Figure 9

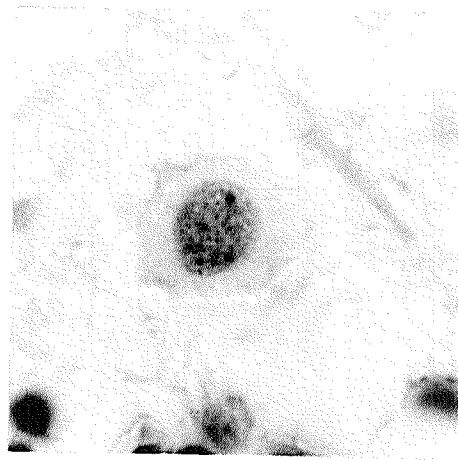


Figure 10

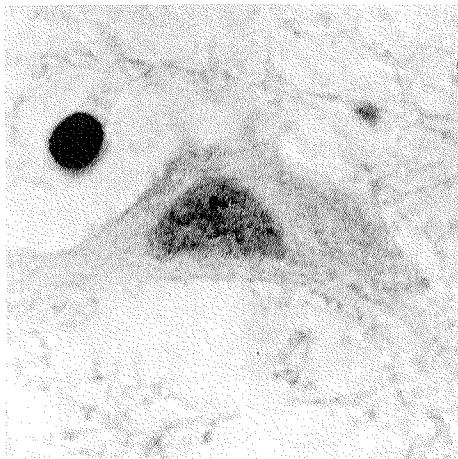


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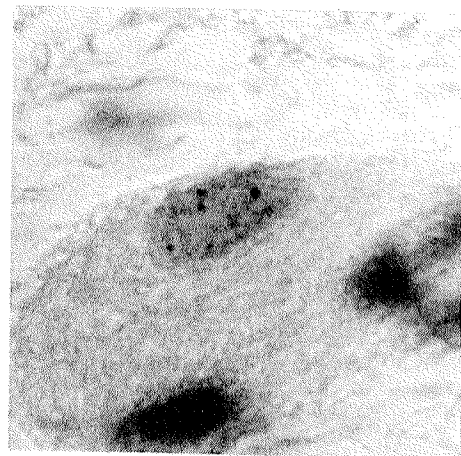


Figure 12

FIGURE

13. Rainbow trout. Kidney tubule, female. Small chromocenters are visible in nuclei, but one nucleus contains a distinct chromatin mass which is free in the nucleoplasm. 6μ , Feulgen reaction, X1600.
14. Rainbow trout. Kidney tubules, male. Coarse chromocenters are present in nuclei. It is difficult to identify distinct masses. 6μ , Feulgen reaction, X1600.
15. Rainbow trout. Liver, female. Nuclei contain irregular chromocenters. In some nuclei it is possible to identify distinct chromatin masses which are usually near nucleoli. 6μ , Feulgen reaction, X1600.
16. Rainbow trout. Liver, male. Nuclei are similar to those in liver from the female. 6μ , Feulgen reaction, X1600.
17. Rainbow trout. Intestinal epithelium, female. Irregular chromatin masses are visible in nuclei. 6μ , Feulgen reaction, X1600.
18. Rainbow trout. Intestinal epithelium, male. nuclei are similar to those of intestinal epithelium from the female. 6μ , Feulgen reaction, X1600.

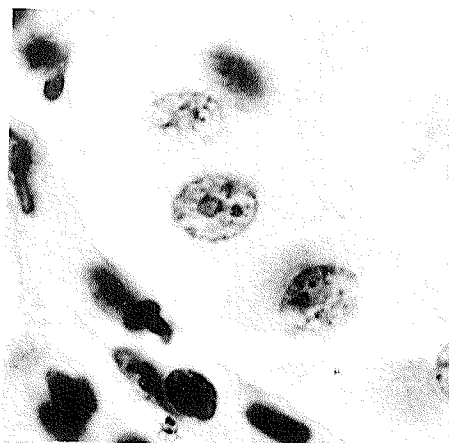


Figure 13

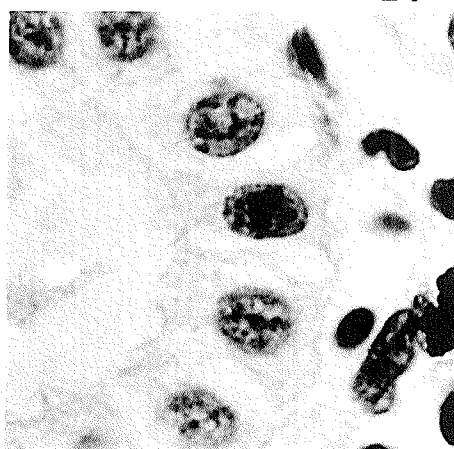


Figure 14

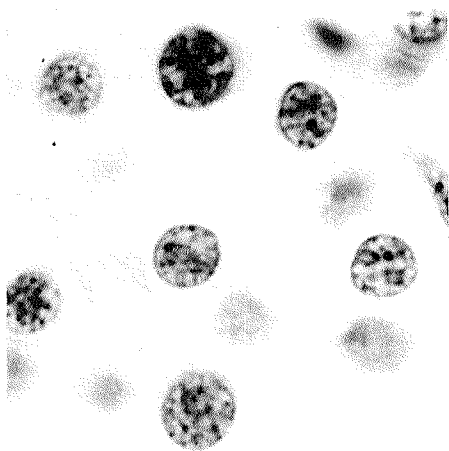


Figure 15

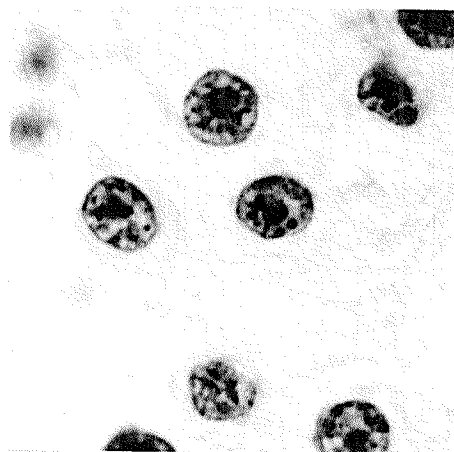


Figure 16

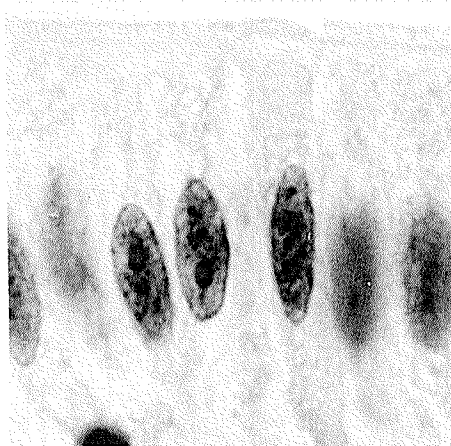


Figure 17

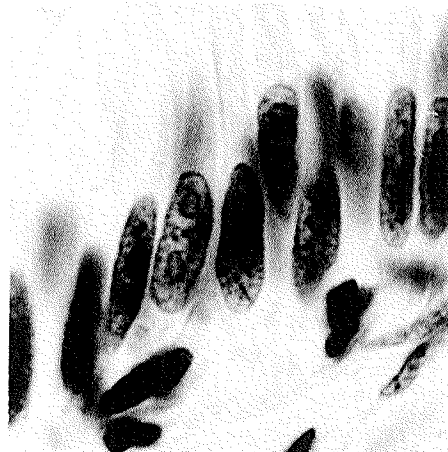


Figure 18

FIGURE

19. Spot tail shiner. Cells from optic lobe, female. A chromatin mass is present adjacent to the nucleolus. 10μ , Feulgen reaction, X2560.
20. Mottled sculpin. Cells from cerebral hemisphere, male. Distinct chromatin masses are visible in nuclei. A chromatin mass on the nuclear membrane is present in one of the nuclei. 10μ , Feulgen reaction, X1600.
21. Mottled sculpin. Neuron from cerebellum, female. The nucleus contains two small chromatin masses which are on the nuclear membrane. 10μ , Feulgen reaction, X1600.
22. Stickleback. Neurons from cerebellum, female. Irregular chromocenters are present in nuclei. 10μ , Feulgen reaction, X1600.
23. Spot tail shiner. Liver, female. Coarse chromatin masses are present. In one nucleus, a chromatin mass is seen on the nuclear membrane. 6μ , Feulgen reaction, X2560.
24. Spot tail shiner. Kidney tubule, male. Fine chromocenters are present in nuclei, no distinct chromatin masses are visible. 6μ , Feulgen reaction, X2048.

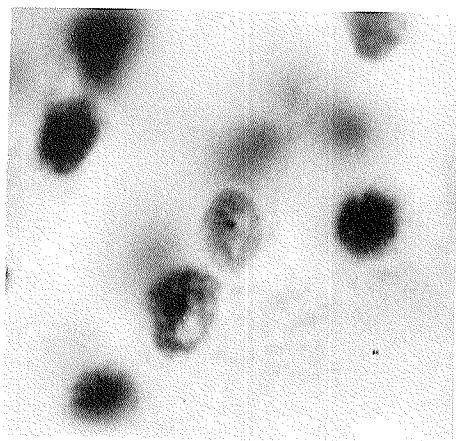


Figure 19

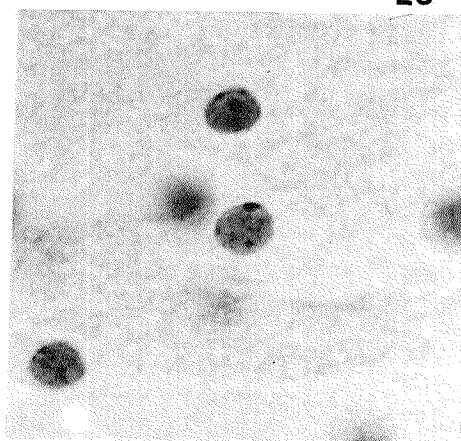


Figure 20

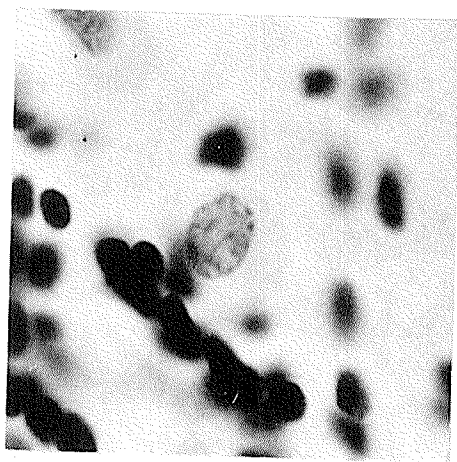


Figure 21

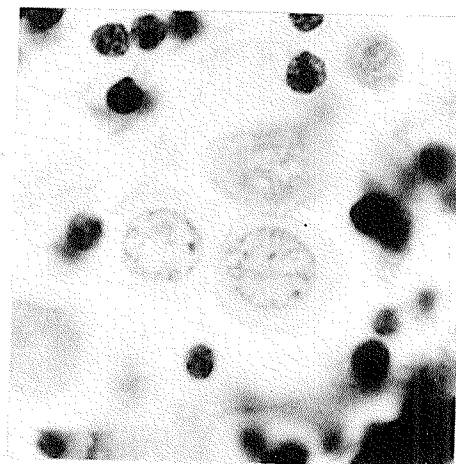


Figure 22

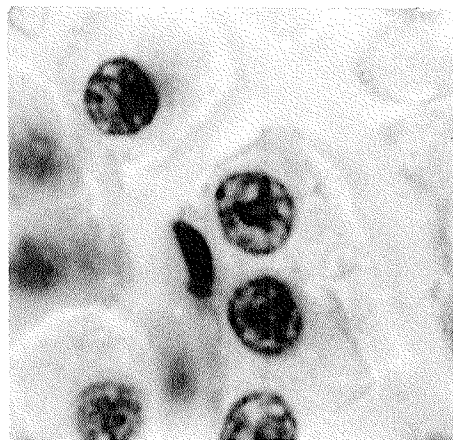


Figure 23

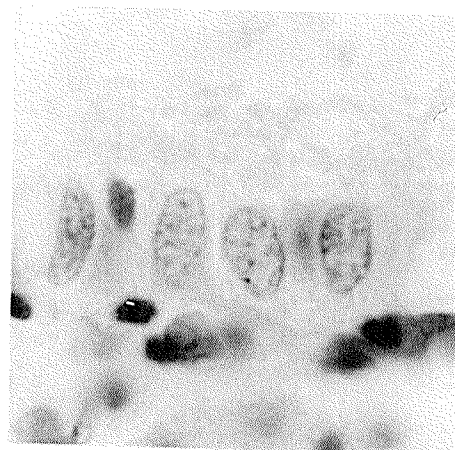


Figure 24

Amphibia

Rana pipiens (leopard frog), Bufo hemiophrys (Manitoba toad),
Ambystoma tigrinum diaboli (tiger salamander).

