THE SEX CHROMATIN IN MAMMALIAN CELLS

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ABSTRACT

Representative regions of the nervous system and various other tissues and organs of the timber wolf, pig, prairie dog, porcupine and armadillo were studied with respect to nuclear morphology according to sex. Only non-nervous cell types of the skunk and mouse were examined as nerve cells of these species had previously been studied (Moore and Barr, 1953).

A nuclear sex difference was observed in the nervous and non-nervous tissues of the timber wolf. Only neuronal nuclei of the pig showed a sex difference; in other tissues of both sexes, multiple chromatin masses obscured any sex difference present. In the porcupine, nuclear sexual dimorphism was observed in cells of stellate ganglion, skeletal, smooth and cardiac muscle, adrenal cortex and medulla. In the skunk, the latter four cell types and also epithelial cells of the urinary bladder showed a distinct sex difference. A sex difference in nuclear morphology could not be detected in tissues of the armadillo, prairie dog and mouse; multiple chromatin masses were observed in nuclei of both sexes.

The observations made in this study are compared with those previously reported for other mammalian species. The

sex characteristics of the nucleus are discussed with reference to chromosome constitution, probable derivations of sex chromatin and its possible relationship to cell metabolism.

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INTRODUCTION

A morphological sex difference in the mammalian nucleus was first observed by Barr and Bertram (1949) in hypoglossal neurones of the cat. In nuclei of the female, a special chromatin mass was frequently seen adjacent to the nucleolus whereas a comparable nuclear component was rarely observed in those of the male. Its close association with the nucleolus earned it the name of "nucleolar satellite". As other tissues were studied, this chromatin mass was found to occur in other positions and was present when the nucleolus was small or even absent. The term "nucleolar satellite" was therefore inappropriate. In view of the relation of this chromatin mass to the sex of the animal and its postulated derivation from the XX sex chromosomes of the female, it is now referred to as sex chromatin.

The presence of a nuclear sex difference in mammalian tissues has various applications in diverse fields of medical and biological science. For example, development of the skin biopsy technique (Moore, Graham and Barr, 1953) and the oral smear technique (Moore and Barr, 1955a) introduced a new approach to the diagnosis and understanding of intersex

states and sexual anomalies. The sex difference in nuclear morphology has also been adopted by tumour cytologists as a new research method in the study of tumour aetiology.

Research in this new cytological field has advanced greatly in the past decade. Study of various tissues of many different species has produced information on the morphological characteristics of sex chromatin, species variations, and has indicated a possible phylogenetic trend in sex chromatin patterns. An association between sex chromatin and cell metabolism has been observed (Barr and Bertram, 1951; Crouch and Barr, 1954), indicating a relationship to nuclear and cytoplasmic function.

Further investigation is necessary to determine the role of the sex chromosomes and autosomes in forming the chromatin pattern of the interphase nucleus, and the relation of chromosomes to cell metabolism. Extension of comparative studies to additional species is needed before a phylogentic trend can be established. The present study was undertaken with these views in mind and to advance our knowledge of the sex characteristics of the metabolic nucleus.

CHAPTER II

HISTORICAL REVIEW

1. The Mammalian Nucleus

a) Chromosomes. It is assumed that the sex difference in metabolic nuclei is based on the sex chromosomes. A brief discussion of chromosome structure and composition is presented with respect to this relationship.

Darlington (1942) gave an account of chromosome structure. He stated that the desoxyribonucleic acid (DNA) molecules which give the aldehyde reaction in the Feulgen test, are attached to the protein fibers which constitute the framework of the chromosomes. These DNA molecules undergo a cycle of attachment and detachment during cell division. Certain regions of some chromosomes, the heterochromatic regions, retain their high DNA content in the metabolic nucleus. The remaining portions of the chromosomes lose DNA molecules in the interphase state and are termed euchromatic.

b) Sex chromosomes. Henking (1891) observed that in the meiotic division of the male germ cells of some insects, a chromosome-like body was passed along to some cells and not to others, thus producing two types of spermatozoa. McClung (1902) postulated the relationship between this peculiar

chromosome and sex determination. In subsequent studies on various species it was shown that one pair of chromosomes consisted of members of differing morphology in contrast to the homologous nature of the pairs of autosomes. The former are the sex chromosomes, the larger element being called the X-chromosome, the smaller, the Y-chromosome.

It is generally agreed that female mammals possess an XX sex chromosome complex whereas the male has an XY chromosome constitution; however, the argument about chromosome numbers and type of sex chromosomes of the various species still continues. The Y-chromosome is generally very small and its existence has been disputed in many cases. For example, Painter (1924) found an exceedingly minute unpaired element in human male germ cells which he called the Y-chromosome. Oguma (1937) in a similar study found no Y-chromosome, in which case, the sex determining mechanism would be XO. Lists of chromosome numbers and the sex chromosome constitution of various species are given by Wilson (1925) and Oguma (1934).

c) <u>The nucleolus</u>. A brief discussion of the nucleolus is pertinent in view of the relationships between sex chromatin, cell metabolism, and the nucleolus. Gates (1942) presented an account of the nucleolus of plants and insects. He stated that it was formed during the telophase, arising

from a definite region of specific or nucleolar chromosomes. These are frequently the sex chromosomes. Little is known concerning the mammalian nucleolus. Schultz and St. Lawrence (1949) observed that the nucleolus in man is formed from an autosome, but noted that a nucleolus is formed from the X-chromosome, differing in its staining properties from the true nucleolus.

d) Interrelations. Mirsky (1943) presented some views on cellular interrelations. Heterochromatin, though generally lacking in genes, is not inert as was previously believed. It is associated in some way with the properties of the nucleolus. The latter structure contains ribonucleic acid (RNA) and is somehow connected with the quantity of RNA in the cytoplasm. It would seem then, that heterochromatin is related to the nucleic acid metabolism of both the nucleus and the cytoplasm.

2. Sex Chromatin

The heterochromatic properties of the sex chromosomes have long been recognized, but it is only relatively recently that this feature has been used in the identification of sex. Smith (1944) used the presence of a large heterochromatic mass in nuclei of somatic cells of the female to determine the primary sex ratio of the spruce budworm Archips fumiferana. In this species, the female is heterogametic and

the male homogametic, but the sex chromosomes of the female form a large heterochromatic mass, whereas a comparable structure is not present in male nuclei. A "nucleolar satellite" of chromatin was observed in neurones of the female cat but was absent in those of the male (Barr and Bertram, 1949); it was thought to represent the nucleolar chromosomes. In view of its presence when the nucleolus was small or absent and its occurrence in other positions, this interpretation seemed unlikely. As stated in the Introduction, this sex-specific chromatin mass was renamed sex chromatin. On the basis of indirect evidence, the idea was put forth that this mass was derived from heterochromatic regions of the XX chromosomes, whereas the XY chromosomes of the male did not form a mass of comparable size. This is the current theory of the origin of sex chromatin.

Brusa (1952) confirmed the presence of a sex difference in cat neurones but was unable to observe a sex difference in neuronal nuclei of the pigeon. He maintained that one could just not look at this structure and say that it represented the XX chromosome complex. He believed that when it was located adjacent to the nucleolus it represented the basophil clot of Levi, and when free of the nucleolus it was the accessory body of Cajal. This idea was refuted by Lindsay and Barr (1955). They stated that no relationship had been demonstrated between the size of the basophil clot of Levi

and the sex of the animal; they were also able to demonstrate the sex chromatin and the accessory body of Cajal in the same nucleus by staining with protargol followed by a basic dye which stained the sex chromatin.

The characteristics of sex chromatin were described by Barr, Bertram and Lindsay (1950). In neuronal nuclei of the female cat, the sex chromatin is located adjacent to the nucleolus, free in the nucleoplasm, or adjacent to the nuclear membrane, changing its shape to conform to adjacent structures, yet retaining its individuality. It is approximately one micron in diameter. Feulgen and methyl green pyronin staining show that it is composed of DNA. Investigations on sex chromatin were extended to non-nervous tissues of the cat and a similar sex difference was found to occur (Barr, 1951; Graham and Barr, 1952). The age of the animal, castration, or administration of the opposite sex hormones was found to have no bearing on the sex characteristics of the nuclei (Coidan, 1951; Moore and Barr, 1953; Moore, Graham and Barr, 1953; Graham, 1954b).

An important study by Klinger (1957a) revealed valuable information on the finer structure of the sex chromatin body in human tissues. He found that at high magnifications, the sex chromatin could be resolved into two components which formed a bipartite or diplococcus structure. The total size of the two masses was in the order of that described for the

single sex chromatin mass. These observations strengthen the theory that the sex chromatin is derived from the close association or fusion of portions of the XX chromosomes. The bipartite form was more frequently observed in embryonic membranes; this may possibly be due to the fact that these tissues do not need embedding or sectioning. The sex chromatin was sometimes found to adopt a spiral-like form. Vacuoles were sometimes seen in the sex chromatin suggesting the association of a small nucleolus; this may be homologous with the nucleolus formed by the sex chromosomes in man (Schultz and St. Lawrence, 1949).

The origin of sex chromatin from a single X-chromosome has been suggested. Evidence for this is presented in a recent communication from Grumbach et al. (1959). They report a case of gonadal dysgenesis in which the interphase nuclei contained sex chromatin but chromosome studies revealed an XO sex chromosome constitution. Sex chromatin has also been demonstrated in somatic nuclei of the female domestic chicken, the heterogametic sex (XO or XY), but is not observed in the male (XX) (Kosin and Ishizaki, 1959). Ashley and Theiss (1959) were unable to confirm this observation.

A relationship between cell metabolism and the position of the sex chromatin was shown by Barr and Bertram (1951) and Crouch and Barr (1954). The sex chromatin, usually located adjacent to the nucleolus in cat neurones,

enlarged slightly and moved toward the nuclear membrane during axon reaction of hypoglossal neurones. As the cell recovered, the sex chromatin returned to its original position. Normally the sex chromatin in male neurones is too small to permit accurate identification but it is seen more frequently in cells undergoing axon reaction suggesting a slight enlargement under these conditions.

Davidson and Smith (1954) observed a nuclear appendage in the form of a drumstick in the neutrophil leucocytes of females, its incidence being about 6 per 500 neutrophils; such a structure is absent in those of the male. This drumstick has a well-defined solid round head about 1.5 micra in diameter, and is joined to one lobe of the nucleus by a single fine chromatin thread. They were tempted to think that the drumstick represented the XX chromosome complex as was suggested for the sex chromatin in other tissues, however, discrepancies between the findings of this and other methods have resulted in the idea that this is a sex characteristic rather than an indication of sex chromosome constitution (Ashley and Jones, 1958).

The observations on sex chromatin in various species are very extensive and will be summarized in tabular form (Table I).

TABLE I

OCCURRENCE OF SEX CHROMATIN IN VARIOUS SPECIES

Species	Sex Difference	Tissues*	Reference
		Amphibia	
frog	absent	spinal cord & mesencephalon	Brum et al. (1959)
		Reptilia	
snakes	absent	non-nervous	Ashley & Theiss (1959)
		Aves	
domestic fowl	present	duodenum & feathers	Kosin & Ishizaki (1959)
	absent	non-nervous	Ashley & Theiss (1959)
pigeon	absent	nervous system	Brusa (1952)
ducks	absent	non-nervous	Ashley & Theiss (1959)
parrots and parakeets	absent	non-nervous	Ashley & Theiss (1959)
		Mammalia	
Marsupialia			
opossum	present	nervous & non-nervous	Graham (1956)
	present	liver	Brum et al. (1959)
	absent	skin & intestine	Brum et al. (1959)
Artiodactyla			
cattle	present	nervous system	Moore, Graham & Barr (1957)
	present	liver, pancreas & adrenal	Lang & Hansel (1959)
	absent	non-nervous	Moore, Graham & Barr (1957)
	absent F	epithelial tissues	Sachs & Danon (1956)
pig	present	nervous system	Moore & Alyede (1958), Cantwell et al. (1958)
	absent	non-nervous	Moore & Alyede (1958), Cantwell et al. (1958)

*Unless otherwise indicated, the tissues examined were from mature animals.
Those preceded by "F" indicate fetal tissues.
Those preceded by "E" indicate embryonic tissues.

TABLE I (continued)

Species	Sex Difference	Tissues	Reference
		Mammalia (continued)	
Artiodactyla (continued)			
sheep	absent	epithelial tissues	Sachs & Danon (1956)
goat	present	nervous system	Moore & Barr (1953)
deer	present	nervous system	Moore & Barr (1953)
Lagomorpha			
rabbit	absent	nervous system	Moore & Barr (1953)
	present	neutrophils	Lüers (1956), Carpentier et al. (1957)
Rodentia			
rat	present	spinal cord neurones	Klinger (1952)
	present	ameloblasts	Castro et al. (1956)
	present	liver	Reitalu (1958), Ohno et al. (1959)
	absent	spinal cord neurones	Coïdan (1951)
	absent	nervous system	Moore & Barr (1953)
	absent	neutrophils	Lüers & Lüers (1958)
guinea pig	absent	nervous system	Moore & Barr (1953)
	absent	neutrophils	Krueger & Dihlmann (1957)
hamster	absent	nervous system	Moore & Barr (1953)
	absent	liver, pancreas, smooth muscle & adrenal	Walsh (1955)
	present	spinal cord neurones	Walsh (1955)
ground hog	absent	nervous system	Moore & Barr (1953)
albino mouse	absent	spinal cord neurones	Coïdan (1951)
cotton rats (castrate & non-castrate)	absent	spinal cord neurones	Coïdan (1951)
voles (castrate & non-castrate)	absent	spinal cord neurones	Coïdan (1951)
white footed jumping mouse	absent	spinal cord neurones	Coïdan (1951)
mouse	absent	nervous system	Moore & Barr (1953)
house mouse	absent	neutrophils	Lüers & Lüers (1958)

TABLE I (continued)

Species	Sex Difference	Tissues	Reference
		Mammalia (continued)	
Carnivora			
mink	present	nervous system	Moore & Barr (1953)
marten	present	nervous system	Moore & Barr (1953)
ferret	present	nervous system	Moore & Barr (1953)
raccoon	present	nervous system	Moore & Barr (1953)
skunk	present	nervous system	Moore & Barr (1953)
red fox	present	nervous & non-nervous	Moore & Aiyede (1958)
black bear	present	nervous & non-nervous	Moore & Aiyede (1958)
timber wolf	present	nervous & non-nervous	Moore & Aiyede (1958)
coyote	present	nervous & non-nervous	Moore & Aiyede (1958)
dog	present	nervous system	Moore & Barr (1953)
	present	neutrophils & eosinophils	Porter (1957)
cat	present	hypoglossal neurones	Barr & Bertram (1949, 1951), Crouch & Barr (1954)
	present	nervous system	Barr et al. (1950), Brusa (1952)
	present	nervous & non-nervous	Graham (1954b)
	present	non-nervous	Graham & Barr (1952)
	present	neuroglia	Barr (1951)
	present E	embryonic membrane	Graham (1954a)
	present E	nervous & non-nervous	Graham (1954b)
	present E	embryonic tissues in the 2nd week	Austin & Amoroso (1957)
	absent	liver & pancreas	Graham & Barr (1952)
Primates			
monkey	present	nervous & non-nervous	Prince et al. (1955)
	present	neutrophils	Girod (1958)
	present E	occasionally in trophoblast at 10th day	Park (1957)
	present E	embryo proper from 19th day	Park (1957)
	absent	spinal cord neurones	Coidan (1951)

TABLE I (continued)

Species	Sex Difference	Tissues	Reference
		Mammalia (continued)	
Primates (continued)			
man	present	non-nervous	Moore & Barr (1954)
	present	gingiva	Marwah & Weinmann (1955)
	present	ocular tissues	Pedlar & Ashton (1955)
	present	epidermis	Moore, Graham & Barr (1953), Emery & McMillan (1954)
	present	urinary sediment cells	Castro et al. (1957)
	present	oral mucosa cells	Moore & Barr (1955a), Marberger et al. (1955), Greenblatt et al. (1956)
	present	frontal cortex & sympathetic ganglia	Rylle & Graham (1954)
	present	neutrophils	Davidson & Smith (1954), Briggs & Kupperman (1956), Läers (1956)
	present	peripheral lymphocytes	Riis (1957)
	present	neutrophils & eosinophils	Riis (1955), Tenczar & Streitmatter (1956)
	present E	heart mesenchyme & adrenal	Witschi (1957)
	present E	at 12th day in trophoblast	Park (1957)
	present E	at 16th day in embryo	Park (1957)
	present FE	trophoblast	Glenister (1956)
	present F	cells in amniotic fluid	Serr, Sachs & Danon (1955), Makowski et al. (1956), Sachs, Serr & Danon (1956)
	present F	fetal portions of the placenta	Klinger (1957b)
	present F	chorionic villi	Stevenson & McClarin (1957)
	present F	umbilical cord, fetal membranes & placenta	Sohval et al. (1959)
	absent	spinal cord neurones	Coidan (1951)
	absent	Purkinje cells	Barr et al. (1950)

3. Applications of Sex Chromatin

a) Intersex states and sexual anomalies. Sex chromatin has become a valuable aid in the diagnosis of sexual anomalies and intersex states, and has augmented understanding of the biology of these conditions. The chromosomal sex of an individual is determined at fertilization; however, genetic or hormonal balance may produce an individual whose phenotypic sex is in disagreement with the chromosomal sex.