Twelve tissues were studied, six from the nervous system, five from viscera and the epidermis. Large chromatin masses were present in nuclei of tissues from both sexes. Figures 25 to 36 illustrate selected cells from various tissues. The chromatin masses were spherical when free in the nucleoplasm (Figure 25), formed a cap when adjacent to a nucleolus (Figure 28) and a planoconvex mass or triangle when on the nuclear membrane (Figures 25 and 30). Frequently a smaller chromatin mass in addition to a larger mass was seen in the same nucleus (Figure 30), but no significance could be attributed to its occurrence. Chromatin masses appeared occasionally as caps on opposite poles of a nucleolus (Figure 35).

Although many chromatin masses did fit the description for sex chromatin, sexual dimorphism could not be detected microscopically. The incidence of chromatin masses was high in all tissues (Appendix D, Tables 27 to 31). Counting was possible in tissues from the nervous system for all amphibians studied and in tissues from viscera and the epidermis only in Manitoba toad. In leopard frog and tiger salamander, nuclei in tissues from viscera and the epidermis were not satisfactory for counting as they contained too many coarse chromatin masses.

Statistical analysis on observations from Manitoba toad and tiger salamander did not reveal any nuclear differences between sexes in either species (Table 1). F values for sex were not significant. In tiger salamander, only tissues from the nervous system were analysed; therefore, a nonsignificant F value for tissue was expected. There was no interaction. Observations of tissues from leopard frog did not warrant analysis.

Figure

25. Manitoba toad. Cells from cerebral hemisphere, female. Several chromatin masses are present in each nucleus. One of the chromatin masses located on the nuclear membrane is similar to sex chromatin found in mammalian cells. 10μ , Feulgen reaction, X1600.
26. Leopard frog. Cells from cerebral hemisphere, male. Two distinct chromatin masses are present on the nuclear membrane. 10μ , Feulgen reaction, X1600.
27. Manitoba toad. Neuron from optic lobe, female. Two chromatin masses are visible, one adjacent to a nucleolus and the other is on the nuclear membrane. 10μ , Feulgen reaction, X1600.
28. Tiger salamander. Cell from cerebral hemisphere, male. A large chromatin mass forms a cap on the nucleolus. 10μ , Feulgen reaction, X1600.
29. Leopard frog. Neuron from spinal cord, female. The nucleus contains a chromatin mass on the nuclear membrane. 10μ , Feulgen reaction, X1600.
30. Tiger salamander. Neuron from medulla, female. Three chromatin masses are present, two on the nuclear membrane, and one large free in the nucleoplasm. 10μ , Feulgen reaction, X1600.

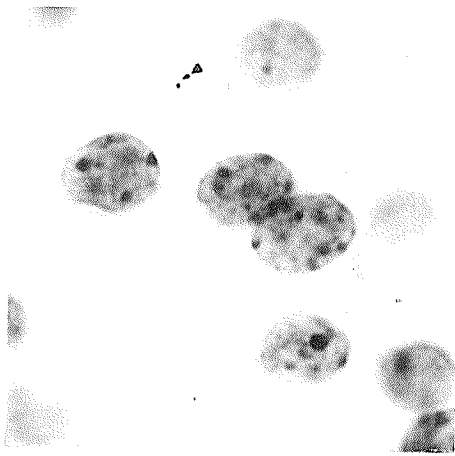


Figure 25



Figure 26

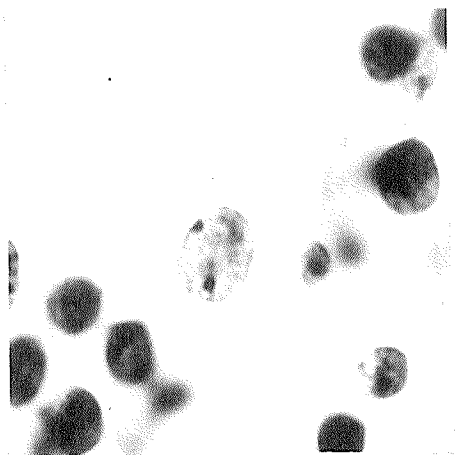


Figure 27

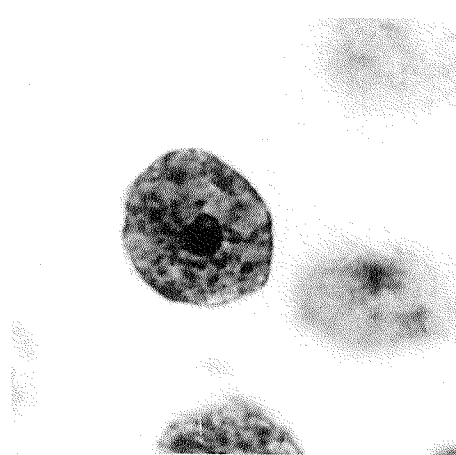


Figure 28

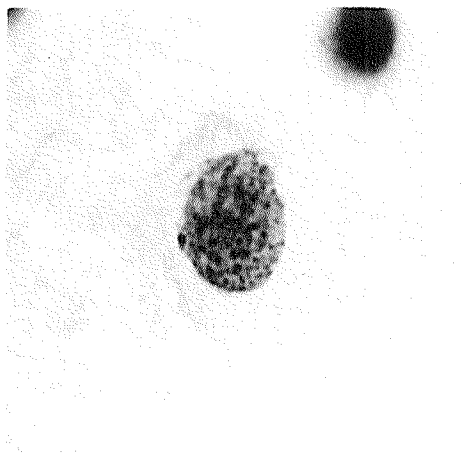


Figure 29



Figure 30

FIGURE

31. Tiger salamander. Liver, female. The nucleus contains coarse and irregular chromatin masses. One chromatin mass on the nuclear membrane is similar to sex chromatin in mammalian cells. 6μ , Feulgen reaction, X1600.
32. Leopard frog. Liver, male. Nuclei contain coarse and irregular chromatin masses. 6μ , Feulgen reaction, X1600.
33. Leopard frog. Smooth muscle, female. The nucleus contains irregular chromocenters. 6μ , Feulgen reaction, X1600.
34. Tiger salamander. Kidney tubule, male. The nucleus contains fine chromocenters and several distinct chromatin masses. Three chromatin masses appear next to the nucleolus. 6μ , Feulgen reaction, X1600.
35. Tiger salamander. Intestinal epithelium, male. Large chromatin masses are present and some appear on opposite poles of nucleoli. 6μ , Feulgen reaction, X1600.
36. Tiger salamander. Epidermis, male. Nuclei contain irregular chromocenters but distinct chromatin masses are present also. 6μ , Feulgen reaction, X1600.



Figure 31

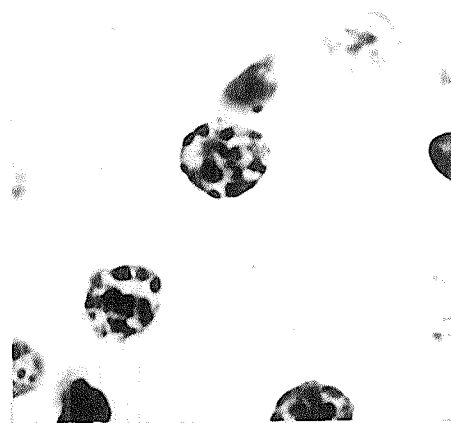


Figure 32

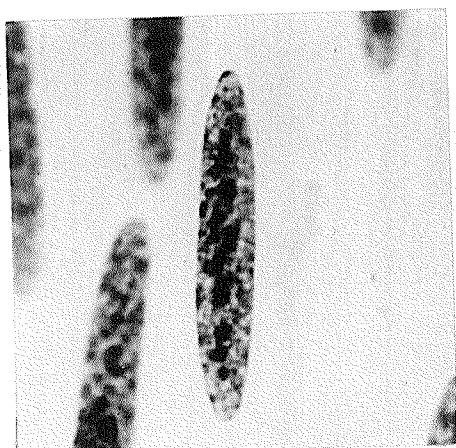


Figure 33



Figure 34



Figure 35

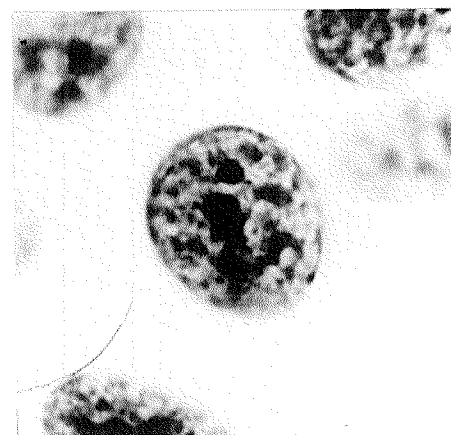


Figure 36

Reptilia

Thamnopsis sirtalis ssp. (garter snake), Crotalus horridus horridus (timber rattler).

Eleven tissues were studied, five from the nervous system, five from viscera, and the epidermis. Chromatin masses were present in nuclei of these tissues from females and males of both species. Figures 37 to 54 are selected cells from garter snake, and Figures 55 to 60 from timber rattler. The chromatin masses were similar to sex chromatin in mammalian cells, and sexual dimorphism of nuclei could be detected microscopically in garter snake, but not in timber rattler. In garter snake, there was a greater incidence of chromatin masses, total and on the nuclear membrane, in most tissues from females (Appendix D, Tables 32 to 35). Also there was a size difference between the chromatin masses of females and males, those in females being larger (see Figures 37 and 38, 45 and 46). Ten nuclei from each of medulla, kidney, and liver were measured by a micrometer for each female and male garter snake (Appendix E). A student "t" test was applied, and for each tissue the "t" value was significant (Table 2). These differences in nuclear morphology could not be detected in timber rattler (Appendix D, Tables 36 and 37).

Statistically there was a significant difference ($P < 0.01$) between nuclei of tissues from female and male garter snakes and timber rattlers (Table 1). In both species, for most tissues the incidence of intranuclear masses was

greater in females than males. The F values for tissue was significant ($P < 0.01$) also in both species; this reaffirms the assumption that tissues are different. In garter snake, the F value for interaction was significant ($P < 0.01$). This can be accounted by the much greater incidence of chromatin masses in females than males, and by the reversal of rank in intestinal epithelium; the incidence in males exceeded that in females. In timber rattler, the F value for interaction also was significant ($P < 0.01$). This can be accounted by the much greater incidence of chromatin masses in pancreas from females than from males.

Table 2. Comparison of mean size of sex chromatin between male and female garter snake.

Tissue	Sex	Mean (N=20)	t value	P
medulla	female	0.2230	8.023	<0.01
	male	0.1655		
kidney	female	0.1865	9.473	<0.01
	male	0.1320		
liver	female	0.2035	9.522	<0.01
	male	0.1380		

FIGURE

37. Garter snake. Neurons from optic lobe, female.
Nuclei contain distinct chromatin masses which are adjacent to nucleoli. 10 μ , Feulgen reaction, X2048.
38. Garter snake. Neurons from optic lobe, male.
Chromatin masses are adjacent to nucleoli, but are smaller than those in nuclei from females. 10 μ , Feulgen reaction, X2560.
39. Garter snake. Neurons from medulla, female.
Nuclei contain distinct chromatin masses. A chromatin mass appears on the nuclear membrane in one nucleus. 10 μ , Feulgen reaction, X2048.
40. Garter snake. Neuron from medulla, male.
Distinct chromatin masses are not visible. 10 μ , Feulgen reaction, X1600.
41. Garter snake. Neuron from spinal cord, female.
The nucleus contains a large chromatin mass. 10 μ , Feulgen reaction, X1600.
42. Garter snake. Neuron from spinal cord, male. A small chromatin mass is present, but is smaller than that in female spinal cord nucleus. 10 μ , Feulgen reaction, X2048.

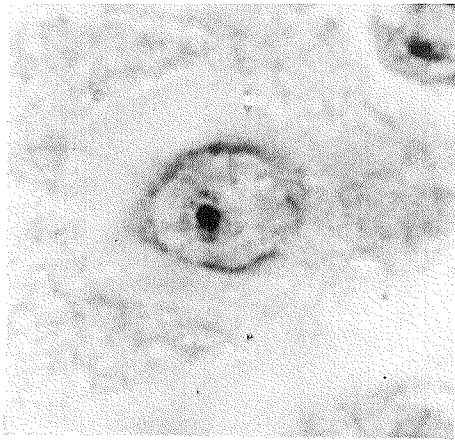


Figure 37

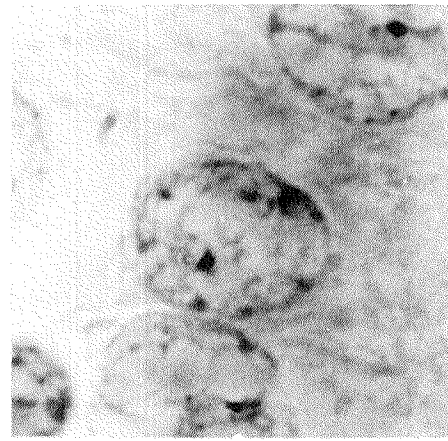


Figure 38

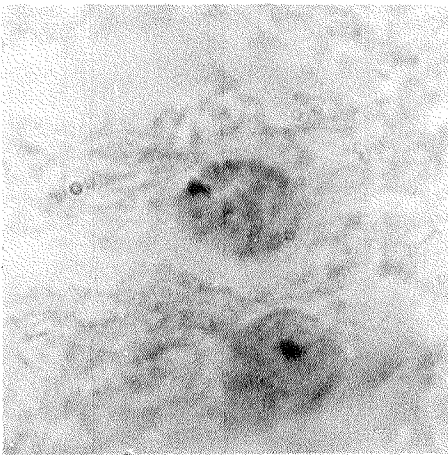


Figure 39

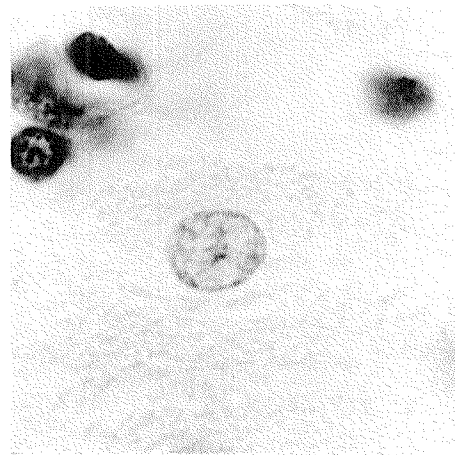


Figure 40

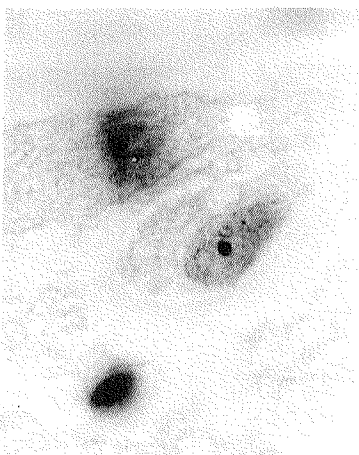


Figure 41

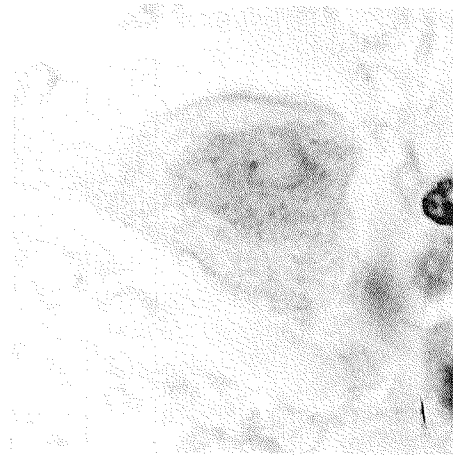


Figure 42

FIGURE

43. Garter snake. Epidermis, female. A small chromatin mass appears adjacent to a nucleolus. 6μ , Feulgen reaction, X1600.
44. Garter snake. Epidermis, male. A small chromatin mass similar to that in female epidermis appears adjacent to a nucleolus in one nucleus. 6μ , Feulgen reaction, X1600.
45. Garter snake. Kidney tubule, female. Nuclei contain irregular chromocenters but distinct chromatin masses are present also. In one nucleus, the chromatin mass is on the nuclear membrane and is similar to sex chromatin found in mammalian cells. 6μ , Feulgen reaction, X1600.
46. Garter snake. Kidney tubule, male. Nuclei contain irregular chromocenters, but distinct chromatin masses are present also. These are smaller than those found in females. 6μ , Feulgen reaction, X2048.
47. Garter snake. Liver, female. Distinct chromatin masses are visible and on the nuclear membrane frequently, resembling mammalian sex chromatin. 6μ , Feulgen reaction, X1600.
48. Garter snake. Liver, male. Chromatin masses are visible, but are smaller than those in female liver cells. 6μ , Feulgen reaction, X1600.

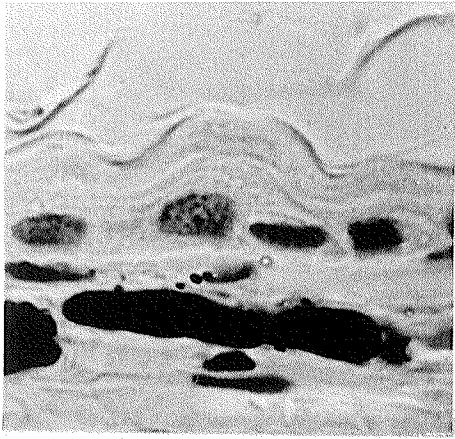


Figure 43

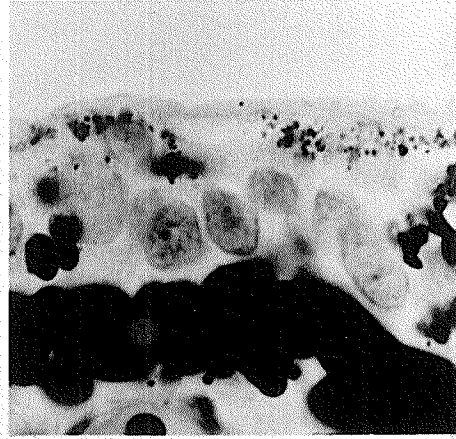


Figure 44

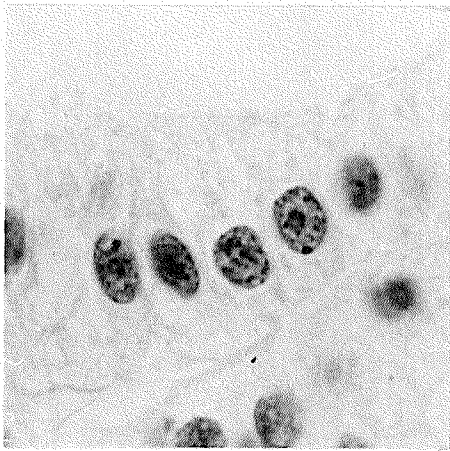


Figure 45

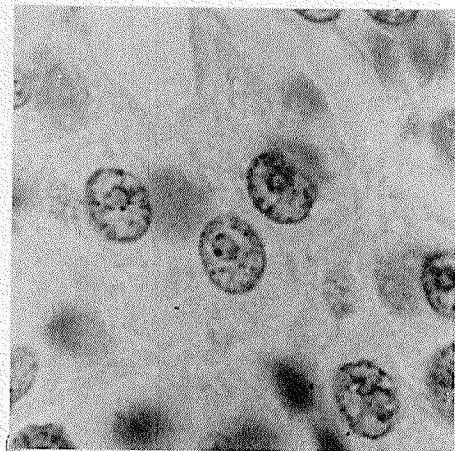


Figure 46

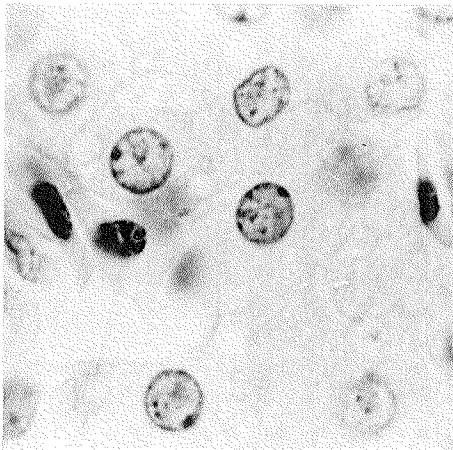


Figure 47

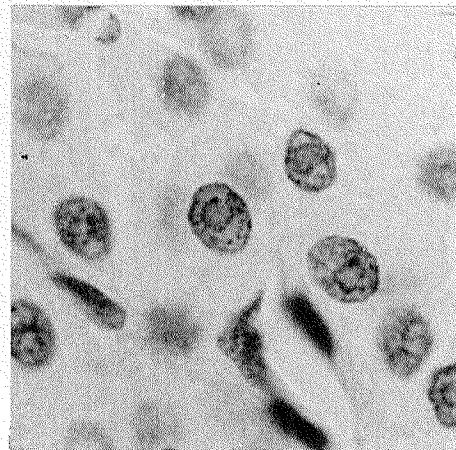


Figure 48

FIGURE

49. Garter snake. Intestinal epithelium, female. A distinct chromatin mass is visible in one of the nuclei. It appears surrounded by a clear halo. 6μ , Feulgen reaction, X1600.
50. Garter snake. Intestinal epithelium, male. Fine chromocenters are present in the nuclei. 6μ , Feulgen reaction, X1600.
51. Garter snake. Pancreas, female. Nuclei contain distinct chromatin masses, some are on the nuclear membrane and others appear next to nucleoli. 6μ , Feulgen reaction, X1600.
52. Garter snake. Pancreas, male. Distinct chromatin masses are not visible in many nuclei. 6μ , Feulgen reaction, X1600.
53. Garter snake. Smooth muscle, female. Chromatin masses are present, but they are not as distinct as those in other tissues of female origin. 6μ , Feulgen reaction, X1600.
54. Garter snake. Smooth muscle, male. No chromatin masses are visible. 6μ , Feulgen reaction, X1600.