In the past, the only method by which chromosomal sex could be determined was by examination of the chromosomes of dividing germ cells or somatic cells in tissue culture. These methods necessitate considerable skill and experience. Development of the skin biopsy technique by Moore, Graham and Barr (1953) provided a simple and accurate method of determining chromosomal sex. The sex chromatin present in female nuclei is believed to be derived from the XX chromosomes thus indicating female chromosomal sex. These nuclei are termed chromatin positive. A comparable mass is not formed in nuclei of the male, and these chromatin negative nuclei indicate male chromosomal sex. The oral smear method of Moore and Barr (1955a) is now more widely advocated in view of its simpler procedure. The intersex conditions in which chromatin tests have proved of value will be described briefly.

The chromatin test is most valuable in differentiating male and female pseudohermaphroditism. In all cases of female

pseudohermaphroditism the nuclei are chromatin positive and the gonads are ovaries. The majority of these cases are due to hyperplasia of the fetal adrenal cortex. The hormonal imbalance results in varying degrees of masculinization, and an electrolyte imbalance may also be present. Masculinization of the female fetus has resulted in some cases where certain hormones have been administered to the mother in the course of pregnancy (Wellhaus, 1958; Wilkins et al., 1958; Bongiovanni et al., 1959). Few cases of female pseudohermaphroditism are of non-adrenal origin. Reconstructive surgery and hormonal therapy may enable these individuals to function as females. Male pseudohermaphrodites are individuals with testes, but the remainder of the reproductive system is of an intersexual nature. The nuclei are always chromatin negative. The testes are usually undescended and at puberty may produce estrogens that will increase feminization, or androgens may be produced and result in masculinization. In cases where male or female pseudohermaphroditism is suggested but cannot be established by chromatin and other tests, the possibility of true hermaphroditism must be considered. True hermaphrodites are rare. Both ovarian and testicular tissue are present, with varying morphology of the internal and external genitalia. As one would expect, these individuals may have either chromatin positive or chromatin negative nuclei and histological examination of the gonads is necessary

to confirm the diagnosis.

Chromatin tests have proved valuable in confirming or suggesting the diagnosis of gonadal dysgenesis. About 80% of phenotypically female patients with gonadal dysgenesis have chromatin negative nuclei. Jost (1950) showed that early removal of the gonads in rabbit fetuses resulted in feminization of the internal and external genitalia. It would appear, therefore, that functional testes are necessary to counteract the tendency of the fetus to feminize. The finding of chromatin positive nuclei does not exclude a diagnosis of gonadal dysgenesis since some patients proven to have this condition are chromosomal as well as anatomical females.

Some phenotypically male patients with seminiferous tubule dysgenesis have chromatin positive nuclei. These patients are usually considered sterile, but if such a chromatin positive individual were fertile, all the offspring should be females as only X-type spermatozoa could likely develop. The diagnosis of this condition is not necessarily excluded by the presence of chromatin negative nuclei.

An excellent review of the literature in this field is found in Grumbach and Barr (1958).

The presence or absence of drumsticks in neutrophil leucocytes is also used in clinical diagnosis. However, discrepancies between the results obtained from blood smears

and those of oral smears and skin biopsies have been reported (Plunkett and Barr, 1956; Ashley and Jones, 1958). It is now thought that the drumstick of female neutrophils is a sex characteristic rather than an indication of chromosomal sex. A study by Caratzali, Phelps and Turpin (1957) showed that the frequency of drumsticks in female blood smears varies with the stages of the menstrual cycle. This seems to indicate that this structure is susceptible to hormonal influence and this may be the cause of the differing results of the various methods.

b) Benign and malignant tumours

i. Benign tumours. Benign tumours, hyperplastic and hypertrophic tissues, inflammations, and squamous metaplasia have been studied and were shown to have a nuclear sex in agreement with that of the host (Moore and Barr, 1955b; Sohval and Gaines, 1955).

ii. Malignant tumours. Moore and Barr (1957) in their study of malignant tumours from female patients found typical female nuclear morphology present. The frequency of sex chromatin was higher in well differentiated tumours, being lower in those that were poorly differentiated or undifferentiated. Sohval and Gaines (1955) recorded similar results. In a study of mammary carcinoma, Kimel (1957) found the incidence of sex chromatin was lower than in normal

tissues. He also found evidence that high count tumours are more likely to metastasize than those of low count.

With the exception of teratomas, nuclear sex in agreement with that of the host was found in malignant tumours from male patients (Sohval and Gaines, 1955; Moore and Barr, 1957). Weinmann et al. (1955) found sex-chromatin-like particles in nuclei of basal cell carcinoma of eleven male patients, its incidence being about half that found in a corresponding series from females. They suggested that this represented an alteration in the chromatin related to the development of the cancer rather than a sex reversal. Study of basal cell carcinomata by Moore and Barr (1957) and Ashley (1958) showed no discrepancy between the nuclear sex and that of the host.

Montenegro-Ortíz and Silva-Inzunza (1958) have used the incidence of sex chromatin in tumour cells as a means of determining the type of hormonal therapy which should be used. They maintain that the nuclear sex of a tumour falls into three groups: it agrees with that of the host, it is contra-sex, or it is intersex. Using hormones opposite to the sex indicated by the tumour and a mixture of hormones in the case of intersex tumours, they claim that improvements were noted. They stated, however, that it was necessary to recheck the incidence of sex chromatin at intervals as prolonged hormonal therapy altered the sex chromatin pattern. This fact is incongruous with the results of other studies in which it has

been shown that the presence or incidence of sex chromatin is not affected by hormonal influence.

iii. Teratomas. Investigations on teratomas have revealed that in those from females, the nuclear sex and that of the host are always identical, whereas in teratomas from males, a female nuclear sex pattern may be present (Hunter and Lennox, 1954; Tavares, 1955; Cruickshank, 1955; Moore and Barr, 1955b, 1957). Combining the results of these authors, about 48% of these tumours in the male exhibit host-teratoma heterosexuality. Hunter and Lennox explain this by suggesting the possible fusion of two gametes or two haploid cells, whereas Tavares postulates that these female tumours in the male be due to the parthenogenetic division of haploid cells followed by reduplication of the chromosomes.

c) Other applications. Prenatal diagnosis of sex has long been a source of scientific and non-scientific speculation. An accurate method is now available whereby cells present in aspirations of amniotic fluid are examined for the presence of sex chromatin. These cells are of fetal origin and hence one may determine the chromosomal sex of the unborn child (Serr, Sachs and Danon, 1955; Makowski et al. 1956). This application of sex chromatin has more theoretical than practical value as a certain amount of danger is involved and the procedure is not advisable unless it is necessary to

obtain a sample of amniotic fluid for other purposes such as antigen studies.

Sex chromatin studies have been undertaken on individuals with psychosexual disorders. Barr and Hobbs (1954) found typical male nuclear morphology in a series of five male transvestites. Pare (1956) and Haboch and Nedoma (1958) investigated the chromosomal sex of homosexual men and found no discrepancies between the nuclear and anatomical sex. These findings seem to exclude an abnormal sex chromosome constitution as a basis for these conditions, but a genic aetiology may be possible. Studies on male and female schizophrenics (Lasersohn and Rowland, 1958) showed the nuclear sex to be consistent with the anatomical sex. From the results of these investigations, it would appear that the psychological and behavioural aspects of these conditions are not related to the sex chromatin pattern.

A preponderance of female anencephalics is born at or near term. In view of the wide difference between the theoretical and obtained sex ratios, sex chromatin studies have been done on several series of anencephalics to determine whether a sex reversal in the female direction results in the greater number of females born. All specimens studied showed a nuclear sex in agreement with the morphological findings of the internal and external genitalia (Polani and Claireaux, 1957; Perrin and Benirschke, 1958; Bearn, 1959;

Polani, 1959). It is possible that there is an early selective loss by miscarriage of the male anencephalic embryos.

Acardiac monsters in twin pregnancies are thought to occur only in cases of single ovum twinning. This hypothesis has been strengthened by the results of a study on five specimens of holoacardii amorphi, a type of acardiac monster, which showed a nuclear sex corresponding to that of the normal twin (Benirschke, 1959).

A recent application for sex chromatin has been found in transplantation experiments. For example, a piece of tissue from a female donor is transplanted into a male host. By subsequent examination for the presence of sex chromatin, one can determine the degree of replacement by host tissues or the proliferation and survival of the donated tissues. These studies have been undertaken on transplants of skin (Peer et al., 1957), cartilage (Peer, 1958), and cornea (Basu and Ormsby, 1959).

CHAPTER III

MATERIALS AND METHODS

Tissues from seven mammalian species were examined in this study. One male and one female of each species was obtained. The animals were purchased through animal supply houses or received from other sources as noted in the acknowledgements.

1. Obtaining the Tissues

a) Sacrifice. Small animals such as the prairie dog, were given an overdose of ether. Larger animals such as the timber wolf, were given a lethal dose of Nembutal (Abbot) intraperitoneally.

b) Fixation. The non-nervous tissues were fixed in modified Davidson's solution. Nervous system tissues were fixed in 10% formalin. Fixation was continued for a minimum of 24 hours; the tissues were then transferred to 70% ethyl alcohol.

2. Preparation of the Tissues for Study

a) Embedding and sectioning. Following dehydration in graded alcohols (70%, 95%, absolute), the tissue blocks were cleared in xylol and embedded in paraffin. Sections of the non-nervous tissues were cut at 5μ. Nervous tissues were

sectioned at 10μ.

b) Staining techniques. Non-nervous tissues were stained with haematoxylin (Harris's) and eosin and by the Feulgen method. Cresyl echt violet and Feulgen stains were used for the nervous tissue sections.

The procedures in the various staining methods are as follows.

1. Haematoxylin and eosin: (H & E)

1. remove paraffin in xylol
2. hydrate in graded alcohols (absolute, 95%, 70%)
3. running tap water--5 minutes
4. haematoxylin--10 minutes
5. tap water until blue--5 minutes
6. acid alcohol--4 to 5 seconds
7. running tap water--15 to 20 minutes
8. eosin--15 seconds
9. wash excess eosin from slides
10. dehydrate in graded alcohols (70%, 95%, absolute)
11. clear in xylol
12. mount in picolyte

The sex chromatin stains deeply with the haematoxylin and is distinguished by its morphology from other chromatin material present in the nucleus. Other nuclear constituents are shown by the eosin.

11. Cresyl echt violet: (CEV)

1. remove paraffin in xylol
2. hydrate in graded alcohols (absolute, 95%, 70%)
3. distilled water--5 minutes
4. cresyl echt violet--5 minutes
5. dehydrate in graded alcohols (70%, 95%, absolute)
6. clear in xylol
7. mount in picolyte

The nucleolus and Nissl substance stain a light blue. The chromatin is stained a dark blue and the sex chromatin, when present, is readily distinguished.

iii. Feulgen technique:

1. remove paraffin in xylol
2. hydrate in graded alcohols (absolute, 95%, 70%)
3. distilled water--5 minutes
4. rinse in cold N HCl--1 minute
5. hydrolyze in N HCl for 20 minutes in oven at 50°C
6. rinse in cold N HCl--1 minute
7. stain in fuchsin sulphurous acid reagent--2 hours
8. drain and pass quickly to the first of 3 closed coplin jars containing acid bleaching

solution. Leave 10 minutes in each jar

9. rinse in tap water--5 minutes
10. rinse in distilled water--5 minutes
11. counterstain in 0.05% aqueous solution of Light Green--10 seconds
12. dehydrate in graded alcohols (70%, 95%, absolute)
13. clear in xylol
14. mount in picolyte

This stain is specific for DNA; the chromatin is stained a purple or violet colour. The nucleolus and cytoplasm are not stained but are shown by the Light Green counterstain.

3. Examination of Tissues

Nuclei of the cell types listed below were examined in detail.

a) Non-nervous tissues: unless otherwise stated, the parenchymatous cells of the tissue were studied.

adrenal cortex
adrenal medulla
thyroid (follicle cells)
kidney (convoluted tubules)
spleen (large lymphocytes)
liver
pancreas (acinar cells)
pancreatic islets
duodenum (surface epithelium)
stomach (surface epithelium)
urinary bladder (surface epithelium)
pars distalis of the pituitary

epidermis
cartilage
skeletal muscle
cardiac muscle
smooth muscle of the stomach or duodenum
testes (interstitial cells)
prostate (glandular epithelium)
ovary (follicle cells and stromal cells)
uterus (glandular epithelium)

b) Nervous system tissues:

stellate ganglion cells
dorsal root ganglion cells
anterior horn cells of the spinal cord
Betz cells of the motor cortex
neurones of the hypoglossal nucleus
Purkinje cells of the cerebellum

For each of the above cell types, a minimum of 200 nuclei were examined with an oil immersion objective for the presence of sex chromatin. For each of the non-nervous tissues, 100 nuclei were studied using the haematoxylin and eosin method. One hundred nuclei of each region of the nervous system were studied using the cresyl echt violet method. Feulgen preparations of both types of tissue were examined in order to determine the type of nucleic acid present, 100 nuclei of each cell type being studied.

When sex chromatin was present, its position was recorded as adjacent to the nucleolus, free in the nucleoplasm, or adjacent to the nuclear membrane. The chromatin distribution

was also recorded for those species in which no sex difference was observed.

CHAPTER IV

OBSERVATIONS

1. Carnivora

a) Timber wolf (Canis nubilis)

1. Nervous system tissues.

Female (Figs. 1, 3, 5)

The neurones of six regions of the nervous system showed a nuclear sex difference consistent with that previously reported for other carnivores (see Table I). The incidence and position of the sex chromatin are recorded in Table II.

The sex chromatin of the female is a nuclear component approximately one micron in diameter, its size being relatively constant for the several cell types. The sex chromatin is most frequently found adjacent to the nucleolus (Fig. 3). In this position, it appears as a swelling on the nucleolus or a cap-like structure, and is distinguishable from the nucleolus only by its differential staining. Sex chromatin stains a darker blue than the nucleolus with cresyl echt violet and is Feulgen positive in contrast to the nucleolus which is Feulgen negative. When the sex chromatin is free in the nucleoplasm, it may be spherical (Fig. 5) or irregular in outline. Located adjacent to the nuclear membrane, the sex chromatin presents a planoconvex or triangular outline (Fig. 1);

TABLE II

TIMBER WOLF - INCIDENCE AND POSITION OF SEX CHROMATIN (NERVOUS SYSTEM TISSUES)

Region of Nervous System	Sex	POSITION OF SEX CHROMATIN (%) Adjacent to nucleolus	Free in nucleo-plasm	Adjacent to nuclear membrane	Total (%)
Dorsal Root Ganglion	F	76	9	7	92
	M	2	1	2	5
Stellate Ganglion	F	80	8	6	94
	M	3	1	1	5
Anterior Horn Cells	F	63	11	16	90
	M	3	2	3	8
Motor Cortex	F	52	7	35	94
	M	4	1	3	8
Hypoglossal Neurones	F	69	14	11	94
	M	2	1	1	4
Purkinje Cells	F	84	4	6	94
	M	5	1	3	9

occasionally it is spherical.

Several nucleoli are present in dorsal root ganglion cell nuclei, but only one sex-influenced body is observed. In many nuclei the sex chromatin is very slightly removed from the nucleolus but is not exactly free in the nucleoplasm; the position of the sex chromatin in such cases was recorded

as being adjacent to the nucleolus. Feulgen staining reveals a small chromatin particle at one nucleolus, possibly related to the nucleolar chromosomes, while the sex chromatin is located at another nucleolus.

The sex chromatin is located adjacent to the nuclear membrane in 35% of neurones of the motor cortex. In 12% of these nuclei, the nucleolus is eccentric in position and the sex chromatin is situated between the nucleolus and the nuclear membrane.

The nucleolus and Nissl substance are Feulgen negative and are shown by the counterstain. Sex chromatin and other chromatin particles are Feulgen positive; this cytochemical reaction indicates that they are composed of DNA.

Male (Figs. 2, 4, 6)

Small chromatin particles are seen in the nuclei of male tissues, but seldom does one encounter a structure comparable in size to the sex chromatin of female cells. It is easier to distinguish the male sex chromatin from unrelated chromatin by the Feulgen method. It is believed that all nuclei of the male contain sex chromatin, but in most cells it is too small to permit accurate identification with standard optical equipment. Thus, the data for the male in Table II are only approximate.

Theoretically, one would expect the male (XY) sex chromatin to be one-half the size of the female (XX) sex

chromatin. The opossum is the only animal in which this has been found to occur (Graham, 1956). In other species, the sex chromatin of the male appears to be smaller than predicted. This will be referred to in the Discussion.

ii. Non-nervous tissues.

Female (Figs. 7, 9, 11)

A nuclear sex difference was observed in all non-nervous tissues examined. The incidence of the sex chromatin is recorded in Table III. The sex chromatin is consistently found as a planoconvex or triangular mass adjacent to the nuclear membrane (Figs. 7, 9, 11). In haematoxylin and eosin sections, the sex chromatin can be distinguished from other chromatin particles by its morphological characteristics. Feulgen preparations show that it is Feulgen positive and therefore composed of DNA. Chromatin associated with the nucleolus is small in volume and probably represents segments of the nucleolar chromosomes.

Cells of the adrenal cortex and medulla, cartilage, and pancreatic islets are particularly favourable for study as the general chromatin of nuclei of these tissues is in the form of fine particles. Nuclei of pancreatic acini, liver and kidney are less suitable as the chromatin particles are large and coarse. In cells of the gastric and duodenal mucosa, the nuclei frequently show a nucleolus-associated chromatin mass slightly smaller than the sex chromatin which is located at

TABLE III

TIMBER WOLF - INCIDENCE OF SEX CHROMATIN
(NON-NERVOUS TISSUES)

Tissue	INCIDENCE OF SEX CHROMATIN (%)	
	Female	Male
adrenal cortex	77	7
adrenal medulla	72	8
pancreatic islets	72	9
pancreatic acini	73	10
liver	72	6
kidney (convoluted tubules)	68	8
stomach (surface epithelium)	72	8
duodenum (surface epithelium)	75	9
urinary bladder (surface epithelium)	74	6
epidermis	71	7
cartilage	72	6
spleen (large lymphocytes)	67	10
pars distalis of the pituitary	66	9
cardiac muscle	72	8
skeletal muscle	71	9
smooth muscle (duodenum)	76	6
uterus (glandular epithelium)	74	-
prostate (glandular epithelium)	-	9
testis (interstitial cells)	-	6

the nuclear membrane.

Muscle nuclei are relatively good for study provided they are not too contracted; in this condition, one must exercise care in distinguishing between sex chromatin and shrinkage artifacts. In skeletal and smooth muscle nuclei the sex chromatin is adjacent to the nuclear membrane at the side of the nucleus (Fig. 9). It is polar in position in most cardiac muscle nuclei (Fig. 11). Many nuclei show the sex chromatin lying in the curve of the nucleus, being neither definitely polar nor on the side.

Male (Figs. 8, 10, 12)

The smaller size of nuclei of non-nervous tissues makes it difficult to distinguish the male sex chromatin from unrelated chromatin material. Therefore, data for the male in Table III are only approximate. Feulgen staining is a valuable aid in the identification of sex chromatin in male tissues. The observations regarding the suitability of various tissues for nuclear sexing in the female, also apply to those of the male.

b) Skunk (Mephitis mephitis)

Moore and Barr (1953) found nuclear sexual dimorphism present in five regions of the nervous system of this species. Only non-nervous tissues were examined in this study. With respect to sex characteristics of the nuclei, the tissues of the skunk can be classified into four groups.

1. Sex difference present

Female (Fig. 13)

A sex difference in nuclear morphology was observed in cells of the adrenal cortex and medulla, urinary bladder epithelium, cardiac and smooth muscle. The incidence of sex chromatin in the female is recorded in Table IV. The sex chromatin is approximately one micron in diameter and usually appears as a triangular or planoconvex mass adjacent to the nuclear membrane (Fig. 13); seldom is it found in other positions. It is composed of DNA as shown by the Feulgen technique.

The sex chromatin is polar in about two-thirds of smooth muscle nuclei. Nuclei of the cardiac muscle were exceedingly contracted and not suitable for study and this possibly accounts for the low incidence found in these cells; the sex chromatin is frequently polar in position.

In cells of the adrenal cortex, the sex chromatin is located adjacent to the nucleolus in 15% of the nuclei. When the nucleolus is eccentric in position, the sex chromatin may be associated with both the nucleolus and the nuclear membrane. This is also a feature of nuclei of the adrenal medulla.

Male (Fig. 14)

Observations on the sex chromatin of the male skunk are similar to those previously recorded for the male timber wolf. The sex chromatin, when present, is usually located

TABLE IV

SKUNK - INCIDENCE OF SEX CHROMATIN
(NON-NERVOUS TISSUES)

Tissue	INCIDENCE OF SEX CHROMATIN (%)	
	Female	Male
adrenal cortex	65	6
adrenal medulla	64	17
urinary bladder (surface epithelium)	65	9
cardiac muscle	45	8
smooth muscle (stomach)	77	5

adjacent to the nuclear membrane, and is seldom found in other positions.

In the male, the sex chromatin is generally located at the side of the nucleus in cardiac and smooth muscle cells. Thickenings of the nuclear membrane in adrenal medulla nuclei produce the high incidence recorded for this tissue in Table IV. These thickenings are Feulgen positive, and give the impression that the sex chromatin is exceedingly flattened against the nuclear membrane.

2. No distinct sex difference present (Figs. 15,16)

No distinct nuclear sex difference was observed in nuclei of epidermis, skeletal muscle, duodenal mucosa, and

large lymphocytes of the spleen.

In 63% of epidermal nuclei of the female, one or more particles resembling sex chromatin are present (Fig. 15) although their morphology is not always as typical as in the tissues of the first group. Similar particles are seen in 43% of male nuclei (Fig. 16). One to three chromatin particles are present in 69% of female skeletal muscle nuclei. Cells of the male show one chromatin mass, sometimes several, in 23% of these nuclei; the chromatin is most frequently located adjacent to the nuclear membrane. Female duodenal mucosal cells show one or more particles resembling sex chromatin in 64% of the nuclei; these particles are generally located at the nuclear membrane. These observations apply to 45% of these cells in the male. A similar pattern is found in 68% of the large lymphocytes in the female, and in 26% in the male.

These tissues are not practically suitable for nuclear sexing. A random assortment of slides cannot be accurately sorted according to sex. Typical sex chromatin is not a constant feature of either sex, and multiple chromatin particles make its accurate identification difficult.

3. No sex difference present (Figs. 17, 18)

No sex difference in nuclear morphology was observed in cells of pancreatic islets and acini, liver, renal convoluted tubules, cartilage, and the pars distalis of the

pituitary. With the exception of the islet cells, multiple chromatin particles of varying size and position mask any sex difference that may be present in these tissues. In some nuclei, a more prominent particle is seen, but it is not a constant feature and does not appear to be related to the sex of the animal. Nuclei of pancreatic islet cells are generally devoid of chromatin. One or more particles resembling sex chromatin are present in 14% of these nuclei of the female, and 11% of those of the male.

4. Specific male and female tissues (Figs. 19, 20)

Sex chromatin is found adjacent to the nuclear membrane in 45% of nuclei of the uterine glands of the female skunk. One or more particles resembling sex chromatin are seen in varying positions in 78% of the follicle cells of the ovary (Fig. 19). In ovarian stromal cells, the sex chromatin is located adjacent to the nuclear membrane in 38% of the nuclei, free in the nucleoplasm in 3% and adjacent to the nucleolus in 15%. The sex chromatin is more readily discernable with the Feulgen technique. In haematoxylin and eosin sections, unrelated nuclear material makes it difficult to accurately identify the sex chromatin.

Interstitial cells of the testes of the male skunk show a particle with the characteristics of sex chromatin in 68% of the nuclei; it is located adjacent to the nuclear membrane. In 17% of the cells, two such structures are

observed (Fig. 20), and as many as four may be present. The possibility of the animal being a natural case of sex reversal was excluded on the basis of the sex difference in the tissues of the first group. Moore and Barr (1953) showed a sex difference to be present in the sympathetic ganglion cells of this species; in this animal, no sex chromatin was observed in parasympathetic ganglion cells of the gastro-intestinal tract.

2. Artiodactyla

a) Pig (Sus scofra)

i. Nervous system tissues

Female (Fig. 21)

Neurones of the pig showed a consistent sex difference in nuclear morphology similar to that observed in the timber wolf. The incidence and position of the sex chromatin is shown in Table V. Additional chromatin particles are present in most nuclei, but the sex chromatin is usually easily recognized by its morphology. Its characteristics are similar to those described for the timber wolf.

Multiple nucleoli are present in cells of the stellate and dorsal root ganglion; only one sex-influenced body is present. It is located adjacent to the nuclear membrane in 70% of stellate ganglion cell nuclei, whereas it is almost equally distributed at the nuclear membrane and adjacent to

TABLE V

PIG - INCIDENCE AND POSITION OF SEX CHROMATIN
(NERVOUS SYSTEM TISSUES)

Region of Nervous System	Sex	POSITION OF SEX CHROMATIN (%) Adjacent to nucleolus	Free in nucleo-plasm	Adjacent to nuclear membrane	Total (%)
Dorsal Root Ganglion	F	46	2	41	89
	M	1	0	1	2
Stellate Ganglion	F	21	1	70	92
	M	1	0	3	4
Anterior Horn Cells	F	78	11	4	93
	M	5	1	0	6
Motor Cortex	F	54	1	37	92
	M	5	0	2	7
Hypoglossal Neurones	F	87	3	5	95
	M	1	1	1	3
Purkinje Cells	F	74	3	13	90
	M	15	1	1	17

the nucleolus in dorsal root ganglion cells. The sex chromatin is located adjacent to the nucleolus in the majority of Purkinje and anterior horn cells (Fig. 21) and hypoglossal neurones. Chromatin particles larger than the general chromatin but a little smaller than the sex chromatin are frequently seen in the Purkinje cells.

Male (Fig. 22)

Nuclei of the nervous tissues of the male pig seldom

contain a structure comparable to the female sex chromatin. The frequent presence of unrelated chromatin particles in this species makes identification of the sex chromatin in the male difficult, and hence the results for the male in Table V are only approximate.

The Purkinje cells of the cerebellum show a chromatin mass similar to sex chromatin in 17% of the nuclei; it is usually located adjacent to the nucleolus. Other smaller particles are also present in this position in the nuclei of both sexes; this tissue was found less suitable for nuclear sexing than other regions of the nervous system. A sex difference is present, but careful observation is necessary to distinguish the sex chromatin from larger particles of unrelated chromatin.

ii. Non-nervous tissues (Figs. 23, 24, 25, 26)

No sex difference in nuclear morphology was observed in cells of non-nervous tissues of this species. Nuclei of both the male and female show multiple clumps of chromatin. The chromatin pattern of pancreatic islet cells was not determined as a sufficient number of cells could not be found.

A polar chromatin mass is more frequently observed in cardiac muscle nuclei of the female, but other chromatin particles resembling sex chromatin are found at the nucleolus, free in the nucleoplasm, and at the sides of the nucleus in both sexes.

In some nuclei, a particle with the characteristics of sex chromatin is observed; however, this is not a constant feature of either sex. Probably one of these chromatin masses represents the sex chromatin, but it is impossible to distinguish it from other chromatin particles of similar morphology.

3. Rodentia

a) Mouse (Mus musculus) - (Figs. 27, 28, 29, 30)

In a study of four regions of the nervous system of this species, Moore and Barr (1953) observed two relatively large clumps of chromatin on opposite sides of the nucleolus in nuclei of the male and female. A nuclear sex difference was not present.

No sex difference in nuclear morphology was found in the non-nervous tissues examined in this study. Multiple chromatin particles, varying in size, position and number are present in nuclei of both sexes. Frequently they are associated with the nuclear membrane and/or nucleoli.

In certain tissues, adrenal medulla, gastric mucosa, thyroid, and urinary bladder epithelium, one receives the impression that one of the chromatin particles is more prominent in nuclei of the female. This is not a constant feature however, and several particles of similar size may appear equally prominent. It is not an indication of the

sex of the animal as this pattern can also be seen in nuclei of these tissues from the male.

b) Prairie dog (Cynomys ludovicianus)

1. Nervous system tissues (Figs. 31, 32, 33, 34, 35, 36)

A sex difference in nuclear morphology was not observed in neuronal nuclei of the prairie dog. With the exception of stellate ganglion nuclei, one or two large chromatin masses are generally present in association with the nucleolus (Figs. 31, 32). Other smaller masses may be present in this position and/or free in the nucleoplasm or adjacent to the nuclear membrane. One forms the impression that some of these large chromatin masses are derived from an aggregation of smaller chromatin particles, as their outline often suggests a berry-like structure. These large masses may assume the form of a cap on the nucleolus (Figs. 31, 32), a horseshoe-shaped structure, or a large sphere which partially overlaps the nucleolus.

In contrast to the above picture, nuclei of stellate ganglion cells contain one to three, seldom four, chromatin masses of varying size. These particles are considerably smaller than those observed in other regions of the nervous system. Multiple nucleoli are common, and often occur adjacent to the nuclear membrane. The chromatin is usually located in this position also and is frequently associated

with the nucleoli, as well as the nuclear membrane (Fig. 33, 34). A nucleolar cap of chromatin may also be present (Fig. 34).

Odd-shaped lighter-staining bodies may be encountered in neuronal nuclei of this species (Figs. 35, 36). The exact nature of these structures is not known. They are only slightly Feulgen positive in contrast to the rest of the chromatin material. Possibly they are traces of chromatin left in the path of migration of other chromatin masses, or they may represent a chemically altered form of chromatin.

ii. Non-nervous tissues (Figs. 37, 38, 39, 40, 41, 42)

Nuclei of non-nervous tissues of the prairie dog did not show a difference in nuclear morphology according to sex. Nuclei of most tissues contain multiple large chromatin masses (Figs. 39, 40). These are frequently adjacent to the nuclear membrane, but may vary in position. Variations in size and staining properties are also observed. A vacuole, or clear central area, is seen in some large chromatin masses. It is probable that some of these represent nucleoli with a very dense layer of perinucleolar chromatin; this is quite obvious in liver and skeletal muscle nuclei (Figs. 39, 40).

The nuclei of cells composing the upper layers of urinary bladder epithelium are almost devoid of chromatin; nuclei in the basal layers show multiple chromatin masses.

In some cells, such as those of the duodenal and gastric mucosa, adrenal cortex, and basal layers of the urinary bladder, the chromatin masses seem slightly smaller; it may be that variations in nuclear volume create this impression. Cells of the pars distalis of the pituitary generally contain only one large chromatin mass which is usually centrally located in the nucleus, but may be located adjacent to the nuclear membrane (Figs. 41, 42).

Muscle cell nuclei of this species present an interesting series of chromatin patterns. Smooth muscle nuclei are almost devoid of chromatin except for structures which appear as thickenings of the nuclear membrane (Figs. 37, 38). They tend to be small, but may vary in size and also in number. A larger particle is present in cardiac muscle nuclei; sometimes more than one is observed. In nuclei of the female, this particle is frequently polar in position; it is more prominent and has a higher incidence than in the male. This feature is not a reliable indication of the sex of the animal however, as a selection of unknown slides cannot be accurately sorted according to sex. Nuclei of skeletal muscle of both the male and female show the multiple large chromatin masses observed in other tissues (Figs. 39, 40).

Small sex-chromatin-like particles may be present in addition to the above chromatin patterns of large chromatin masses. They occur in tissues of the male and female, and do

not appear to be related to the sex of the animal.

c) Porcupine (Erethizon epixanthum)

Before enumerating the results obtained for this species, it is necessary to point out that the tissues of the female were considerably superior in their staining affinities to those of the male. Since the staining of male and female tissues was carried out simultaneously, it is possible that this difference arose during the process of fixation and/or embedding the tissues of the male.