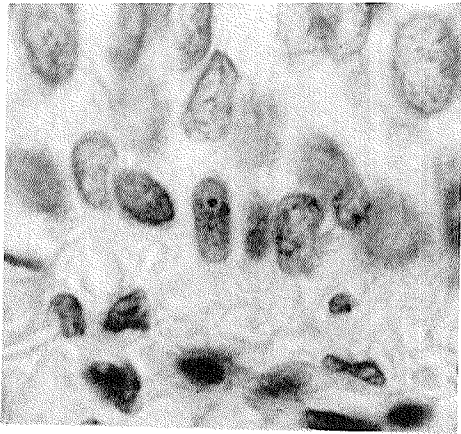


Figure 49

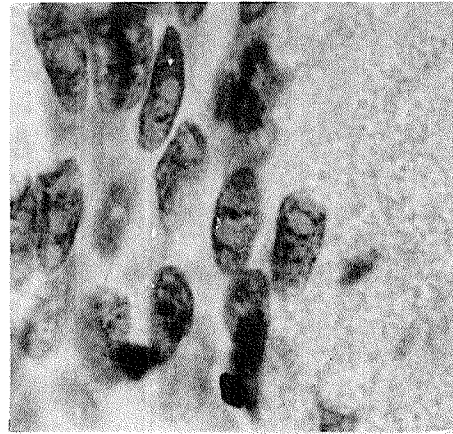


Figure 50

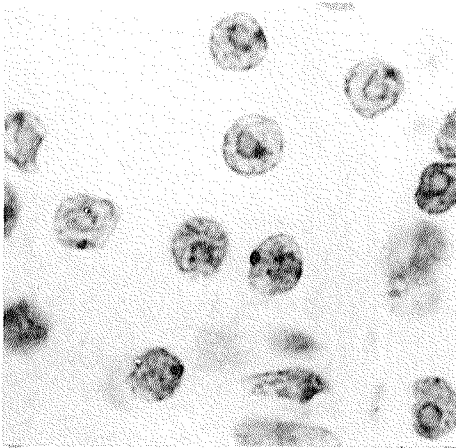


Figure 51

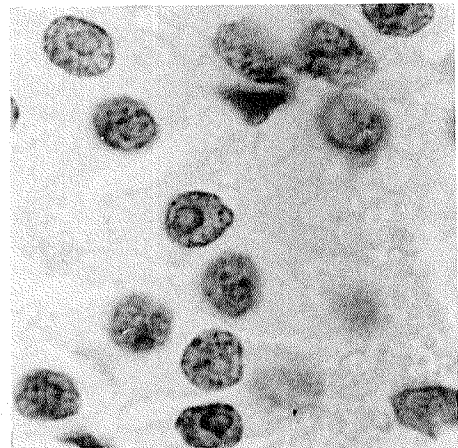


Figure 52

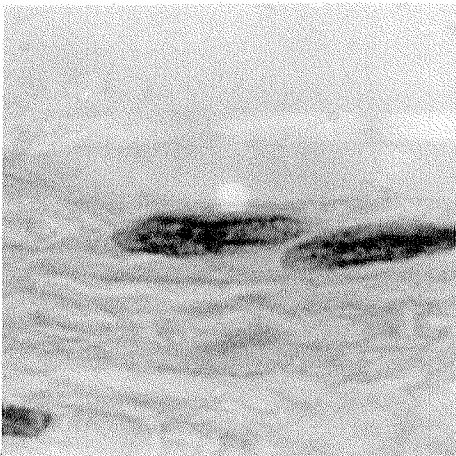


Figure 53



Figure 54

FIGURE

55. Timber rattler. Cell from optic lobe, female. A large chromatin mass is seen free in the nucleoplasm. 10μ , Feulgen reaction, X1600.
56. Timber rattler. Cells from optic lobe, male. No distinct chromatin masses are visible. 10μ , Feulgen reaction, X1600.
57. Timber rattler. Liver, female. Chromatin masses of different sizes are visible in nuclei, some appear free in the nucleoplasm and others are on the nuclear membrane. 6μ , Feulgen reaction, X1600.
58. Timber rattler. Liver, male. One nucleus contains a chromatin mass on the nuclear membrane; others have fine chromocenters. 6μ , Feulgen reaction, X1600.
59. Timber rattler. Kidney tubule, female. Chromatin masses are present in nuclei; they appear on the nuclear membrane and free in nucleoplasm. 6μ , Feulgen reaction, X1600.
60. Timber rattler. Kidney tubules, male. Nuclei are similar to those in female kidney tubule. 6μ , Feulgen reaction, X1600.

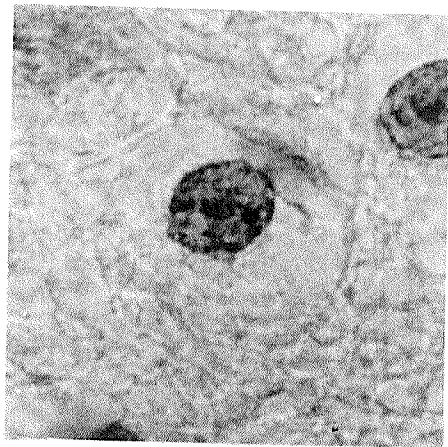


Figure 55

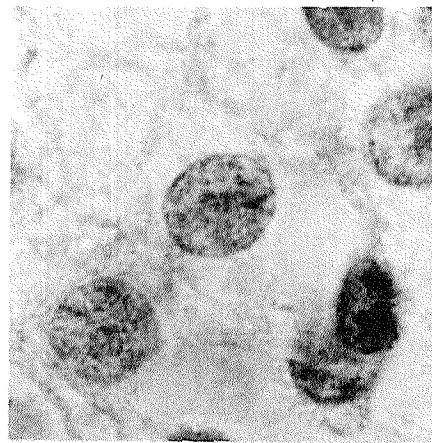


Figure 56

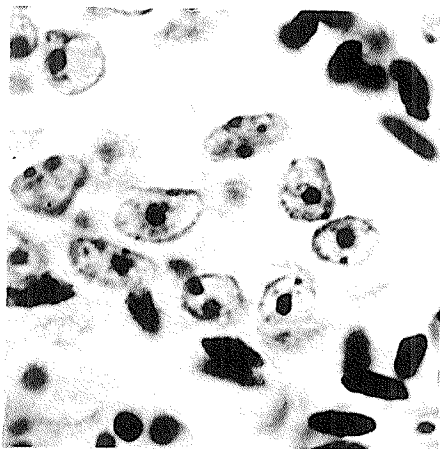


Figure 57

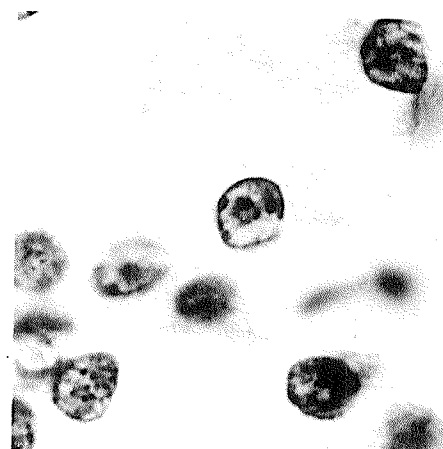


Figure 58

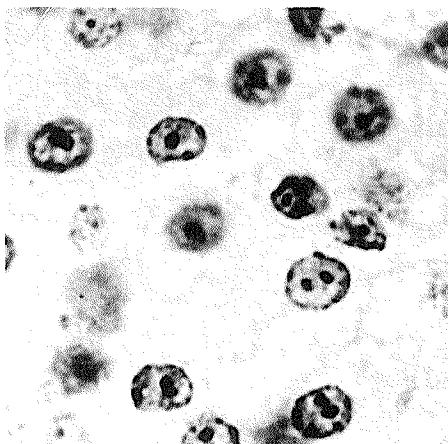


Figure 59

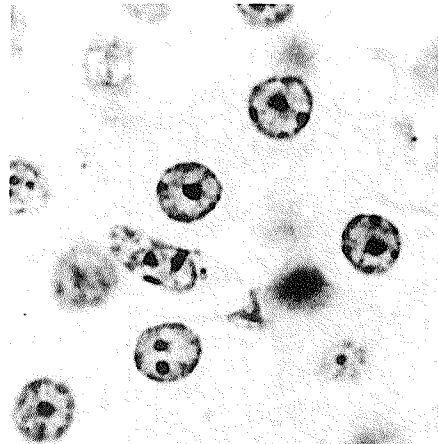


Figure 60

Aves

Columba livia (pigeon), Anas platyrhynchos (mallard duck).

Nine tissues were studied, three from the nervous system, five from viscera, and the epidermis. An additional tissue from the nervous system was included in the study on the mallard duck. Chromatin masses were present in nuclei of tissues from both sexes of the two species. The masses were larger than those seen in other species in this investigation. Figures 61 to 78 illustrate selected cells from female and male pigeons. In tissues from the nervous system, chromatin masses were frequently found adjacent to the nucleolus (Figures 61, 64 and 66). In the non-nervous tissues several masses per nucleus were more common than in tissues from the nervous system. Also there was a greater number of nuclei in which the chromatin masses were on the nuclear membrane (Figures 67 to 72).

Incidence of chromatin masses was high in most tissues from females and males of both species (Appendix D, Tables 38 to 42). In pigeon, smooth muscle and the epidermis had counts lower than those in other tissues. A nuclear sexual dimorphism could not be detected microscopically.

Statistical analysis on incidence of chromatin masses did not reveal any sexual dimorphism in pigeon (Table 1). The F value for sex was not significant. The significant F value for tissue ($P < 0.01$) verifies that the incidence of chromatin masses is different from tissue to tissue. There

was no interaction. Since there was a higher per cent of chromatin masses on the nuclear membrane in cells from female tissues than from male tissues, these observations were analysed. The difference was significant (Table 1). A nuclear sexual dimorphism was revealed when only the number of chromatin masses on the nuclear membrane were considered; F value for sex was significant ($P < 0.01$). The F value for tissue was significant ($P < 0.01$) and there was interaction ($P < 0.01$). A much greater number of chromatin masses on the nuclear membrane were observed in cells of liver and kidney tubules from the females than from the males. This could account for the significant interaction term.

In mallard duck, observations were not analysed because there were not enough females. But, there appears to be a difference between sexes similar to that in pigeons. The number of chromatin masses on the nuclear membrane in cells of female origin was greater, 17.1%, than that, 5.8%, in cells of male origin (Table 1).