1. Nervous system tissues (Figs. 43, 44, 45, 46, 47, 48)

With the exception of stellate ganglion cells, no morphological sex difference was observed in neuronal nuclei of the porcupine. Nuclei of anterior horn, Purkinje, and dorsal root ganglion cells of both sexes show one to four chromatin masses of varying size and position; there is frequently a larger chromatin mass adjacent to the nucleolus. Sometimes this large mass appears to have two components or consist of an aggregation of smaller chromatin particles. This nucleolus-associated chromatin mass appears to be of smaller volume in nuclei of dorsal root ganglion cells. Motor cortex neurones tend to show fewer chromatin particles, but in the majority, there is at least one nucleolus-associated chromatin mass (Figs. 43, 44). When the nucleolus is eccentric, this mass often lies between the nucleolus and

the nuclear membrane.

Nuclei of stellate ganglion cells show a morphological difference according to sex. In nuclei of the female, a chromatin mass occurs in 86% of the cells, and is located adjacent to the nucleolus in 10%, free in the nucleoplasm in 2%, and adjacent to the nuclear membrane in 74% (Figs. 45, 47). The sex chromatin, though not always well defined in outline, is usually easily distinguished from additional chromatin particles of similar morphology which are occasionally present. The characteristics and histochemistry of the sex chromatin are similar to those described for the timber wolf. In the male, a comparable structure is observed adjacent to the nucleolus in 6% of the cells and adjacent to the nuclear membrane in 9%. Seldom is it recognized in cresyl echt violet stained sections (Fig. 46), but is readily distinguished in Feulgen preparations (Fig. 48). With this procedure, two such chromatin masses are sometimes observed; one may be located adjacent to the nucleolus, the other at the nuclear membrane.

ii. Non-nervous tissues

Pancreatic islet cells are not included in the results as, in this species, they are not organized into distinct islets, and a sufficient number of cells could not be found on which to base an accurate observation.

The non-nervous tissues of the porcupine can be classified into four groups according to their nuclear sex

characteristics.

1. Sex difference present

In nuclei of adrenal cortex and medulla, smooth, skeletal and cardiac muscle, several chromatin particles are present; nucleolus-associated chromatin is common. In tissues from the female, however, one of these particles showed the characteristics of sex chromatin, whereas a comparable structure was absent in nuclei of the male.

Female (Figs. 49, 51)

The incidence of sex chromatin is shown in Table VI. Cells of the adrenal medulla show the sex chromatin adjacent to the nuclear membrane in most nuclei (Fig. 49). It is not as well defined in adrenal cortex cells and is found adjacent to the nucleolus in 12% of the nuclei, free in the nucleoplasm in 1%, and adjacent to the nuclear membrane in 51%.

The sex chromatin occurs in the polar position in the majority of cardiac muscle nuclei. Smooth and skeletal muscle nuclei show the sex chromatin at the side of the nucleus and at the pole, being slightly more frequent in the polar position (Fig. 51).

Male (Figs. 50, 52)

The incidences recorded for male tissues in Table VI are only approximate as it is difficult to distinguish the male sex chromatin from unrelated chromatin particles present

TABLE VI

PORCUPINE - INCIDENCE OF SEX CHROMATIN
(NON-NERVOUS TISSUES)

Tissue	INCIDENCE OF SEX CHROMATIN (%)	
	Female	Male
adrenal cortex	64	18
adrenal medulla	68	16
skeletal muscle	70	16
smooth muscle (duodenum)	77	11
cardiac muscle	69	6

in nuclei of this species. Even with Feulgen staining, differentiation is not always accurate. The position of the sex chromatin in the male is similar to that described for the female.

2. No distinct sex difference present (Figs. 53, 54)

Gastric and duodenal mucosa, epidermis, thyroid, cartilage, and large lymphocytes of the spleen, showed no clear cut sex difference in nuclear morphology. Nuclei of both sexes contain one or several chromatin particles, one of which is often larger. This particle sometimes seems to be more prominent in nuclei of the female, but it is not a constant feature and may be a result of the technical

variations previously described; a selection of unknown slides of these tissues cannot be accurately sorted according to sex.

3. No sex difference present (Figs. 55, 56)

No sex difference in nuclear morphology was observed in nuclei of liver, pancreatic acini, renal convoluted tubules, urinary bladder epithelium, and pars distalis of the pituitary. Nuclei of these tissues contain one to several chromatin masses of varying size and position. One of these masses often appears larger, but this is found in tissues of both the male and female. This mass is frequently adjacent to the nuclear membrane and may also be associated with the nucleolus when the latter is eccentric.

4. Specific male and female tissues

Intersitial cells of the testes, ovarian stromal and follicle cells and glandular epithelium of the uterus, contain one to several chromatin particles which vary in size and position. A larger particle similar to that observed in the tissues of group three, is often located adjacent to the nuclear membrane.

4. Edentata

a) Armadillo (Dasypus novemcinctus)

1. Nervous system tissues (Figs. 57, 58, 59, 60)

A distinct sex difference was not observed in the five regions of the nervous system studied.

Dorsal root ganglion cells of both sexes show irregularly outlined chromatin masses intimately associated with the nucleolus (Figs. 57, 58). Often they seem to be almost a part of the nucleolus, or they may appear as a slight swelling of the nucleolar surface. The chromatin in hypoglossal neurones is generally associated with the nucleolus; these chromatin masses vary in size and number. Other smaller chromatin granules are also present at the nucleolus, or they may occur in other positions.

Accurate chromatin patterns are difficult to determine in motor cortex neurones as the chromatin lies in several focal planes. Numerous small chromatin particles are found adjacent to the nucleolus, but may also be free in the nucleoplasm and there is often a particle adjacent to the nuclear membrane. One or more larger masses may be present at the nucleolus (Figs. 59, 60) and these are perhaps composed of an aggregation of smaller particles. These larger particles frequently seem to be more prominent in the female, but this is not a constant feature.

Purkinje cell nuclei of both sexes generally have several chromatin masses of varying size associated with the nucleolus. Chromatin particles of smaller dimensions are also found here or in other positions. The chromatin is not always well-defined in outline, and lies in several focal planes, hence it is hard to obtain a true picture of the

chromatin distribution.

One to three large chromatin masses occur in anterior horn cell nuclei; these are very closely applied to the nucleolus and seldom occur in other positions. Smaller particles may be observed adjacent to the nuclear membrane or free in the nucleoplasm and are often present at the nucleolus. Chromatin particles are, in general, slightly more frequent in these nuclei from the female.

The nucleoli of many cells are of an odd shape in contrast to their spherical nature as observed in other species. In some cases, these odd-shaped structures can be resolved into two very proximal nucleoli. The exact relationships of the chromatin are difficult to determine when the nucleoli occur in this form. Feulgen staining reveals little additional information on the chromatin patterns of this species. The outline of the chromatin tends to be irregular, and variations in the size and number of particles are observed as in the cresyl echt violet preparations.

ii. Non-nervous tissues (Figs. 61, 62, 63, 64, 65, 66)

A nuclear sex difference was not observed in the non-nervous tissues of the armadillo. Nucleolus-associated chromatin of varying volume is a relatively prominent feature in nuclei of most tissues of both sexes. Chromatin other than the nucleolar chromatin is also generally present, and

some variations in pattern are observed.

Nuclei of epidermis, pancreatic acini and islets, pars distalis of the pituitary, thyroid, kidney and duodenal mucosa contain multiple chromatin particles. In addition to the nucleolar chromatin, one to several particles resembling sex chromatin occur at the nuclear membrane.

The lymphatic tissue of the spleen is not organized into distinct nodules, differing in this respect from the other species studied. In large lymphocytes of this organ, the chromatin is generally associated with the nucleolus, but some nuclei show particles at the nuclear membrane. Nuclei of the undifferentiated chondrocytes contain one or more chromatin particles, whereas the mature cells often show only one large mass per nucleus. In gastric mucosa cells of the female, a particle resembling sex chromatin is found more frequently adjacent to the nuclear membrane than in the male, but the difference is not practical, as one cannot accurately sort a selection of unknown slides according to sex; other chromatin particles are also present.

Liver nuclei of both sexes tend to lack chromatin particles with the characteristics of sex chromatin (Figs. 63, 64). In the female, approximately 11% of the nuclei show sex-chromatin-like particles at the nuclear membrane, whereas they occur in 8% of nuclei of the male. With Feulgen staining these particles can be more readily recognized, but in

general, the chromatin material is indistinctly Feulgen positive. Other small chromatin particles are present at the nuclear membrane and nucleolus-associated chromatin is common. Nuclei of the upper layers of cells of urinary bladder epithelium tend to be devoid of chromatin. Particles resembling sex chromatin in these nuclei are more frequently found in the female than in the male, but there is no clear cut sex difference. In both sexes, nuclei of the basal layers contain one or more particles resembling sex chromatin.

Adrenal cortical nuclei tend to lack particles with the characteristics of sex chromatin. Sex-chromatin-like particles adjacent to the nuclear membrane are slightly more frequent in nuclei of the female, but no distinct sex difference is present. Nucleolus-associated chromatin is common to both sexes and when the nucleolus is adjacent to the nuclear membrane, it is difficult to determine the exact relationship of the chromatin. One to several chromatin particles, similar in morphology to sex chromatin, are present in the zona reticularis of both sexes, but appear to be more prominent in the male. The chromatin of adrenal medulla nuclei is coarse, and the incidence of sex-chromatin-like particles at the nuclear membrane is somewhat higher in both sexes than in nuclei of the adrenal cortex.

Smooth and cardiac muscle nuclei tend to lack chromatin structures resembling sex chromatin; nucleolus-associated

chromatin may be present, but it is less prominent than in some of the other tissues. Smooth muscle nuclei of the female were exceedingly contracted and the shrinkage artifacts resulted in some structures similar in appearance to sex chromatin; these appeared as indistinct masses of Feulgen positive material with the Feulgen technique. A polar chromatin mass with the characteristics of sex chromatin is more frequently observed in cardiac muscle nuclei of the female (Fig. 65) than in the male (Fig. 66), but this does not appear to be reliable indication of the sex of the animal. Nuclei of skeletal muscle cells of both sexes contain one to several chromatin masses; a polar particle may also be present. Nucleolus-associated chromatin of varying volume is again relatively prominent.

Nuclei of uterine gland epithelium contain several chromatin masses, one or more of which are adjacent to the nuclear membrane. Nuclear detail of the ovarian stromal cells was poor, but they appeared to contain multiple chromatin particles. Follicle cells of the ovary show multiple perinucleolar chromatin masses often resembling sex chromatin, and one or more masses of similar morphology are frequently present at the nuclear membrane. One or more chromatin masses are present at the nuclear membrane in nuclei of the glandular epithelium of the prostate, in addition to the prominent nucleolar chromatin. The chromatin of the

interstitial cells of the testes tends to be coarse. The nucleolus-associated chromatin is the most prominent feature, small chromatin masses sometimes occurring at the nuclear membrane; however, the chromatin of these cells seldom resembles sex chromatin in morphology.

CHAPTER V

DISCUSSION

There appears to be a phylogenetic trend in sex chromatin patterns, that is, members of the same order tend to show similar nuclear sex characteristics. In view of this feature, the observations recorded in this study will be correlated and compared with the observations on other mammalian species.

The presence of a consistent nuclear sex difference in tissues of the timber wolf is in accordance with observations on other members of the order Carnivora (see Table I). The sex chromatin present in nuclei of the female is believed to be derived from the fusion or close association of heterochromatic regions of the two X-chromosomes. One would therefore expect to find a chromatin mass one-half the size of the female sex chromatin in nuclei of the male which has XY chromosome constitution; this has been observed only in the opossum, a marsupial (Graham, 1956). Observations on the timber wolf and other carnivorous species show that the sex chromatin of the male is smaller than would be expected on this basis. A mass one-half the size of the female sex chromatin would lie at the limits of resolution of standard optical equipment; thus it is seldom

possible to identify, with confidence, sex chromatin in nuclei of the male. It may be that association with the Y-chromosome detracts from the heterochromatic properties of the X, whereas these properties are enhanced when associated with another X-chromosome. Sex chromatin is thought to be present in all nuclei of the female. The incidence of sex chromatin in sectioned material is usually considerably less than 100% as it may be excluded from the plane of sectioning. By examination of the entire nucleus, for example in whole mounts of embryonic membranes, the incidence approaches 100% (Graham, 1954b).

These comments would apply to the tissues of the skunk in which a sex difference is present. Absence of a sex difference in some cells and lack of a clear-cut sex difference in other cells of this species may be due to a greater contribution of heterochromatin by the autosomes, possibly related to the metabolic state of the cell.

The presence of a sex difference in neuronal nuclei of the pig, and its absence in nuclei of other tissues, conforms to observations recorded for certain other members of the order Artiodactyla (see Table I). Purkinje cells of the pig contain multiple small chromatin masses associated with the nucleolus. It is difficult to distinguish between the sex chromatin and other particles of similar morphology; this has also been observed by Cantwell et al. (1958). Moore

and Barr (1957) recorded similar observations for these cells in cattle. Crabb and Kelsall (1957) stated that the absence of typical sex chromatin in Purkinje cells of the hamster and man may be due to a more complete fusion of the DNA in forming the nucleolus-associated chromatin. This may be a possible explanation of the chromatin pattern in the Purkinje cells of the pig.

Absence of a sex difference in tissues of the pig, other than those of the nervous system, may be due to the formation of large chromocenters by the autosomes, resulting in multiple chromatin masses of varying size and position. If this is so, why are these multiple chromatin masses absent in the nuclei of the nerve cells? It may be that the metabolic processes of neurones inhibit manifestation of large amounts of heterochromatin from the autosomes in the interphase nucleus.

Lang and Hansel (1959) report the presence of a sex difference in nuclei of liver, adrenal and pancreas of cattle following strong acid hydrolysis that removed much of the unrelated autosomal chromatin. The photomicrographs presented in their paper are somewhat unconvincing, however, this method is worthy of further investigation as it may prove to be a means of unmasking the sex chromatin in those species in which the sex chromatin cannot be distinguished from multiple, unrelated chromatin particles.

A large Y-chromosome has been shown for some members of the order Rodentia (Koller, 1938; Sachs, 1953). Makino (1951) showed that in the developing germ cells of five species of mice, the large Y-chromosome of the male formed a heterochromatic mass as large as the heterochromatic portion of the X-chromosome. These observations are thought to account for the absence of a sex difference in the nuclei of rodent species (see Table I and Moore and Barr, 1953). It appears that the heterochromatic mass formed by the XY complex in the male is comparable in size to that produced by the XX chromosomes of the female thus making it impossible to accurately assign sex by nuclear morphology. Contributions of heterochromatin from the autosomes are believed to be responsible for the presence of multiple chromatin masses in nuclei of these species. This explanation would also apply to the mouse and prairie dog in which sex chromatin cannot be distinguished from unrelated chromatin masses.

The presence of a sex difference in nuclei of stellate ganglion cells, adrenal cortex and medulla, cardiac, smooth and skeletal muscle of the porcupine, a rodent, was unexpected. The possibility of a technical factor cannot be excluded, and attempts are being made to secure another pair of animals in order to check these observations. As these animals were obtained and killed at different times, it is possible that some chemical change in the fixative resulted in the unmasking

of the sex difference in these tissues; this seems unlikely however.

A sex difference has been reported for spinal cord neurones of the hamster (Walsh, 1955), and ameloblasts of the rat (Castro et al., 1956). We have been unable to confirm these observations in this laboratory. Reitalu (1958) and Ohno et al. (1959) have observed a nuclear sex difference in rat liver. The study of non-nervous tissues of this species is not yet complete, but we have found liver nuclei to be generally unsuitable for study, as the chromatin tends to be coarse and abundant. Discrepancy between the postulated origin of the sex chromatin by these authors raises some doubt as to their findings. Reitalu observed many chromatic bodies in rat liver nuclei, but in the female he noted a mass composed of two symmetric parts which he interpreted as the heterochromatic segments of two X-chromosomes; these bodies were often attached to the nucleolus. In male nuclei a rod-shaped chromatin structure, similar in nature to one of the XX elements, is sometimes present either free, or attached by a thread to one of the nucleoli. He concludes that this represents the X-chromosome in the male. Modifications in the stainability of the sex chromatin and other chromatin bodies were found to occur in relation to the age and sex of the animal. Ohno et al. state that the sex chromatin in nuclei of the female rat is formed by the heterochromatic portion of

one X-chromosome only, whereas the other X-chromosome does not exhibit positive heteropycnosis. If this is so, it is possible that at fertilization, an ovum with a 'heterochromatic X-chromosome' could be fertilized by a Y-bearing sperm; thus, on the basis of the above explanation, nuclei of the male (XY) could contain sex chromatin. A selection factor could be involved however, and it is possible that female sex hormones are necessary for the manifestation of the heterochromatic properties of the X-chromosome. Conversely, an ovum with a 'heterochromatic X-chromosome' fertilized by a sperm bearing a similar chromosome, could result in a chromatin mass in nuclei of the female that is twice the size of that formed by the heterochromatic and non-heterochromatic X-chromosomes. Lowe and Salmon (1951) have shown, by chemical analysis, a sex difference in alkaline phosphatase activity and DNA content in rat liver. The DNA content per gram of wet tissue was found to be higher in the female than the male. No explanation is given for this difference, although it is known that tissue phosphatase activity can be influenced by sex hormones. Further investigation on chromosome constitution and composition in relation to cell metabolism is necessary before the sex characteristics of nuclei can be clearly understood.

Little information is available concerning the chromosomes of the armadillo. Painter (1925) counted 60

chromosomes in this species, but was unable to identify the sex chromosomes. The pattern of multiple chromocenters observed in many tissues is probably the result of contributions of heterochromatin from the autosomes. The nucleolar chromosomes would appear to have relatively large heterochromatic segments in view of the prominent nucleolus-associated chromatin present in nuclei of most tissues. Cell metabolism may be related to the difference in chromatin configuration in tissues such as the liver and thyroid.

A relationship between sex chromatin and cell metabolism is known to exist. The female sex chromatin becomes slightly enlarged and undergoes a migration from its usual position, adjacent to the nucleolus, toward the nuclear membrane during axon reaction of the hypoglossal nerve in cats (Barr and Bertram, 1951; Crouch and Barr, 1954). The sex chromatin of the male enlarges slightly under these conditions. A similar migration of the sex chromatin from the nucleolus toward the nuclear membrane has been observed by Bertrand and Girard (1956) in cases of chromatolysis of the hypoglossal neurones of the human female; modification of the sex chromatin in the male was not observed.

Similar experiments on the rat are currently in progress in this laboratory. If the migration of chromatin from the nucleolus towards the nuclear membrane is specific reaction of sex chromatin or the XX chromosome complex, it

may be that one of the multiple chromatin masses adjacent to the nucleolus in this species would move from this position in the female, whereas no change in the chromatin pattern would occur in the male.

The sex chromatin of the female is believed to be derived from the heterochromatic regions of the XX chromosomes, and is composed of DNA as shown by the positive Feulgen test. As previously stated, heterochromatin, and therefore DNA, is related to nucleic acid metabolism of the nucleus and cytoplasm. Studies on the nucleic acid content of the superior cervical ganglion in rabbits by Causey and Stratmann (1956) showed nearly a three fold increase in DNA content of the cells following preganglionic stimulation while the total nucleic acid content remained the same. After axon section, there is at first an increase in both DNA and RNA, then later mainly in RNA; this is possibly associated with synthesis of new axoplasm. The relationship between cell activity of the thyroid and the DNA content of the nuclei has been demonstrated in male rats by Roels (1954). Histophotometric measurement following administration of thyroxine, which inhibits thyroid activity, showed a reduced value of DNA compared to the controls; in the controls, the DNA content was less than the theoretical diploid value. Stimulation of thyroid activity by thiouracil resulted in an increase in DNA content approaching the theoretical diploid value.

Variation between the chromatin pattern of stellate ganglion cells and other regions of the nervous system of the pig, prairie dog and porcupine may be related to a difference in cell metabolism. The general absence of chromatin in nuclei of the upper layers of cells of urinary bladder epithelium of the armadillo and prairie dog may be related to the physiological environment of these cells. Manifestation of a chromatin mass similar to sex chromatin, which is more frequently observed in cardiac muscle nuclei of females of these two species and the pig, may be the result of a differential action of the female sex hormones on the metabolism of these cells, although it has been shown that sex hormones do not exert a direct influence on sex chromatin. A sex difference in nuclear morphology is absent in tissues of very early embryos (Austin and Amoroso, 1957; Park, 1957). These investigations suggest that sex chromatin appears at different rates in different tissues, or at different times. The intense metabolic activity of the developing embryo may be related to this differential appearance of sex chromatin. Considerable study is necessary before definite conclusions on the exact relationship of sex chromatin to cell metabolism can be stated.

It is generally accepted that most female mammals have an XX sex chromosome constitution whereas the males have an XY chromosome complex. The sex difference in nuclear

morphology which has been observed in the nuclei of certain mammals is thought to be based on this difference in sex chromosome constitution. It is interesting to postulate the possible chromatin patterns of the interphase nucleus, with reference to a sex difference, in species with multiple sex chromosome mechanisms. In Potorous tridactylus, a marsupial (Sharman, McIntosh and Barber, 1950) and Gerbillus gerbillus gerbillus, a rodent, (Wahrman and Zahavi, 1955) the males have the sex chromosome constitution XYY, the females having XX sex chromosomes. The only marsupial studied to date is the opossum in which the sex chromatin of the male is approximately one-half the size of that observed in the female (Graham, 1956). The sex chromosomes of the opossum were described by Painter (1925), the female having XX chromosomes and the male XY. It would be interesting to study other species of this order. In Potorous trydactylus it is possible that the additional Y-chromosome could contribute to the XY heterochromatic mass in the male forming a structure of comparable size to that of the female, thus making nuclear sexing impossible. If the additional Y-chromosome in Gerbillus gerbillus gerbillus has a large heterochromatic segment as has been shown for the Y-chromosome of some members of the order Rodentia, the combined volume of heterochromatin of the XYY chromosomes may exceed that of the XX chromosomes of the female, making it possible to determine

the sex of the animal by nuclear morphology.

Russell, Russell and Gower (1959) have studied a sex-linked recessive gene in the mouse, and explain their results on the basis of an XO sex chromosome constitution in these females. One would expect the heterochromatin of such a female to be approximately one-half the size of that contributed by the XX chromosomes of the normal female. As the XY of the normal male mouse forms a heterochromatic mass comparable to that produced by the XX of the females, one could possibly differentiate between an XO female and an XY male on the basis of a smaller chromatin mass in the female.

Study of neuronal nuclei of the pigeon (Brusa, 1952) and various non-nervous tissues of the domestic fowl, ducks, parrots and parakeets (Ashley and Theiss, 1959), showed that no sex difference in nuclear morphology was present in these avian species. Kosin and Ishizaki (1959) report a nuclear sex difference in cells of the duodenum and developing feathers of the domestic fowl. Sex chromatin is present in nuclei of the female and is not visible in these tissues from the male. This observation is of considerable interest as in members of the class Aves, the male is homogametic (XX) and the female heterogametic (XY or XO). If the female has XY sex chromosomes, it is possible that the Y-chromosome has a large heterochromatic segment whereas the heterochromatin of the X-chromosomes is small and not visible in the interphase

nucleus under ordinary conditions. The presence of sex chromatin in nuclei of an XO female could mean that the sex chromatin is derived from one X-chromosome only, female sex hormones possibly being necessary for manifestation of its heterochromatic properties. Extension of study to additional members of this class and to other sub-mammalian forms may provide valuable information on the sex characteristics of the metabolic nucleus.

It is generally agreed that sex determination in mammals is genetically controlled. These genetic factors act upon the undifferentiated gonad causing it to develop into a testis or an ovary. The hormones produced by the testis or ovary act to reinforce the genetic factors. The presence of a sex difference in the metabolic nucleus is a valuable aid in the study of sex differentiation; the sex chromatin is not influenced by age, castration, or sex hormones and therefore serves as a reliable indicator of chromosomal sex, the sex determined at the time of fertilization.

Hormonal imbalance may bring about a partial sex reversal, the bovine freemartin being a specific example. The freemartin has been shown to be a chromosomal female by Moore, Graham and Barr (1957). The intersexual characteristics of the freemartin are caused by androgens from the male twin circulating to the female fetus via an

anastamosis of the two placentas. It is theoretically possible that such a condition could arise in the human, but such a study has not yet been undertaken.

Masculinization of the human female fetus has been reported in some instances where certain androgenic hormones were administered to the mother in the course of pregnancy (Hellhaus, 1958; Wilkins et al., 1958; Bongiovanni et al., 1959). Administration of male sex hormones at a critical stage of gonadal differentiation could possibly bring about a complete sex reversal. Experiments of this nature on the rat are in progress in this laboratory.

A discrepancy exists between the theoretical conception ratio, and the sex ratio of humans at birth. By the sex chromatin method, one can determine the chromosomal sex at a stage prior to differentiation of the gonads or the external genitalia. Current studies in this laboratory on fetal tissues and membranes show that the nuclear sex is not always in agreement with the apparent appearance of the external genitalia. Thus legitimate doubts can be raised as to the accuracy of sex ratios previously reported for various gestational ages in which the external genitalia were used as the diagnostic criterion.

CHAPTER VI

SUMMARY

1. Tissues of seven mammalian species (one male and one female) belonging to four orders were studied with respect to a sex difference in nuclear morphology. Representative regions of the nervous system and cell types of various non-nervous tissues of the timber wolf, pig, prairie dog, porcupine and armadillo were examined. Only non-nervous tissues of the skunk and mouse were studied.

2. Order Carnivora: A nuclear sex difference was observed in all tissues studied from the timber wolf. Nuclei of adrenal cortex and medulla, smooth and cardiac muscle, and urinary bladder epithelium of the skunk showed sexual dimorphism. A practical sex difference was absent in other tissues of this species.

3. Order Artiodactyla: Neuronal nuclei of the pig showed a nuclear sex difference. Multiple chromatin masses in nuclei of the non-nervous tissues obscured any sex difference present.

4. Order Rodentia: Tissues of the mouse and prairie dog did not show a sex difference in nuclear morphology; one to several chromatin masses were observed in nuclei of

both sexes. Nuclei of stellate ganglion cells, adrenal cortex and medulla, smooth, cardiac and skeletal muscle of the porcupine showed sexual dimorphism. A sex difference was absent in nuclei of other tissues of this species.

5. Order Edentata: Similar chromatin patterns were observed in nuclei of the male and female armadillo; a practical sex difference was not present.

6. In those species showing a nuclear sex difference, the sex chromatin of the female is larger than that of the male. The female sex chromatin is believed to be derived from heterochromatic portions of the XX sex chromosomes. The Y-chromosome of the male is probably minute and the XY chromosomes do not form a mass of chromatin comparable in volume to the female sex chromatin. Absence of nuclear sexual dimorphism in some species may be related to the presence of a large Y-chromosome in the male. The pattern of multiple chromatin masses may be due to the formation of large chromocenters by the autosomes. Factors contributing to the morphological appearance of chromosomes in the metabolic nucleus are not clearly understood.

7. The results of this investigation are compared with those previously reported for other mammalian species. Reference is made to the possible derivations of the sex chromatin, and its relationship to cell metabolism and chromosome constitution.

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ILLUSTRATIONS

The magnification of
all photomicrographs
is x1728.

FIGURE

1. Timber wolf. Stellate ganglion cell, female. The nucleolus is eccentric in position and the sex chromatin lies between the nucleolus and the nuclear membrane. 10μ, cresyl echt violet stain.
2. Timber wolf. Stellate ganglion cell, male. No sex chromatin is visible. 10μ, cresyl echt violet stain.
3. Timber wolf. Dorsal root ganglion cell, female. The sex chromatin is located adjacent to the nucleolus. A smaller chromatin mass is also present in this position. 10μ, cresyl echt violet stain.
4. Timber wolf. Dorsal root ganglion cell, male. A chromatin mass, smaller than the sex chromatin of the female, is located adjacent to the nuclear membrane; this structure probably represents the male sex chromatin. 10μ, cresyl echt violet stain.
5. Timber wolf. Anterior horn cell (spinal cord), female. The sex chromatin is free in the nucleoplasm. 10μ, cresyl echt violet stain.
6. Timber wolf. Anterior horn cell (spinal cord), male. There is no visible sex chromatin. 10μ, cresyl echt violet stain.

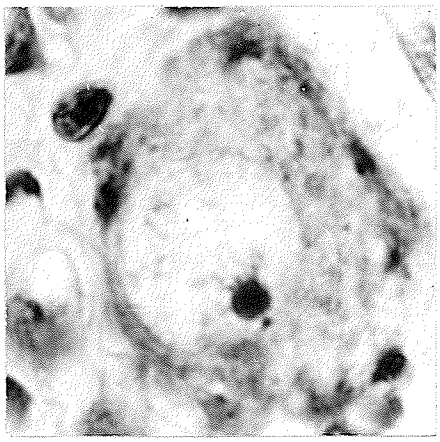


Fig. 1

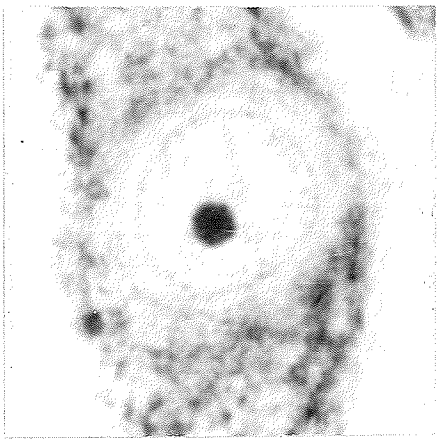
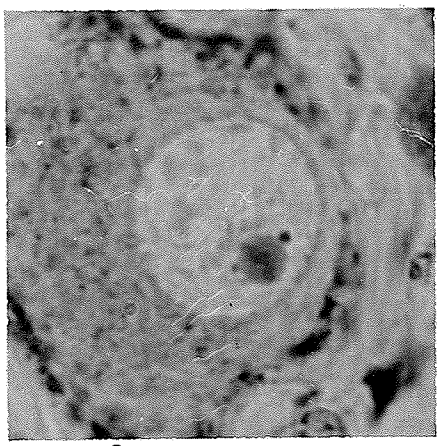


Fig. 2



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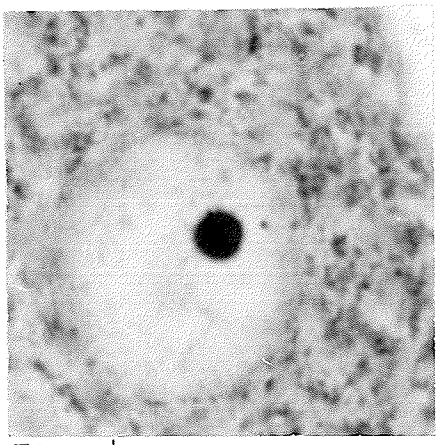

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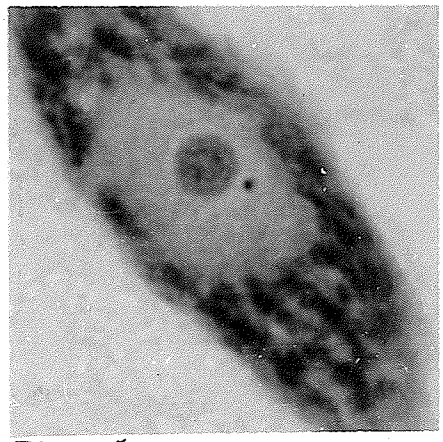

Fig. 5

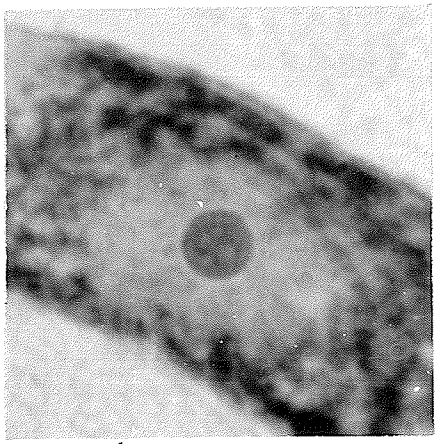

Fig. 6

FIGURE

7. Timber wolf. Adrenal cortex, female. The sex chromatin is located adjacent to the nuclear membrane. It is distinguished from the smaller chromatin masses by its morphology. 5μ, haematoxylin and eosin stain.

8. Timber wolf. Adrenal cortex, male. Fine chromatin granules are present in the nuclei; no sex chromatin is visible. 5μ, haematoxylin and eosin stain.