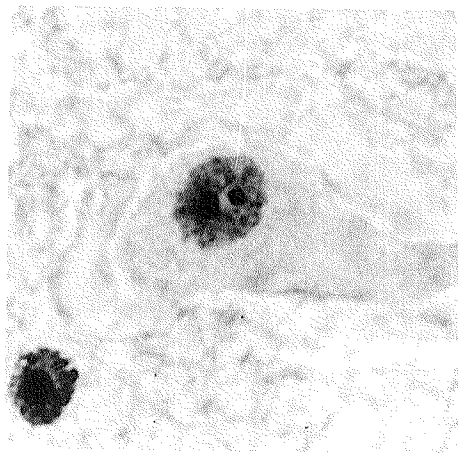


Figure 61

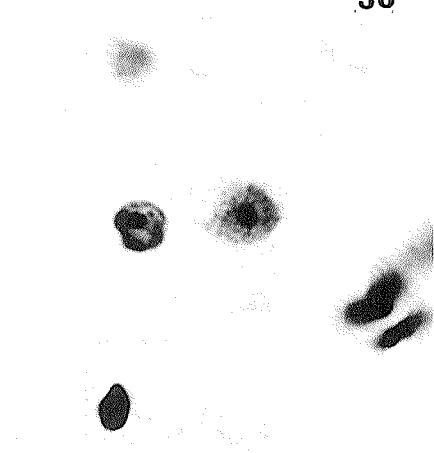


Figure 62

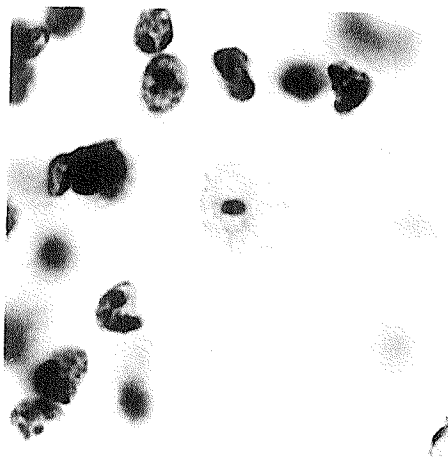


Figure 63

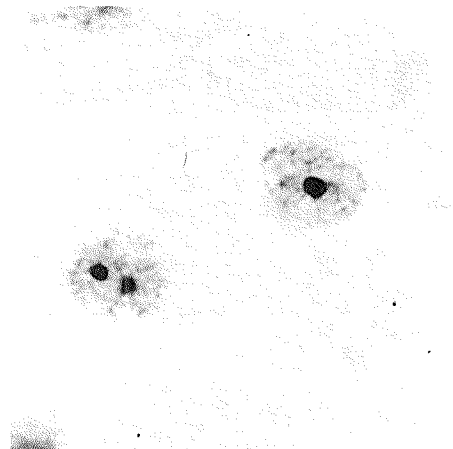


Figure 64

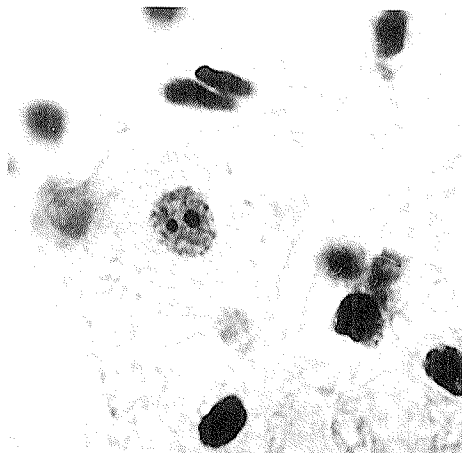


Figure 65

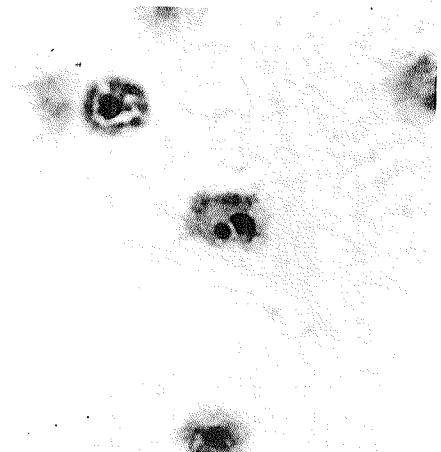


Figure 66

FIGURE

67. Pigeon. Kidney tubule, female. Distinct chromatin masses are present, some are adjacent nucleoli and others are on the nuclear membrane. 6μ , Feulgen reaction, X2048.
68. Pigeon. Kidney tubules, male. Nuclei are similar to those in female kidney tubules. 6μ , Feulgen reaction, X1600.
69. Pigeon. Liver, female. Nuclei contain distinct chromatin masses, some resemble sex chromatin found in mammalian cells. 6μ , Feulgen reaction, X2048.
70. Pigeon, Liver, male. Nuclei are similar to those in female liver. 6μ , Feulgen reaction, X1600.
71. Pigeon. Intestinal epithelium, female. In some nuclei, distinct chromatin masses are visible. 6μ , Feulgen reaction, X1600.
72. Pigeon. Intestinal epithelium, male. In some nuclei, distinct chromatin masses are visible, similar to those in female intestinal epithelium. 6μ , Feulgen reaction, X1600.

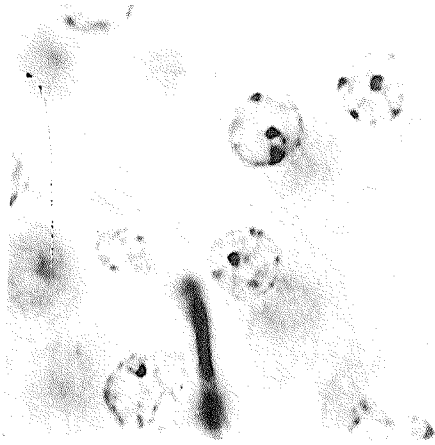


Figure 67

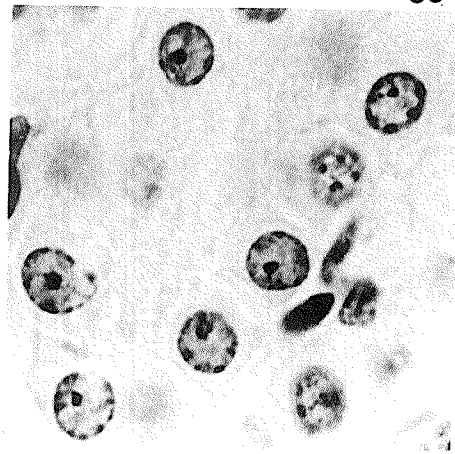


Figure 68

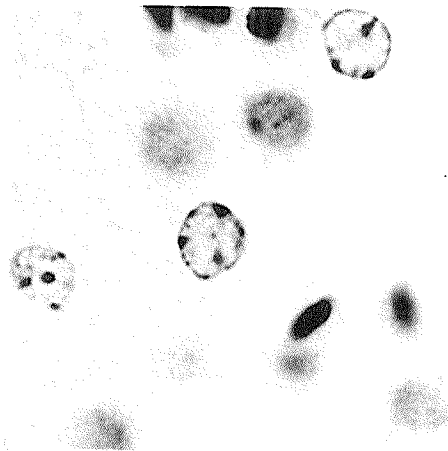


Figure 69



Figure 70

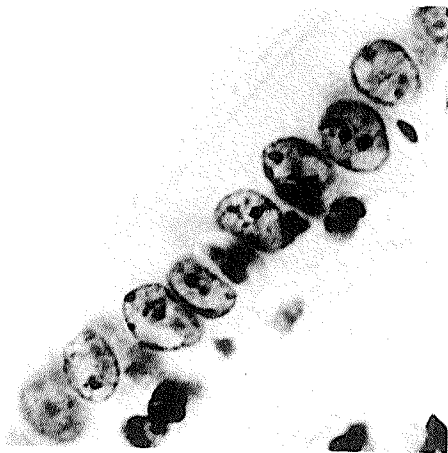


Figure 71

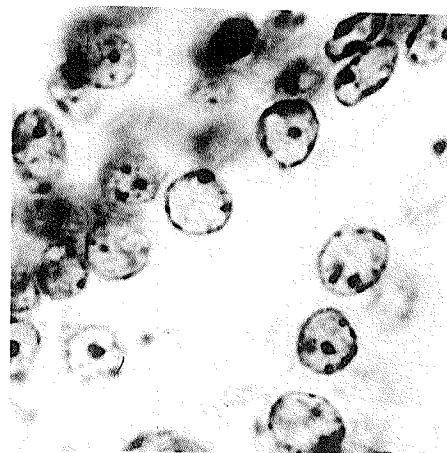


Figure 72

FIGURE

73. Pigeon. Pancreas, female. Nuclei contain coarse and irregular chromatin masses. 6μ , Feulgen reaction, X1600.
74. Pigeon. Pancreas, male. A chromatin mass adjacent to a nucleolus is visible in one nucleus. There are fewer chromatin masses in these nuclei than those of female pancreas. 6μ , Feulgen reaction, X2560.
75. Pigeon. Smooth muscle, female. Distinct chromatin masses are not present. 6μ , Feulgen reaction, X1600.
76. Pigeon. Smooth muscle, male. Distinct chromatin masses are not present. 6μ , Feulgen reaction, X1600.
77. Pigeon. Epidermis, female. A distinct chromatin mass is visible in one nucleus. 6μ , Feulgen reaction, X1600.
78. Pigeon. Epidermis, male. In one nucleus, a crescent-shape chromatin mass appears on the nuclear membrane. 6μ , Feulgen reaction, X1600.

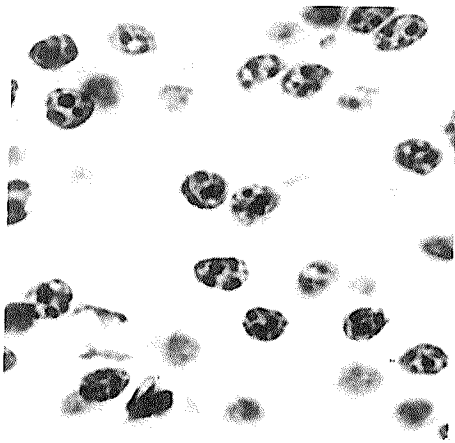


Figure 73

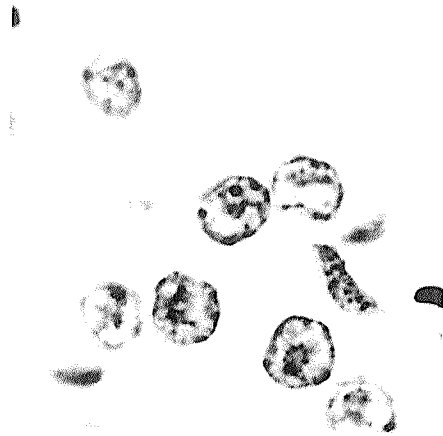


Figure 74

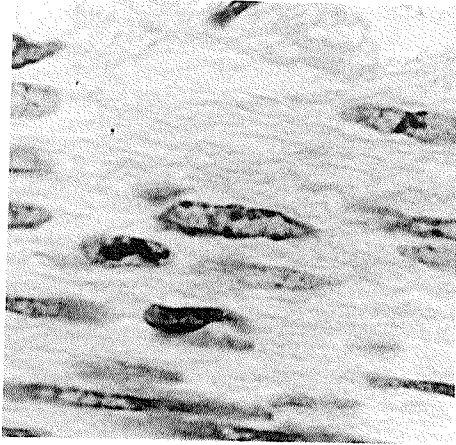


Figure 75

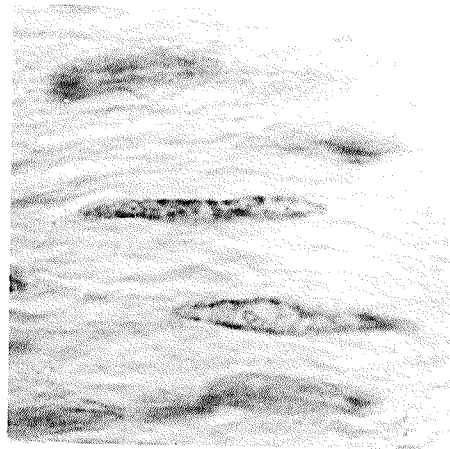


Figure 76



Figure 77

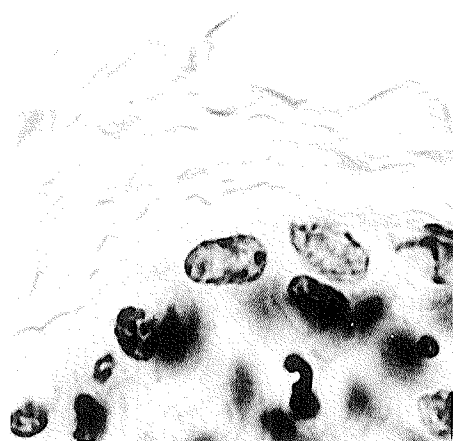


Figure 78

DISCUSSION

Intranuclear chromatin masses were present in tissues of the lamprey and fishes studied. The number per nucleus varied, as did the incidence from tissue to tissue, which was usually greater in non-nervous tissue than in tissue from the nervous system. Although many chromatin masses were similar to sex chromatin described in mammalian tissues, sex-specific chromocenters were not recognized, with exception of rainbow trout. Beckert (1962) also could not distinguish sex chromatin in fishes, with exception of one species in which there was sexual dimorphism in nerve cells. However he does not give the species, and it is assumed that the female is "positive" and the male "negative".