9. Timber wolf. Smooth muscle, female. The sex chromatin is located at the side of the nucleus, adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

10. Timber wolf. Smooth muscle, male. The sex chromatin is not visible. 5μ, haematoxylin and eosin stain.

11. Timber wolf. Cardiac muscle, female. The sex chromatin is adjacent to the nuclear membrane and lies at the pole of the nucleus. Note the prominent nucleoli. 5μ, haematoxylin and eosin stain.

12. Timber wolf. Cardiac muscle, male. No sex chromatin is visible in the nucleus. 5μ, haematoxylin and eosin stain.

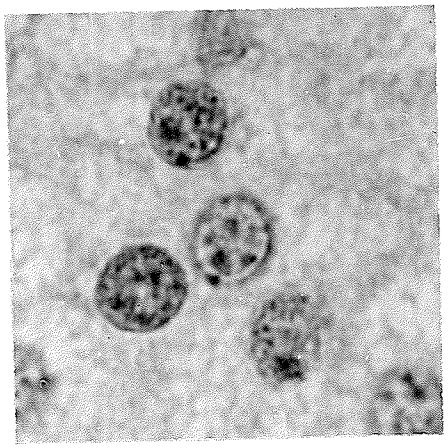

Fig. 7

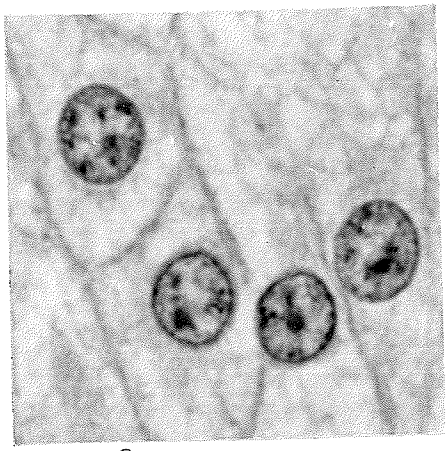

Fig. 8

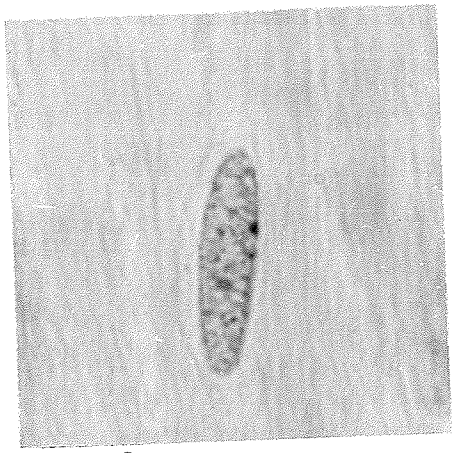

Fig. 9

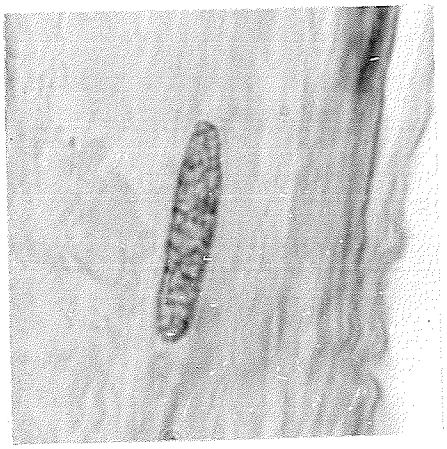

Fig. 10

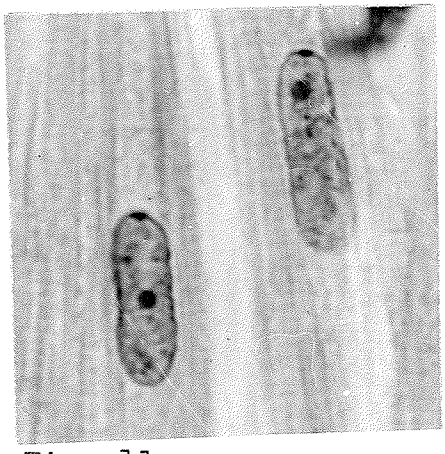

Fig. 11

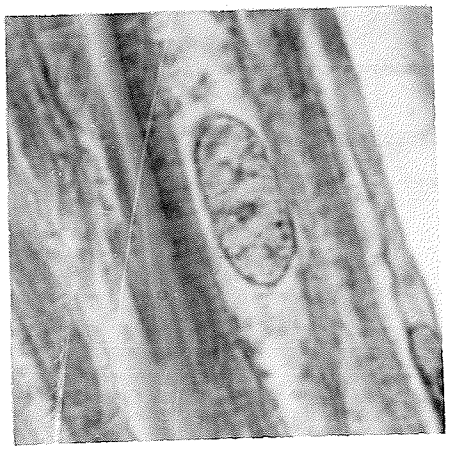

Fig. 12

FIGURE

13. Skunk. Adrenal medulla, female. The sex chromatin is a planoconvex chromatin mass located adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

14. Skunk. Adrenal medulla, male. There is no sex chromatin visible. 5μ, haematoxylin and eosin stain.

15. Skunk. Epidermis, female. The sex chromatin is located adjacent to the nuclear membrane. Additional slightly smaller chromatin masses are also observed in this position. 5μ, haematoxylin and eosin stain.

16. Skunk. Epidermis, male. Chromatin masses similar to the sex chromatin of the female are located adjacent to the nuclear membrane. Note the scattered pigment granules. 5μ, haematoxylin and eosin stain.

17. Skunk. Kidney (convoluted tubules), female. Two chromatin masses, larger than sex chromatin, are located adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

18. Skunk. Kidney (convoluted tubules), male. There are three large planoconvex chromatin masses adjacent to the nuclear membrane. They are similar in morphology to the sex chromatin of the female. 5μ, haematoxylin and eosin stain.

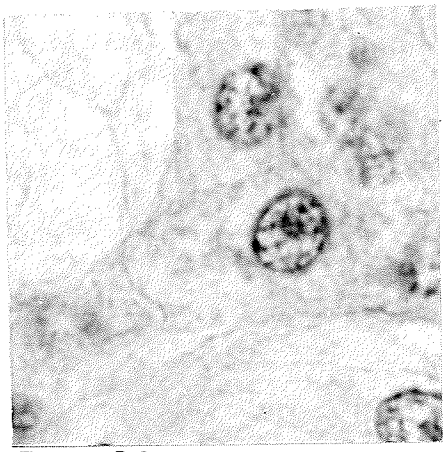

Fig. 13

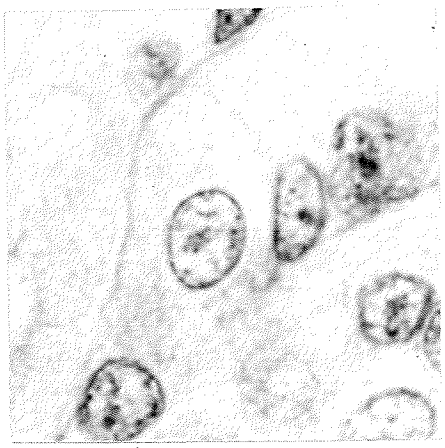

Fig. 14

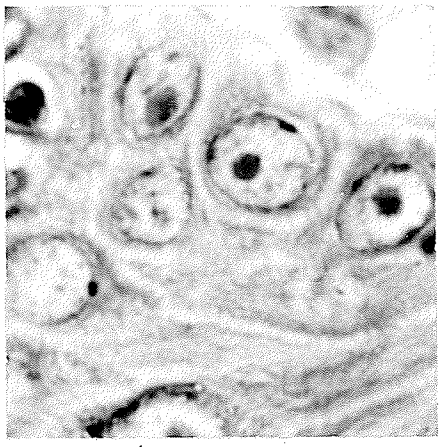

Fig. 15

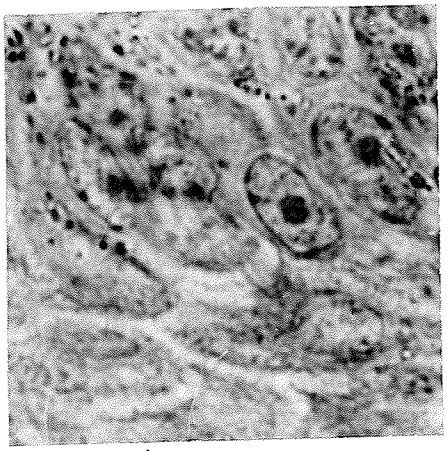

Fig. 16

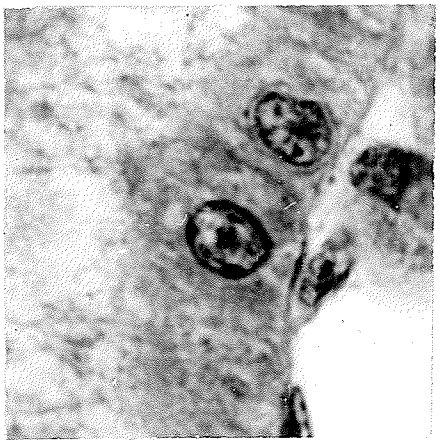

Fig. 17

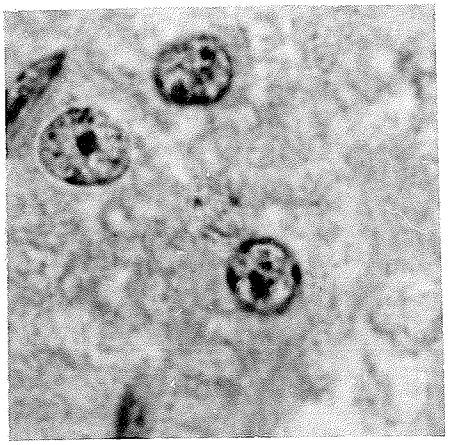

Fig. 18

FIGURE

19. Skunk. Ovary (follicle cells), female. The nuclei contain multiple chromatin masses. Many show a sex-chromatin-like mass adjacent to the nuclear membrane; more than one may be present. 5μ, haematoxylin and eosin stain.

20. Skunk. Testis (interstitial cells), male. One or two chromatin masses similar in morphology to the female sex chromatin are present in the nuclei. They are located adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

21. Pig. Anterior horn cell (spinal cord), female. The sex chromatin is a spherical mass of chromatin very slightly removed from the nucleolus. 10μ, cresyl echt violet stain.

22. Pig. Anterior horn cell (spinal cord), male. No sex chromatin is visible. 10μ, cresyl echt violet stain.

23. Pig. Stomach (surface epithelium), female. The nuclei contain multiple chromatin masses of varying position and size. 5μ, haematoxylin and eosin stain.

24. Pig. Stomach (surface epithelium), male. Multiple chromatin masses of varying size and position are present. 5μ, haematoxylin and eosin stain.

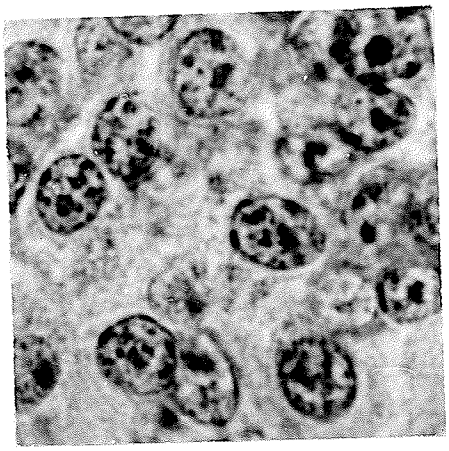

Fig. 19

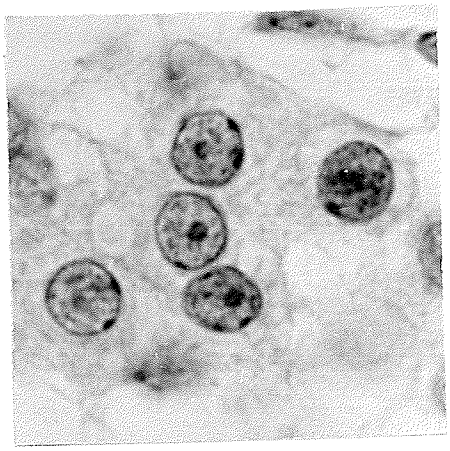

Fig. 20

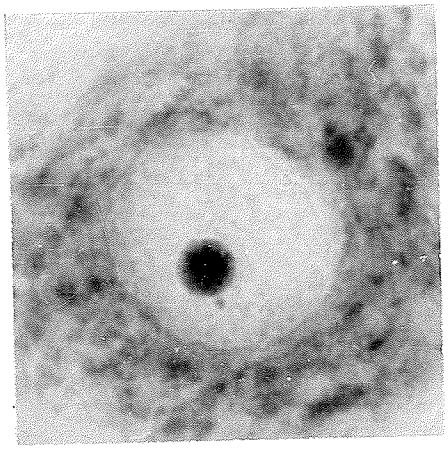

Fig. 21

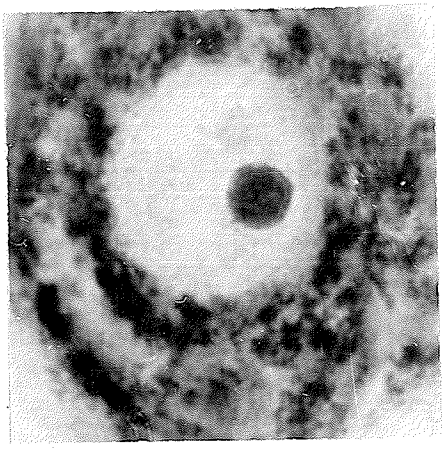

Fig. 22

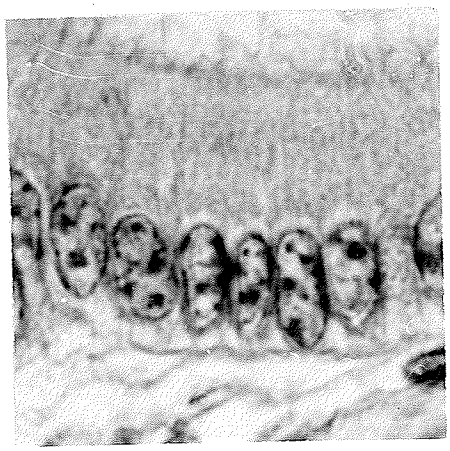

Fig. 23

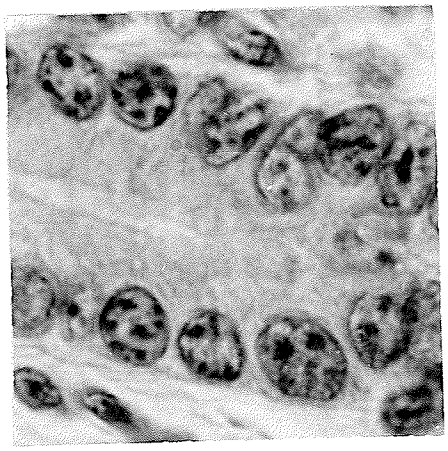

Fig. 24

FIGURE

25. Pig. Adrenal medulla, female. The nuclei contain multiple chromocenters of varying size and position. 5μ, haematoxylin and eosin stain.

26. Pig. Adrenal medulla, male. Chromatin masses of varying size, position and number are present. 5μ, haematoxylin and eosin stain.

27. Mouse. Adrenal cortex, female. Multiple chromatin masses varying in size and position are present. Note the nucleolus-associated chromatin. 5μ, haematoxylin and eosin stain.

28. Mouse. Adrenal cortex, male. The nuclei contain several chromatin masses. Note the sex-chromatin-like masses adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

29. Mouse. Liver, female. Large chromatin masses are present in various positions. 5μ, haematoxylin and eosin stain.

30. Mouse. Liver, male. Multiple large chromocenters are present in the nuclei. 5μ, haematoxylin and eosin stain.

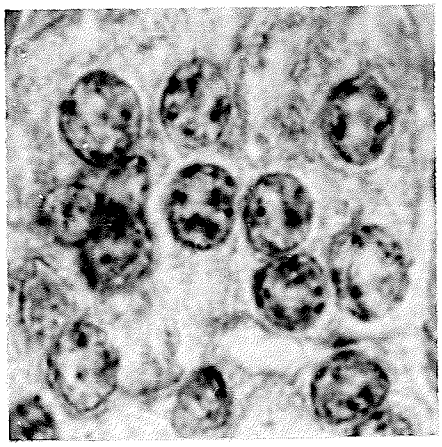

Fig. 25

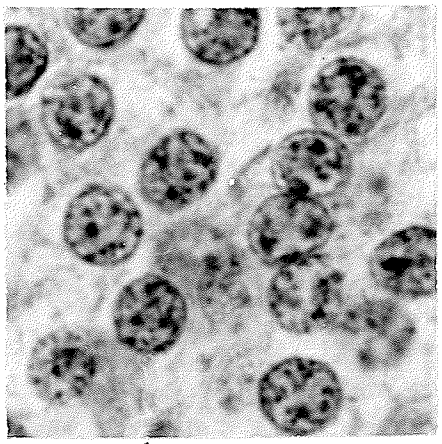

Fig. 26

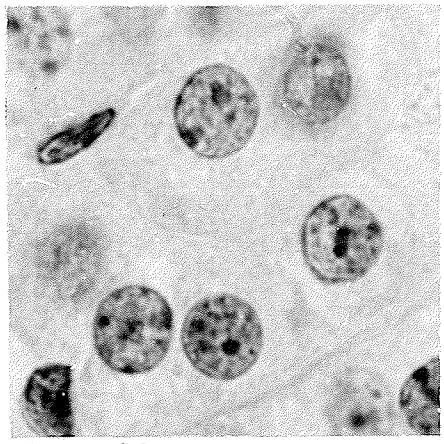

Fig. 27

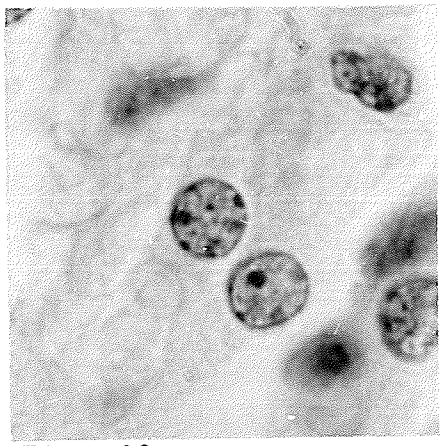

Fig. 28

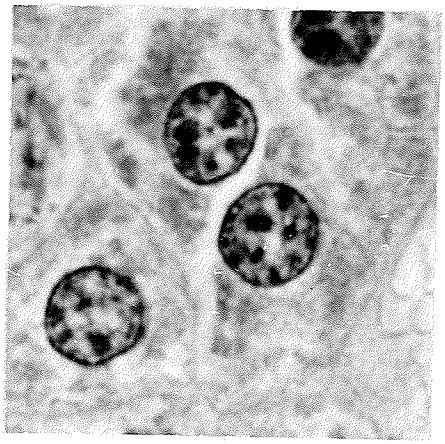

Fig. 29

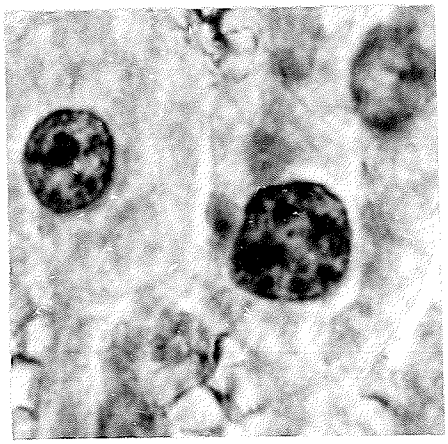

Fig. 30

FIGURE

31. Prairie dog. Dorsal root ganglion cell, female. A large cap-shaped chromatin mass is located adjacent to the nucleolus. 10μ, cresyl echt violet stain.

32. Prairie dog. Dorsal root ganglion cell, male. The chromatin appears in the form of a cap on the nucleolus. 10μ, cresyl echt violet stain.

33. Prairie dog. Stellate ganglion cell, female. Two plano-convex masses of chromatin are located adjacent to the nuclear membrane. Note the lighter-staining nucleolus which is also in this position. 10μ, cresyl echt violet stain.

34. Prairie dog. Stellate ganglion cell, male. The nucleus contains two nucleoli which are located at the nuclear membrane. The chromatin is adjacent to the nucleolus and nuclear membrane at the upper nucleolus. The lower nucleolus has a cap of chromatin and two smaller chromatin masses are associated with both the nucleolus and nuclear membrane. 10μ, cresyl echt violet stain.

35. Prairie dog. Purkinje cell (cerebellum), female. A large chromatin mass overlaps the nucleolus. Odd-shaped lighter-staining bodies are present at the nuclear membrane. 10μ, cresyl echt violet stain.

36. Prairie dog. Purkinje cell (cerebellum), male. Note the odd-shaped lighter-staining structure which surmounts the nucleolar cap of chromatin. 10μ, cresyl echt violet stain.

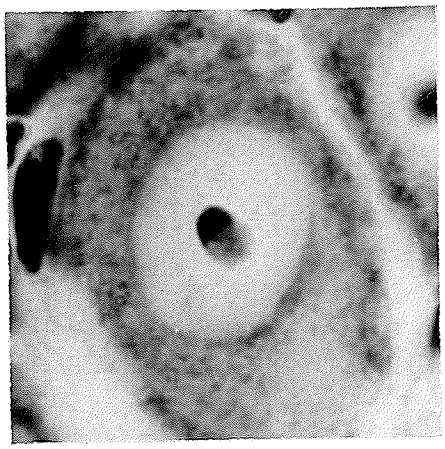

Fig. 31

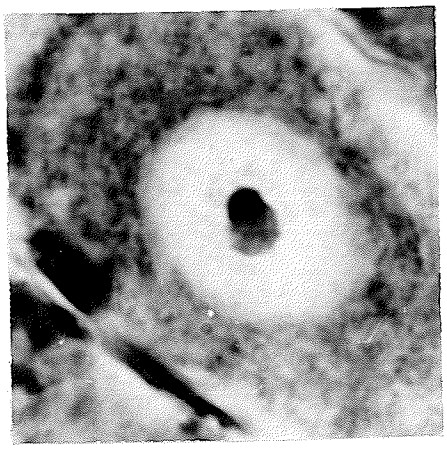

Fig. 32

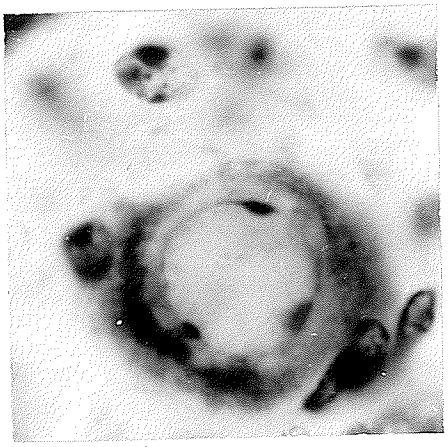

Fig. 33

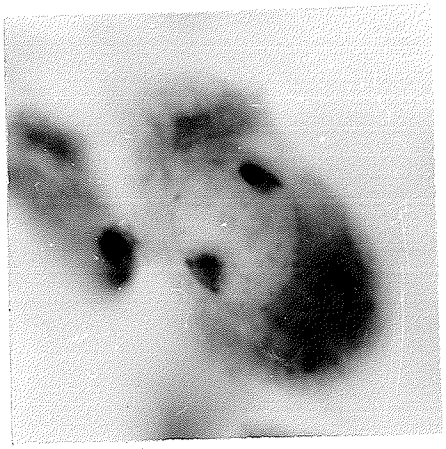

Fig. 34

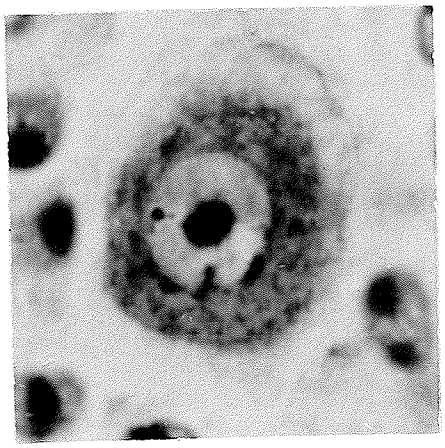

Fig. 35

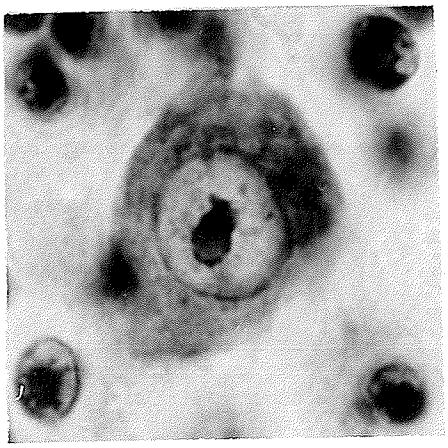

Fig. 36

FIGURE

37. Prairie dog. Smooth muscle, female. The chromatin appears as thickenings of the nuclear membrane at the side of the nucleus. 5μ, haematoxylin and eosin stain.

38. Prairie dog. Smooth muscle, male. The nucleus contains several chromatin masses which appear as thickenings of the nuclear membrane. 5μ, haematoxylin and eosin stain.

39. Prairie dog. Skeletal muscle, female. Several large chromatin masses are present. The lighter-staining structures are nucleoli. 5μ, haematoxylin and eosin stain.

40. Prairie dog. Skeletal muscle, male. The nucleus contains multiple large masses of chromatin which are associated with the nuclear membrane and the nucleoli. 5μ, haematoxylin and eosin stain.

41. Prairie dog. Pituitary (pars distalis), female. A very large chromatin mass is present in the nuclei; it is frequently central, but may be peripheral. Some nuclei contain additional smaller chromatin masses adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

42. Prairie dog. Pituitary (pars distalis), male. A large central or peripheral chromatin mass is present in the nuclei. Small chromatin masses, adjacent to the nuclear membrane, are present in some nuclei. 5μ, haematoxylin and eosin stain.

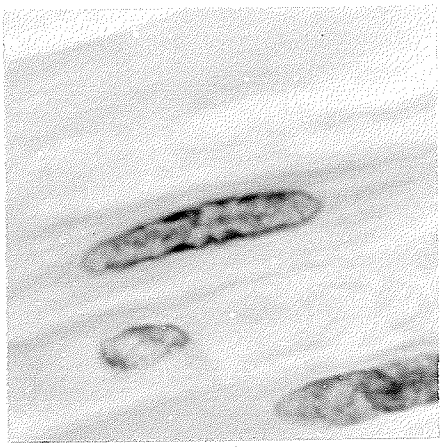

Fig. 37

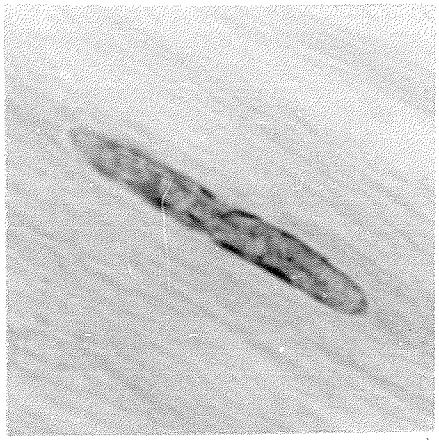

Fig. 38

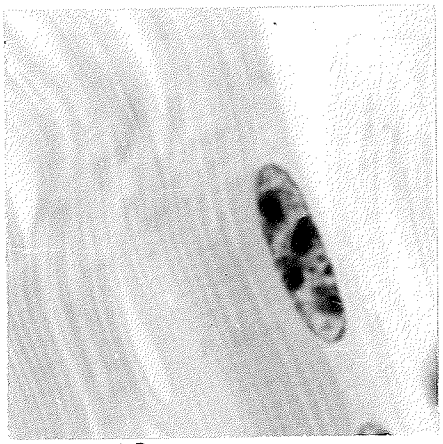

Fig. 39

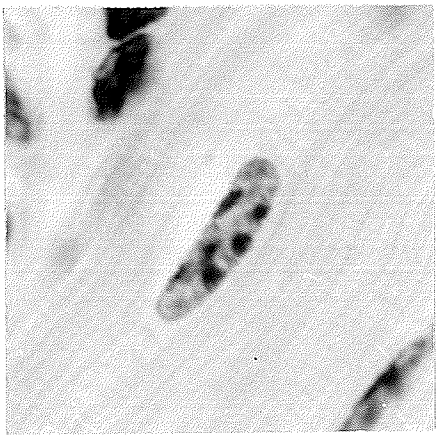

Fig. 40

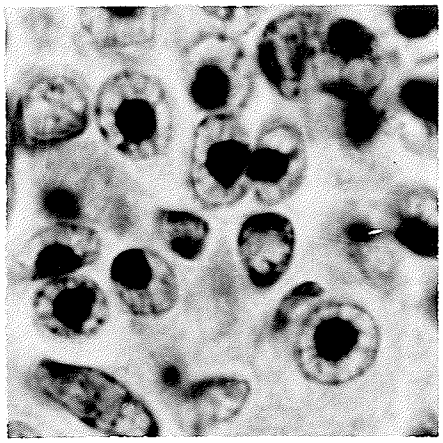

Fig. 41

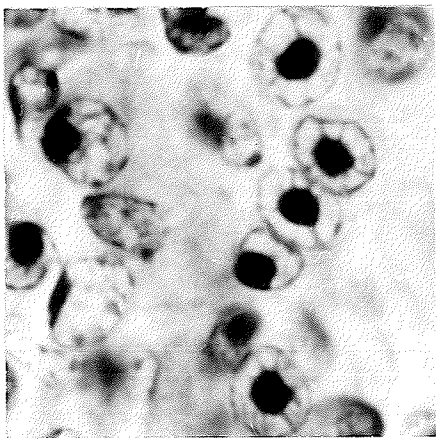

Fig. 42

FIGURES

43. Porcupine. Betz cell (motor cortex), female. The chromatin appears as a cap-shaped structure located adjacent to the nucleolus. 10μ, cresyl echt violet stain.

44. Porcupine. Betz cell (motor cortex), male. A spherical mass of chromatin is associated with the nucleolus. 10μ, cresyl echt violet stain.

45. Porcupine. Stellate ganglion cell, female. The sex chromatin is located adjacent to the nuclear membrane. 10μ, cresyl echt violet stain.

46. Porcupine. Stellate ganglion cell, male. No sex chromatin is visible. 10μ, cresyl echt violet stain.

47. Porcupine. Stellate ganglion cell, female. The sex chromatin is located adjacent to the nuclear membrane and is also associated with the nucleolus which is eccentric in position. 10μ, Feulgen stain.

48. Porcupine. Stellate ganglion cell, male. A chromatin mass slightly smaller than the female sex chromatin is located adjacent to the nucleolus. 10μ, Feulgen stain.