Assuming sex chromatin is formed by sex chromosomes, is the absence of "sex chromatin" due to the lack of sex chromosome differentiation?

There have been two reports on identification of sex chromosomes in fish. Chen and Ebeling (1966) propose male heterogamety in a species of deepwater smelt (order Clupeiformes) based on studies of testis preparation only. The other report, again by Chen and Ebeling (1968), gives evidence to support female heterogamety in the mosquito fish (Cyprinodontiformes) based on gill epithelium and spleen preparations from both sexes, and testis. In this investigation, gill epithelium, spleen and testis were unsuitable for study. Rainbow trout, whitefish and northern pike

belong to the same order as the deep water smelt; interestingly, rainbow trout did illustrate a greater incidence of chromatin masses in tissues from males than from females. What is the basis of the very significant difference found between male and female rainbow trout? Heteromorphic chromosome pairs have not been revealed in the Salmonidae (Chen and Ebeling, 1966; Sasaki et al., 1968). In rainbow trout, the diploid number was found to be 58 to 65, with two to seven distinct chromosome constitutions in each individual fish (Ohno, et al., 1965). There was no identification of a heteromorphic pair of chromosomes. It is reasonable to assume that the sex difference seen here is not due to a pair of heteromorphic chromosomes. Ohno (1960, 1967, 1968) suggests that sex chromosomes are at a primitive state of differentiation and not detectable microscopically in fishes. Sex-linkage does indicate some form of genetic sex determination, but chromosomes bearing sex-determining genes are no different from one another than from autosomes, and like autosomes, may be altered by crossing-over (Mittwoch, 1967).

Sex determination may not be due to a pair of chromosomes but to the diploid number of a fish. Four age

groups were used by Ohno et al. (1965) but the fish were not sorted by sex. Chromosomal analysis by sex may reveal a diploid number for males which is different to that for females. It may be that in the rainbow trout, the females possessed a lower chromosome number than the males and hence a lower incidence of chromatin masses. There is also the possibility that the difference may be a reflection of metabolic activity. All specimens were from the same source, within the same age group (12-15 cm. about 8 month, re: Ohno et al., 1965) and kept under constant laboratory conditions. However female No. 3 and all males were sacrificed in October 1968, while females Nos. 1 and 2 were sacrificed in January 1969 (Appendix B). It was females 1 and 2 in which the counts were lowest. There may have been an inherent metabolic change occurring during this time period. Rainbow trout spawn from late winter to early spring. There may be a higher rate of mitosis prior to spawning that immediately before spawning. The chromatin masses counted may have been due to condensing of chromosomes in preparation for mitosis. However, this would not account for the differences in counts from regions of the nervous system.

There is evidence that environmental factors and hormones alter puffing in chromosomes (Gall, 1963) and hence alter regions of heterochromatin. Some unknown factor or factors are responsible for the expression of chromatin

masses and the great variability from tissue to tissue. This is especially noticeable in fishes. The difference that has been indicated between males and females of rainbow trout should be investigated further. It would be interesting to calculate mitotic rate of various tissues and to see if there is any seasonal variation. Also there should be a comprehensive chromosomal analysis completed on tissues from females and from males, comparing the diploid complement and not searching for a heteromorphic pair of chromosomes.

The amphibians, like the lamprey and fishes, did not possess sex-specific chromocenters. This is similar to previous studies on amphibians (Moore, 1966b). In leopard frog and tiger salamander, chromatin masses in nuclei of tissues from viscera and the epidermis were so large and coarse that accurate enumeration was not possible. Tissues from Manitoba toad were slightly easier to count. Is there an explanation for these large and numerous chromatin masses?

The diploid chromosome number is low relative to that in mammals: Rana sp. 26, Bufo sp. 22, Ambystoma sp. 28 (Cole et al., 1968; Goin and Goin, 1962). There is no record of identification of sex chromosomes in these species. Presence of female heterogamety was reported for the African water frog by Weiler and Ohno (1962) but has been subsequently refuted by Mikamo and Witschi (1966).

The DNA content particularly in salamanders is exceptionally high in diploid cells, 7 to 30 times that in

mammals (Gall, 1963). Gall suggests that the high DNA content in amphibian nuclei is due to serial replication of units. If this is so, and heterochromatin reflects gene inactivity; the abundance of chromatin masses is due to the "turning off" of redundant genetic information. Rather than being redundant, it could represent the information which operated at a previous period during the life cycle. The amphibians have a unique life cycle relative to other vertebrates. Characteristically there is a change in environment, aquatic to terrestrial, accompanied by change in breathing apparatus, locomotory equipment and digestive tract. The life cycle of an amphibian, such as Manitoba toad, provides the experimental material for examination of gene inactivation, heterochromatization and the relationship between the two.

A chromatin mass was found to be specific to female garter snakes, timber rattlers, and pigeons. Ashley and Theiss (1959) investigated several species of snakes, but did not report nuclear sexual dimorphism. The tissues they used were heart, epidermis, liver, epithelium and smooth muscle from the alimentary tract. All, but the heart, were examined in this study and in these tissues the incidence of chromatin masses was low in both sexes. Each tissue alone would not reveal a difference.

In timber rattlers and pigeons the difference was detected statistically, whereas in the garter snakes the

difference was distinct cytologically. The chromatin masses were more frequent and larger in females than in males. A size difference in sex chromatin has been reported for some mammals (Graham and Barr, 1959; Ohno, Becak and Becak, 1964; Perondini and Perondini, 1966). In these mammals, the female is homogametic while in snakes the female is heterogametic. Sex chromosomes have been identified in some species of Colubridae and Viperidae. They are the fourth largest pair of the karyotype and are heteromorphic in the female (Becak, 1968). In Viperidae, the sex chromosomes "WZ" are about the same size except that one (Z) is metacentric and the other (W) is acrocentric. The sex chromosomes in the male are metacentric (ZZ). In Colubridae, there is variability in the sex chromosomes. In some species they cannot be distinguished while in others, they resemble the sex chromosomes described for Viperidae. And in some species of Colubridae the acrocentric (W) chromosome may be larger or smaller than the metacentric chromosome (Becak and Becak, 1969). Karyotypes for the species in this investigation have not been recorded, and therefore the sex chromosome constitution is unknown.

Bianchi et al. (1969) carried out labelling of replicating cells in four species of snakes, they did not find any late replication of sex chromosomes: "Snakes have no mechanism of sex chromosome heterochromatization in either sex. The absence of late replicating Z-chromosome in

the males, favours the hypothesis that no mechanism of sex dosage compensation is acting in the suborder Serpentes". In the light of the report by Bianchi et al. and that sex chromatin in females was so obvious in this study, garter snake should be investigated more extensively: karyotyping, labelling with tritiated thymidine. If in this species the female is heteromorphic as in Colubridae, and sex chromatin is derived from a sex chromosome, further study may reveal new information on heterochromatization.

Sex differences have been ascribed to nuclei of various tissues from birds (Kosin and Ishizahi, 1959; Ohno et al., 1960; Moore and Hay, 1961; Koshida and Kosin, 1968). Other investigators did not detect a sex difference (Ashley and Theiss, 1959; Hammar, 1964). There is an indication of a sex difference in the pigeons examined in this study. The results from mallard duck are incomplete and inconclusive. Brusa (1952) did not find any difference in nerve cells of the pigeon. The same could be reported here. In the nerve cells, chromocenters were present, but they were not adjacent to the nuclear membrane; hence the cells would not show "sex chromatin". The difference was apparent in kidney and liver, if only those chromatin masses on the nuclear membrane were counted.

Galton and Bredury (1966) examined DNA replication patterns of sex chromosomes of the pigeon. In pigeon the diploid number of chromosomes is about eighty, made up of

macrochromosomes and microchromosomes. The female is WZ, and the male ZZ. The Z is the fourth largest macrochromosome and the W is a microchromosome. It appears that the macrochromosomes including the "Z" chromosomes replicate first, followed by the "W" in the female, and the microchromosomes replicate last. The replicating pattern of the sex chromosomes in birds is less striking than that recorded in mammalian tissues. Hsu et al. (1964), Galton and Bredbury (1966) suggest that the sex chromatin in female birds is derived from the "W". As in reptiles, sex chromatin does not appear to be a dosage compensatory mechanism.

From mammalian tissues correlations have been attempted between sex chromosomes, late replication and genetic inactivation to describe significance of sex chromatin. In man, there appears to be a definite relationship between number of sex chromatin bodies in a nucleus and number of X-chromosomes in the karyotype. But heterochromatization is not completely identical with genetic inactivity or late DNA replication (Bianchi et al., 1968; Klinger, 1966; Schmid and Leppert, 1969). Sex chromatin has been observed in species of arthropods and vertebrates, it is a phenomenon of the female sex regardless of heterogamety or homogamety. The incidence of sex chromatin varies in the different tissues of an individual. Significance of sex chromatin is directed toward being a characteristic of females, and an expression of difference between the metabolic state of nuclei of various tissues.

SUMMARY

Tissues of nineteen species of vertebrate were examined for presence of sex chromatin and nuclear sexual dimorphism of somatic cells. The tissues were selected from regions of the nervous system, viscera and skin. Chromatin masses varied in size and number per nucleus and in incidence from tissue to tissue. The data was subjected to factorial analysis of variance.

In lamprey, fishes (with exception of rainbow trout), and amphibians, no sex difference was detected. In rainbow trout, cells in tissues of male origin had a greater incidence of chromocenters than cells in tissues of female origin. Sex chromatin and sexual dimorphism of nuclei were present in garter snakes, timber rattlers and pigeons.

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APPENDICES

	Page
A	57
B	60
C	62
D	64
E	88

Appendix A

Classification of species investigated.

Class	Order	Family	Genus, species and common name
Petromyzones	Petromyzoniformes	Petromyzontidae	<u>Ichthyomyzon castaneus</u> Girard chestnut lamprey
Teleostomi	Clupeiformes	Salmonidae	<u>Salmo gairdneri</u> Richardson rainbow trout
			<u>Coregonus clupeaformis</u> (Mitchill) whitefish
		Esocidae	<u>Esox lucius</u> Linnaeus northern pike
	Cypriniformes	Catastomidae	<u>Catostomus commersoni</u> (Lacépède) common white sucker
		Cyprinidae	<u>Notropis hudsonius</u> (Clinton) spottail shiner
	Gasterosteiformes	Gasterosteidae	<u>Culaea inconstans</u> (Kirkland) brook stickleback
	Percopsiformes	Percopsidae	<u>Percopsis omiscomaycus</u> (Walbaum) troutperch
	Perciformes	Percidae	<u>Percina caprodes</u> (Rafinesque) logperch
			<u>Perca flavescens</u> (Mitchell) yellow perch
			<u>Stizostedion vitreum</u> (Mitchell) walleye
		Cottidae	<u>Cottus bairdi</u> Girard mottled sculpin
Amphibia	Anura	Ranidae	<u>Rana pipiens</u> leopard frog
		Bufo	<u>Bufo hemiophrys</u> Manitoba toad
	Urodela	Ambystomatidae	<u>Ambystoma tigrinum diaboli</u> tiger salamander

Class	Order	Family	Genus, species and common name
Reptilia	Squamata	Colubridae	<u>Thamnopsis sirtalis</u> ssp. garter snake
		Viperidae	<u>Crotalus horridus horridus</u> timber rattler
	Aves	Columbiformes	
		Columbidae	<u>Columba livia</u> pigeon
	Anseriformes		
	Anatidae	<u>Anas platyrhynchos</u> mallard duck	

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- (3) Classification of Vertebrates: Recent Families and Fossil Groups. After L. S. Berg, 1940 (Fishes) and A. S. Romer, 1966 (Tetrapods) with common names from various sources.