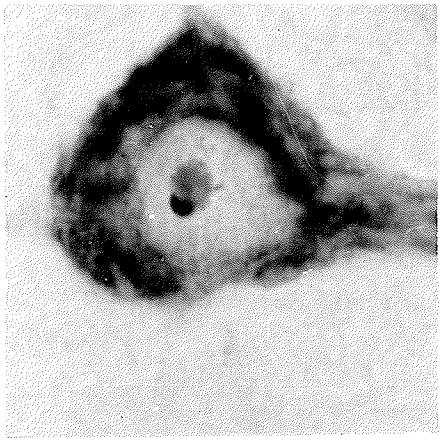

Fig. 43

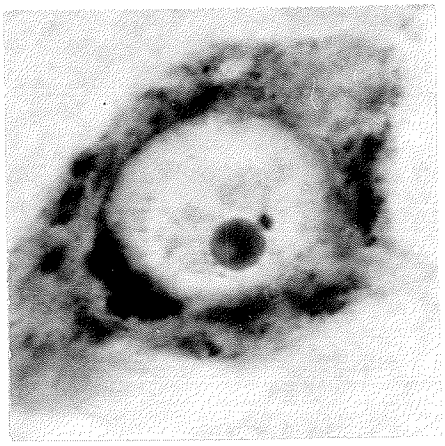

Fig. 44

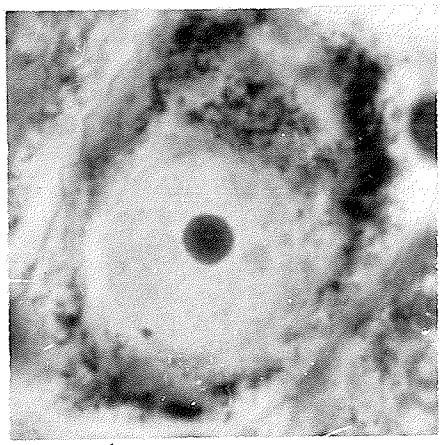

Fig. 45

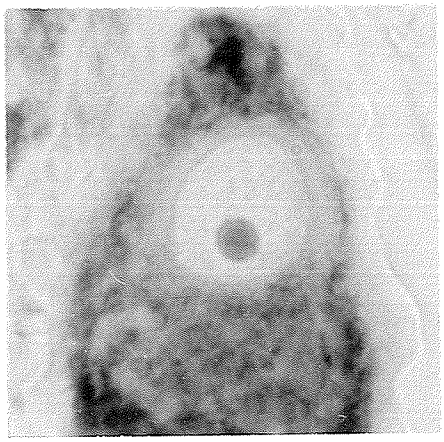

Fig. 46

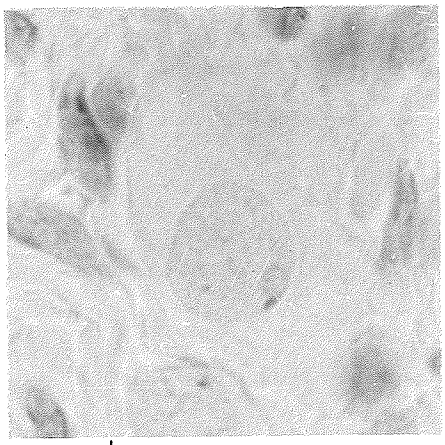

Fig. 47

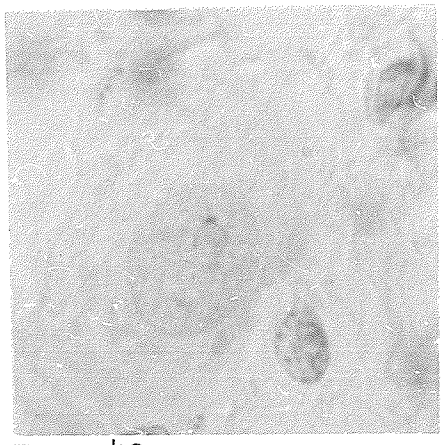

Fig. 48

FIGURE

49. Porcupine. Adrenal medulla, female. The sex chromatin is a distinct planoconvex chromatin mass located adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

50. Porcupine. Adrenal medulla, male. The nuclei contain several small chromocenters; no sex chromatin is visible. 5μ, haematoxylin and eosin stain.

51. Porcupine. Smooth muscle, female. The sex chromatin is located adjacent to the nuclear membrane. It is at the side of the nucleus in the nucleus on the right, and is polar in position in that on the left. 5μ, haematoxylin and eosin stain.

52. Porcupine. Smooth muscle, male. No sex chromatin is visible. 5μ, haematoxylin and eosin stain.

53. Porcupine. Duodenum (surface epithelium), female. The nuclei contain several chromocenters. A chromatin mass with the characteristics of sex chromatin is located adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

54. Porcupine. Duodenum (surface epithelium), male. Multiple chromatin masses of varying size and position are present. Note the sex-chromatin-like masses adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

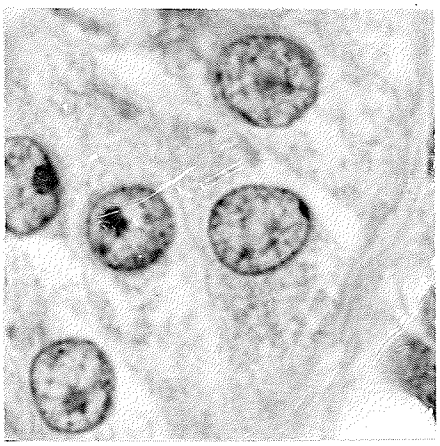

Fig. 49

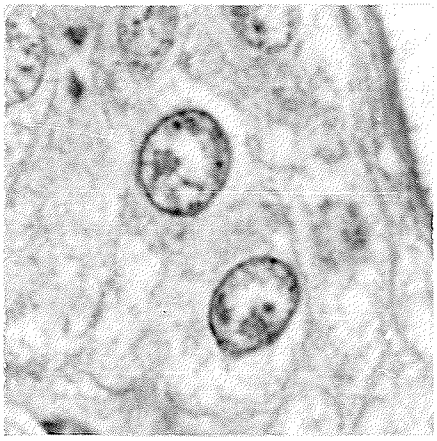

Fig. 50

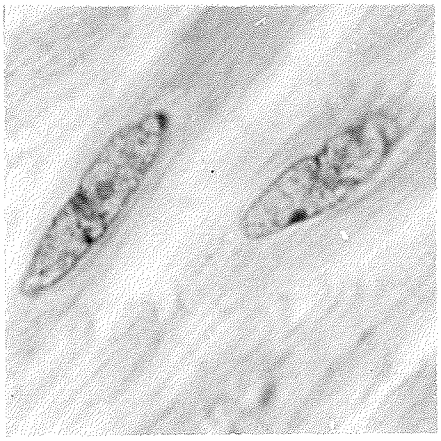

Fig. 51

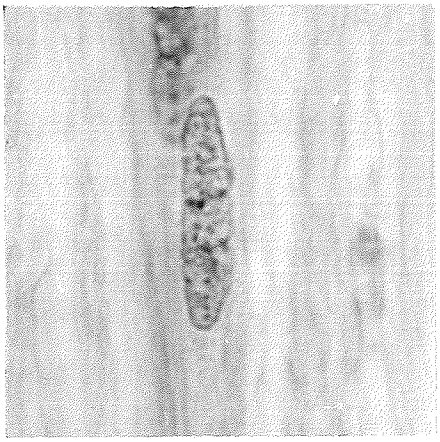

Fig. 52

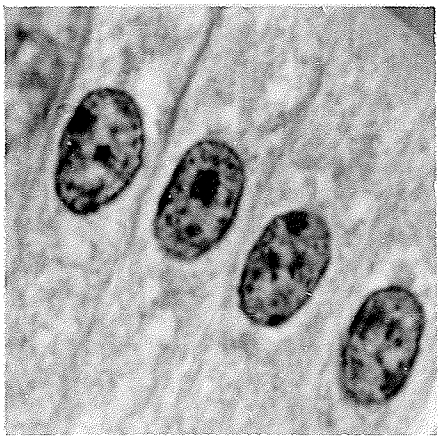

Fig. 53

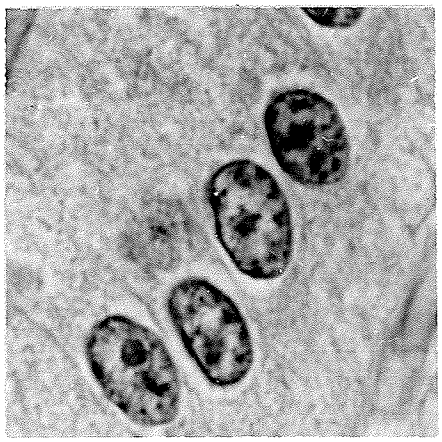

Fig. 54

FIGURE

55. Porcupine. Pituitary (pars distalis), female. Chromatin masses of varying size, position and number are present. 5μ, haematoxylin and eosin stain.

56. Porcupine. Pituitary (pars distalis), male. The nuclei contain multiple chromatin masses varying in size and position. 5μ, haematoxylin and eosin stain.

57. Armadillo. Dorsal root ganglion cell, female. The chromatin masses are irregular in outline and are closely associated with the nucleolus. 10μ, cresyl echt violet stain.

58. Armadillo. Dorsal root ganglion cell, male. The nucleus contains two chromatin masses which are very closely applied to the nucleolus. 10μ, cresyl echt violet stain.

59. Armadillo. Betz cells (motor cortex), female. The two nuclei contain chromatin masses which vary in size, position and number. 10μ, cresyl echt violet stain.

60. Armadillo. Betz cell (motor cortex), male. Three chromatin masses are located adjacent to the nucleolus. A smaller chromatin mass, slightly out of focus, is present at the nuclear membrane. 10μ, cresyl echt violet stain.

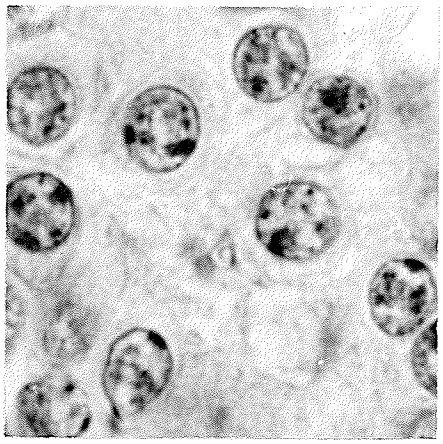

Fig. 55

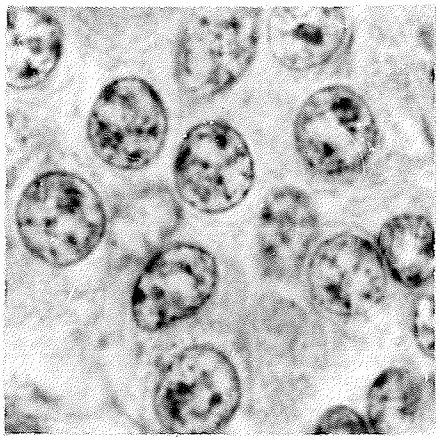

Fig. 56

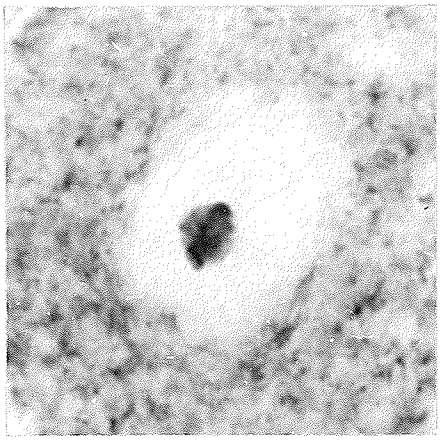

Fig. 57

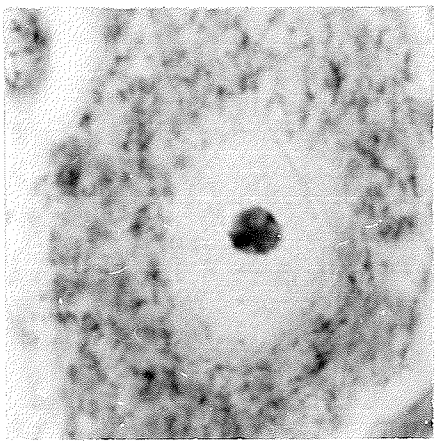

Fig. 58

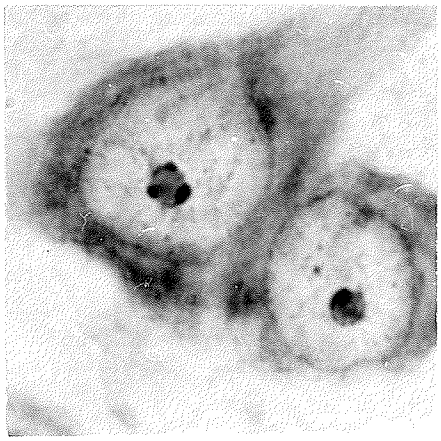

Fig. 59

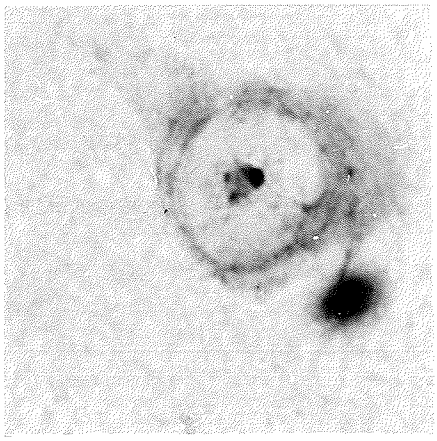

Fig. 60

FIGURE

61. Armadillo. Thyroid (follicle cells), female. Multiple large chromocenters are present. They vary in size, position and number. 5μ, haematoxylin and eosin stain.

62. Armadillo. Thyroid (follicle cells), male. The nuclei contain multiple large chromatin masses of varying size and position. 5μ, haematoxylin and eosin stain.

63. Armadillo. Liver, female. Small chromocenters are present; no sex chromatin is visible. A large nucleolus is eccentric in position and lies adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

64. Armadillo. Liver, male. The nuclei contain small chromatin granules; no sex chromatin is visible. Note the eccentric position of the nucleoli. 5μ, haematoxylin and eosin stain.

65. Armadillo. Cardiac muscle, female. Note the polar sex-chromatin-like mass which lies adjacent to the nuclear membrane. 5μ, haematoxylin and eosin stain.

66. Armadillo. Cardiac muscle, male. Multiple small chromatin masses are present; no sex chromatin is visible. 5μ, haematoxylin and eosin stain.

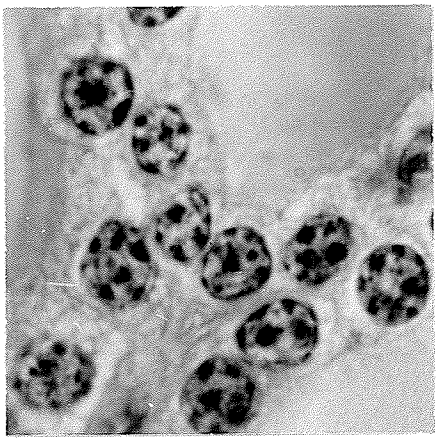

Fig. 61

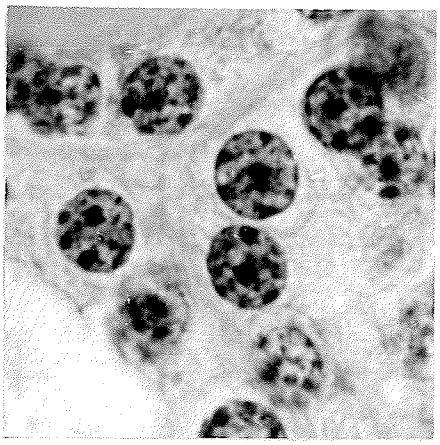

Fig. 62

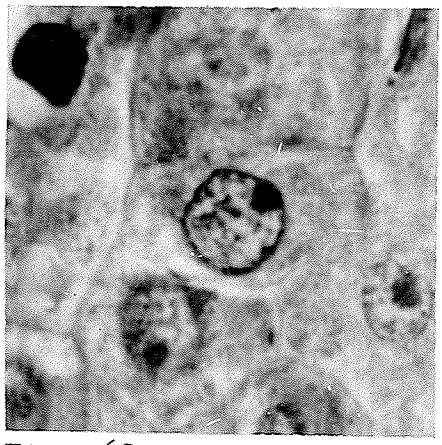

Fig. 63

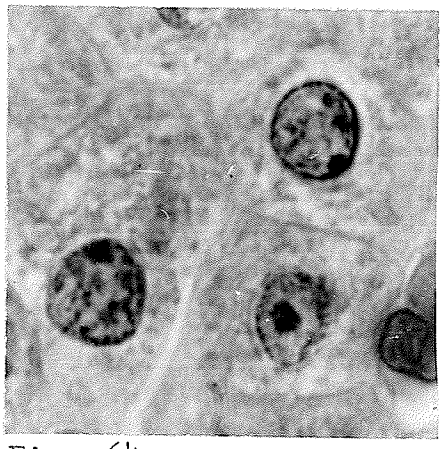

Fig. 64

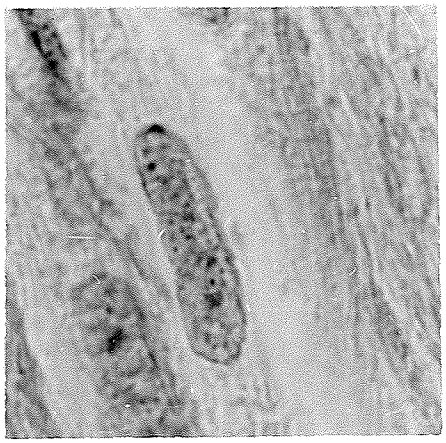

Fig. 65

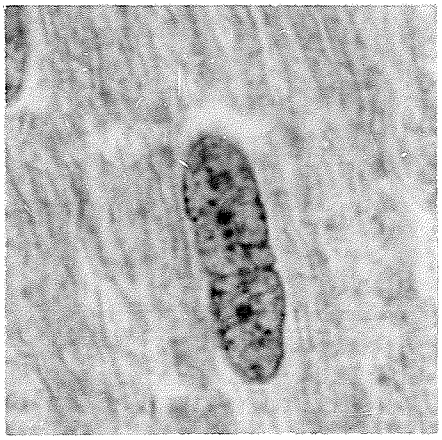

Fig. 66