C.S.W., C.C.L., K.W.S., R.M.E., M.A., Department of Zoology, University of Manitoba, 1969.

Appendix B

Number, length or approximate age, sources, and date of fixation for each species studied.

Source	Date of Fixation
Rat River	8/6/69
Whiteshell Hatchery	2/10/68; 22/1/69
Athapapuskow	23/8/68
Delta; Roseau River; Singoosh Lake, Athapapuskow	20/7/68; 5/8/68; 8/8/68; 20/8/68; 23/8/68
Athapapuskow; Duck Mountains; U. of Man.	23/8/68; 13/10/68; 27/5/69
Delta; Roseau River; Athapapuskow; U. of Man.	5/8/68; 8/8/68; 23/8/68; 6/11/68; 21/5/69
Duck Mountains, Man.	30/6/69; 7/7/69
Delta; Rat River; Hecla Island	23/7/68; 8/6/69; 13/8/69
Roseau River; Hecla Island	8/8/68; 16/8/68
Delta; Athapapuskow	20/7/68; 5/8/68; 23/8/68
West Blue Lake	2/8/68
Hecla Island	13/10/68; 3/7/69; 17/7/69; 21/8/68; 27/5/69
Delta	13/8/69
Delta	28/5/69
Innedosa	20/8/68
Interlake	12/10/68
Delta, U.S.A., U. of Man.	12/8/68; 22/1/69
Winnipeg	2/7/69
Waterfowl Station, Delta	6/9/68

Appendix C

Feulgen Reaction

Reagent

Schiff Reagent (Can. Lab.)

Sulfite Rinses

Prepared daily and/or after a maximum of 120 slides

10 cc 1N HCl

10 cc 10% potassium metabisulfite

200 cc distilled H₂O

Method

1. Bring sections to water.
2. Transfer to 5N HCl and leave for 10 minutes.
3. Rinse well in distilled water.
4. Transfer to Schiff reagent for 20 minutes.
5. Transfer to sulfite rinse for 1 minute.
6. Transfer to a second sulfite rinse for 1 minute.
7. Transfer to a third sulfite rinse for 2 minutes.
8. Rinse in running tap water for 2 minutes and rinse well in distilled water.
9. Counterstain with 0.1% fast green, use 1 dip.
10. Dehydrate, clear and mount in Permount.

This reaction is DNA specific. The chromatin stains bright red to deep purple; the rest of the cell is visible only by counterstain fast green.

Appendix D

Tables of number of nuclei with chromatin masses, number of cells with chromatin masses on nuclear membrane, and analysis of variance of species studied.

Table 1. Lamprey - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3 ^a	1 ^a	2	3		
prosencephalon	18	2	-	-	23	47	4	15
mesencephalon	15	2	-	-	34	15	3	4
metencephalon	27	15	-	49	34	35	5	23
nerve cord	21	13	15	43	30	30	6	19
intestinal epithelium	^a -	22	20	11	18	31	1	3
kidney	22	8	6	4	2	11	10	3
liver	75	62	63	22	11	41	16	13
epidermis	5	5	17	23	6	19	4	4

^a not used for statistical analysis
 - tissue not available

Table 2. Lamprey - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	63.0000	63.0000	0.06	ns.
tissue	6	4025.4297	670.9048	6.41	<0.01
sex tissue	6	2963.0000	483.8333	4.72	<0.01
within cells	14	1465.0000	104.6429		
Total	27	8516.4297			

ns. not significant

Table 3. Rainbow trout - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3	1	2	3		
olfactory ^a	-	-	60	50	66	70	1	3
optic	3	1	58	64	76	82	0	3
cerebellum	7	3	36	55	51	53	1	5
medulla	21	30	69	82	72	62	5	6
nerve cord	23	11	54	65	66	73	3	9
ganglion ^a	19	7	-	88	-	-	1	3
intestinal epithelium	32	21	58	71	56	61	1	4
kidney	38	39	62	63	67	57	1	1
liver	32	36	63	76	68	55	4	5

a not used for statistical analysis
 - tissue not available

Table 4. Rainbow trout - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	10944.8398	10944.8398	40.90	<0.01
tissue	6	2017.6616	336.2769	1.26	ns.
sex tissue	6	1371.4519	228.5753	0.85	ns.
within cells	28	7493.3125	267.6182		
Total	41	21827.3320			

ns. not significant

Table 5. Whitefish - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells					No. cells with chromatin masses on nuclear membrane	
	Female			Male		Female	Male
	1	2	3 ^a	1	2		
optic	24	32	31	29	29	3	0
cerebellum	3	20	31	7	10	0	0
medulla	7	17	16	13	8	1	0
nerve cord	7	10	13	6	13	0	0
ganglion	1	1	6	2	3	0	0
intestinal epithelium	68	57	47	57	68	11	3
kidney	56	63	-	37	54	2	0
liver	65	70	84	52	50	5	4

a not used for statistical analysis
 - tissue not available

Table 6. Whitefish - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	124.0312	124.0312	3.44	ns.
tissue	7	17629.9687	2518.5669	69.78	< 0.01
sex tissue	7	359.7188	51.3884	1.42	ns.
within cells	16	577.5000	36.0937		
Total	31	18691.2187			

ns. not significant

Table 7. Northern pike - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells					No. cells with chromatin masses on nuclear membrane	
	Female			Male		Female	Male
	1	2 ^a	3	1	2		
olfactory ^a	8	-	-	-	-	0	-
optic	17	11	29	9	27	6	2
cerebellum	19	59	28	22	24	53	24
medulla	11	7	25	12	10	3	1
nerve cord	18	16	25	16	18	4	2
ganglion	7	-	26	2	3	7	1
intestinal epithelium	92	89	88	95	95	12	12
kidney ^a	-	49	-	65	-	5	3
liver	57	36	19	57	24	0	2

a not used for statistical analysis
 - tissue not available

Table 8. Northern pike - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	78.8926	78.8926	0.59	ns.
tissue	6	19571.0000	3261.8333	24.57	< 0.01
sex tissue	6	242.8516	40.4753	0.30	ns.
within cells	14	1858.5000	132.7500		
Total	27	21751.2500			

ns. not significant

Table 9. Common white sucker - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3	1	2	3		
olfactory ^a	-	-	-	-	-	1	-	0
optic	13	28	15	7	38	10	2	4
cerebellum	0	24	12	10	28	9	1	0
medulla	14	5	21	18	52	10	0	4
nerve cord	2	5	27	4	15	0	1	1
ganglion ^a	-	-	-	0	-	-	-	0
intestinal epithelium	28	14	29	10	61	34	0	1
kidney ^a	-	25	58	28	33	46	2	1
liver	20	14	46	65	72	27	3	7

a not used for statistical analysis
 - tissue not available

Table 10. Common white sucker - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	650.2434	650.2434	2.58	ns.
tissue	5	3950.4556	790.0911	3.14	<0.05
sex tissue	5	1042.9172	208.5834	0.83	ns.
within cells	24	6044.6602	251.8608		
Total	35	11688.3086			

ns. not significant

Table 11. Spottail shiner - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3	1	2	3		
olfactory	13	9	1	16	33	12	2	7
optic	12	18	5	23	22	20	3	5
cerebellum ^a	18	64	-	38	51	-	8	10
medulla	5	18	7	5	30	11	8	7
nerve cord	17	17	5	12	14	2	3	4
intestinal epithelium	47	65	32	46	71	55	2	20
kidney ^a	-	53	20	49	67	27	7	28
liver	86	89	32	45	78	56	37	26

a not used for statistical analysis
 - tissue not available

Table 12. Spottail shiner - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	143.9993	143.9993	0.76	ns.
tissue	5	16651.5273	3330.3054	17.63	0.01
sex tissue	5	574.6643	114.9329	0.61	ns.
within cells	24	4534.6523	188.9438		
Total	35	21904.8906			

ns. not significant

Table 13. Brook stickleback - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3 ^a	1	2	3 ^a		
olfactory ^a	0	-	-	-	-	1	0	0
optic	3	1	2	3	3	12	2	0
cerebellum	0	6	1	2	1	-	0	0
medulla	3	3	0	1	0	-	0	0
nerve cord	6	0	-	6	5	18	1	7
intestinal epithelium	15	0	2	28	24	19	2	0
kidney	6	11	2	3	9	18	0	0
liver	58	45	14	38	52	86	10	18

a not used for statistical analysis
 - tissue not available

Table 14. Brook stickleback - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	11.5703	11.5703	0.43	ns.
tissue	6	6837.9297	1139.6548	42.78	<0.01
sex tissue	6	394.9297	65.8216	2.46	ns.
within cells	14	373.0000	26.6429		
Total	27	7617.4297			

ns. not significant

Table 15. Troutperch - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3	1	2	3		
olfactory ^a	-	-	-	3	-	-	-	0
optic	2	22	9	1	6	14	18	1
cerebellum	12	28	0	4	1	4	8	2
medulla	2	37	1	1	2	2	6	1
nerve cord ^a	-	-	4	3	-	3	0	0
intestinal epithelium	7	34	41	34	26	27	8	18
kidney ^a	-	-	8	38	31	35	0	18
liver	27	58	57	68	68	66	39	66

a not used for statistical analysis
 - tissue not available

Table 16. Troutperch - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	5.6316	5.6316	0.04	ns.
tissue	4	11142.4531	2785.6133	19.62	<0.01
sex tissue	4	986.8650	246.7162	1.74	ns.
within cells	20	2840.0032	142.002		
Total	29	14974.9687			

ns. not significant

Table 17. Logperch - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3	1	2	3		
olfactory ^a	41	-	37	25	40	-	31	29
optic	41	20	50	10	48	42	47	28
cerebellum	45	9	10	7	39	11	10	6
medulla	26	13	27	12	39	27	33	28
nerve cord	21	20	25	12	12	27	32	11
ganglion ^a	7	25	-	2	2	-	23	4
intestinal epithelium	69	30	36	46	84	51	7	5
kidney	71	17	42	51	63	61	6	8
liver	48	22	45	50	68	36	8	17

a not used for statistical analysis
 - tissue not available

Table 18. Logperch - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	282.8804	282.8804	1.04	ns.
tissue	6	7440.8086	1240.1348	4.56	0.01
sex tissue	6	750.6184	125.1031	0.46	ns.
within cells	28	7606.6523	271.6660		
Total	41	16080.9727			

ns. not significant

Table 19. Yellow perch - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3	1	2	3		
olfactory ^a	0	0	0	2	-	-	0	0
optic	1	2	1	4	7	3	0	0
cerebellum	3	4	3	1	0	0	1	0
medulla	1	8	1	0	2	5	1	0
nerve cord ^a	4	-	1	1	2	5	0	2
ganglion ^a	1	7	-	3	2	3	2	0
intestinal epithelium ^a	-	29	24	51	54	29	0	1
liver	25	41	55	32	26	21	8	2

a not used for statistical analysis
 - tissue not available

Table 20. Yellow perch - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	80.6719	80.6719	2.28	ns.
tissue	3	4267.4883	1422.4961	40.12	<0.01
sex tissue	3	244.9805	81.6602	2.30	ns.
within cells	16	567.3347	35.4584		
Total	23	5160.5000			

ns. not significant

Table 21. Walleye - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells					No. cells with chromatin masses on nuclear membrane	
	Female			Male		Female	Male
	1	2	3 ^a	1	2		
olfactory	0	4	2	0	0	0	0
optic	14	6	12	5	7	1	1
cerebellum	7	2	6	1	1	4	1
medulla	3	1	8	0	4	1	1
nerve cord	0	4	4	1	2	3	0
ganglion	13	5	21	0	7	0	1
intestinal epithelium	55	56	69	59	60	1	1
kidney	41	45	54	53	44	2	2
liver	56	49	66	61	51	3	4

a not used for statistical analysis

Table 22. Walleye - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	0.6876	0.6876	0.05	ns.
tissue	8	19564.0000	2445.5000	173.64	<0.01
sex tissue	8	120.5625	15.0703	1.07	ns.
within cells	18	253.5000	14.0833		
Total	35	19938.7500			

ns. not significant

Table 23. Sculpin - number of nuclei with chromatin masses per 100 cells.

Tissue	Female		Male		
	1	2	1	2	3 ^a
olfactory	46	23	44	41	-
optic	61	38	72	36	52
cerebellum ^a	53	-	56	40	40
medulla ^a	29	-	38	3	-
nerve cord	38	26	55	59	49
intestinal epithelium	33	10	100	19	33
kidney	34	26	96	9	74
liver	41	50	99	43	93

a not used for statistical analysis
- tissue not available

Table 24. Sculpin - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	2542.0415	2542.0415	2.98	ns.
tissue	5	1176.7085	235.3417	0.28	ns.
sex tissue	5	767.7085	153.5417	0.18	ns.
within cells	12	10231.5000	852.6250		
Total	23	14717.9570			

ns. not significant

Table 25. Sculpin - number of cells with chromatin masses on nuclear membrane per 100 cells.

Tissue	Female		Male	
	1	2	1	2
olfactory	37	22	30	36
optic	52	35	59	26
cerebellum ^a	23	-	24	0
medulla ^a	27	-	29	19
nerve cord	24	16	35	32
intestinal epithelium	14	1	91	1
kidney	18	7	72	4
liver	4	4	76	9

a not used for statistical analysis
- tissue not available

Table 26. Sculpin - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	2340.3750	2340.3750	2.92	ns.
tissue	5	1029.8750	205.9750	0.26	ns.
sex tissue	5	1469.8750	293.9749	0.37	ns.
within cells	12	9607.5000	800.6250		
Total	23	14447.6250			

ns. not significant

Table 27. Leopard frog - number of nuclei with chromatin masses per 100 cells.

Tissue	Female			Male		
	1	2	3	1	2	3
olfactory	-	100	96	100	-	93
optic	100	100	100	100	-	100
cerebellum	100	-	-	100	-	-
medulla	-	-	38	100	24	78
nerve cord	47	24	45	45	34	47
ganglion	-	-	42	50	21	56
intestinal epithelium	--	--	--	--	--	--
kidney	--	--	--	--	--	--
liver	--	--	--	--	--	--
smooth muscle	--	--	--	--	--	--
pancreas	--	--	--	--	--	--
epidermis	--	--	--	--	--	--

-- tissue not suitable for counting
 - tissue not available

Table 28. Manitoba toad - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells				No. cells with chromatin masses on nuclear membrane	
	Female		Male		Female	Male
	1	2	1	2		
olfactory	100	100	98	98	131	142
optic ^a	85	100	-	100	130	77
cerebellum ^a	-	-	9	-	-	2
medulla	29	31	30	70	13	7
nerve cord	27	46	16	63	12	4
ganglion ^a	23	10	20	-	12	1
intestinal epithelium	94	81	87	69	39	13
kidney ^a	--	71	--	--	58	-
liver ^a	--	87	87	--	10	13
smooth muscle	16	6	11	9	1	1
pancreas ^a	--	--	--	--	-	-
epidermis	48	63	49	57	12	11

a not used for statistical analysis
 - tissue not available
 -- tissue not suitable for counting

Table 29. Manitoba toad - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	10.6665	10.6665	0.05	ns.
tissue	5	20783.3281	4156.6641	19.72	<0.05
sex tissue	5	499.8281	99.9656	0.47	ns.
within cells	12	2530.0000	210.8333		
Total	23	23823.8359			

ns. not significant

Table 30. Salamander - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3	1	2	3		
olfactory	83	54	59	66	75	90	47	53
medulla	72	72	58	63	63	86	22	17
nerve cord	80	75	65	75	68	72	23	53
ganglion ^a	28	63	62	-	-	20	1	4
intestinal epithelium ^a	--	--	--	--	--	--	-	-
kidney ^a	--	--	--	--	--	--	-	-
liver ^a	--	--	--	--	--	--	-	-
smooth muscle ^a	--	--	--	--	--	--	-	-
pancreas ^a	--	--	--	--	--	--	-	-
epidermis ^a	--	--	--	--	--	--	-	-

a not used for statistical analysis
 - tissue not available
 -- tissue not suitable for counting

Table 31. Salamander - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	88.8867	88.8867	0.76	ns.
tissue	2	37.4414	18.7207	0.16	ns.
sex tissue	2	136.1016	68.0508	0.58	ns.
within cells	12	1399.3318	116.6110		
Total	17	1661.7812			

ns. not significant

Table 32. Garter snake - number of nuclei with chromatin masses per 100 cells.

Tissue	Female			Male		
	1	2	3	1	2	3
optic	78	77	80	77	78	55
cerebellum ^a	23	19	11	-	-	-
medulla	79	63	88	59	43	43
nerve cord	82	80	79	60	44	33
ganglion ^a	75	-	60	-	-	-
intestinal epithelium	1	1	3	4	18	36
kidney	47	50	46	67	31	18
liver	67	54	72	53	33	12
smooth muscle	3	8	11	6	13	8
pancreas	63	68	55	22	11	4
epidermis ^a	1	0	4	3	4	-

a not used for statistical analysis

- tissue not available

Table 33. Garter snake - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	3798.5186	3798.5186	27.98	<0.01
tissue	7	24505.6133	3500.8018	25.78	<0.01
sex tissue	7	5110.6289	730.0898	5.38	<0.01
within cells	32	4344.6680	135.7709		
Total	47	37759.4766			

Table 34. Garter snake - number of cells with chromatin masses on nuclear membrane per 100 cells.

Tissue	Female			Male		
	1	2	3	1	2	3
optic	1	1	0	3	4	4
medulla	1	4	5	0	2	0
nerve cord	0	3	1	0	2	0
intestinal epithelium	0	0	0	0	0	1
kidney	6	26	34	0	0	0
liver	30	32	43	2	1	0
smooth muscle	0	1	0	0	0	1
pancreas	16	42	34	0	2	0

Table 35. Garter snake - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	1386.8555	1386.8555	49.57	<0.01
tissue	7	2301.7051	328.8149	11.75	<0.01
sex tissue	7	2448.1304	349.7329	12.50	<0.01
within cells	32	895.3398	27.9794		
Total	47	7031.9180			

Table 36. Timber rattler - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells						No. cells with chromatin masses on nuclear membrane	
	Female			Male			Female	Male
	1	2	3	1	2	3		
optic	17	11	31	15	14	23	2	6
cerebellum ^a	15	12	13	-	-	9	0	0
medulla	10	7	9	7	23	16	0	0
nerve cord	7	9	12	21	20	10	0	0
ganglion ^a	22	-	-	2	21	4	0	1
intestinal epithelium	15	40	16	3	4	4	4	1
kidney	44	34	14	15	6	2	10	3
liver	34	50	38	25	13	14	14	11
smooth muscle	21	15	7	4	5	3	3	0
pancreas	56	70	40	12	8	7	16	5
epidermis ^a	-	-	-	21	-	4	-	2

a not used for statistical analysis

- tissue not available

Table 37. Timber rattler - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	2310.2109	2310.2109	33.17	<0.01
tissue	7	2870.8269	410.1179	5.89	<0.01
sex tissue	7	3443.2754	491.8965	7.06	<0.01
within cells	32	2228.6641	69.6458		
Total	47	10852.9805			

Table 38. Pigeon - number of nuclei with chromatin masses per 100 cells.

Tissue	Female			Male		
	1	2	3	1	2	3
cerebellum	96	99	96	86	95	79
medulla	90	97	87	95	97	97
nerve cord	90	96	95	97	96	87
intestinal epithelium	97	88	92	98	100	88
kidney	73	89	69	56	80	62
liver	95	100	84	90	83	91
smooth muscle	19	10	8	9	7	4
pancreas ^a	74	-	-	-	-	-
epidermis	35	35	35	34	35	32

a not used for statistical analysis
- tissue not available

Table 39. Pigeon - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	123.6562	123.6562	3.33	ns.
tissue	7	44853.2969	6407.6133	172.50	<0.01
sex tissue	7	357.4922	51.0703	1.37	ns.
within cells	32	1188.6797	37.1462		
Total	47	46522.9805			

ns. not significant

Table 40. Pigeon - number of cells with chromatin masses on nuclear membrane per 100 cells.

Tissue	Female			Male		
	1	2	3	1	2	3
cerebellum ^a	0	0	0	0	0	0
medulla ^a	2	0	0	0	0	0
nerve cord	10	2	0	3	2	0
intestinal epithelium	21	20	23	27	12	15
kidney	35	41	33	13	19	6
liver	53	63	44	46	45	35
smooth muscle	4	5	0	3	6	3
epidermis	7	12	9	9	5	3

a not used for statistical analysis

Table 41. Pigeon - analysis of variance table.

Source of variation	DF	SS	MS	F	P
sex	1	469.4531	469.4531	18.27	<0.01
tissue	5	8848.5469	1769.7092	68.88	<0.01
sex tissue	5	609.8792	121.9758	4.75	<0.01
within cells	24	25.6943			
Total	35	10544.5586			

Table 42. Mallard duck - number of chromatin masses.

Tissue	No. nuclei with chromatin masses per 100 cells				No. cells with chromatin masses on nuclear membrane	
	Female		Male		Female	Male
	1	1	2	3		
cerebellum	-	62	37	34	-	7
medulla	79	80	87	82	1	6
nerve cord	90	79	87	88	4	8
ganglion	-	66	77	72	-	4
intestinal epithelium	100	100	91	97	44	42
kidney	74	93	93	87	8	22
liver	100	100	-	100	33	10
smooth muscle	81	67	-	53	8	10
epidermis	74	61	75	56	22	31

- tissue not available

Appendix E

Size and position of sex chromatin in nuclei of cells from liver, kidney, and medulla of garter snakes.

Measurements and position of sex chromatin

Nucleus	Liver		Kidney		Medulla							
	Female	Male	Female	Male	Female	Male						
1	.17	.21	.14	.13	.18	.16	.15	.11	.22	.20	.18	.15
2	.19	.17	.12	.15	.19	.20	.14	.14	.28	.19	.20	.13
3	.18	.23	.14	.14	.21	.16	.14	.13	.27	.26	.17	.15
4	.20	.22	.14	.15	.17	.16	.12	.13	.22	.21	.19	.17
5	.19	.21	.15	.14	.19	.18	.15	.13	.22	.22	.18	.15
6	.18	.22	.14	.12	.20	.19	.13	.12	.25	.22	.20	.17
7	.16	.26	.14	.14	.24	.16	.15	.13	.22	.22	.16	.13
8	.22	.22	.14	.14	.17	.18	.11	.14	.23	.20	.16	.15
9	.27	.18	.13	.12	.19	.19	.16	.12	.23	.21	.16	.18
10	.18	.21	.15	.14	.21	.20	.14	.10	.20	.19	.18	.15
Mean	.20		.14		.19		.13		.22		.17	
No. on nuclear membrane per 20 nuclei	11		0		13		0		1		0	

1 μ = .2 units

Measured by micrometer across greatest dimension.