SEX CHROMATIN IN DOMESTIC ANIMALS

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By

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Introduction

Since the discovery of the sex chromatin by Barr and Bertram (2) in 1949, a sex difference in the morphology of intermitotic nuclei has been described for several species of the orders Carnivora, Artiodactyla and Primates by Barr, Bertran and Lindsay (3), Graham and Barr (6), Moor and Barr (8), Nakahara (10) and Prince, Graham and Barr (11). They stated that a special mass of chromatin, known as the sex chromatin, was clearly visible only in the nuclei of females, while the nuclei of males rarely contained a mass of chromatin comparable in size, shape, position, and internal structure with the sex chromatin of the female nuclei. Klinger (7) studied the fine structure of the sex chromatin body and reported that the sex chromatin was composed of two portions. Thus it has been known that the sex chromatin of the female nuclei forms a mass which is sufficiently large to be identified readily by heterochromatic portions of the XX chromosomes, while the XY chromosomes of the male nuclei fail to do so.

The present investigation consists of the morphology of the male and female nuclei in the nervous system and the non-nervous tissues of domestic animals, with special reference to the fine structure of the sex chromatin.

Materials and Methods

Three adult female and three adult male cats and pigs, three adult female and two adult male goats, three adult female, one adult male and one adult castrated male cows and horses were used. From each animal was taken the various regions of the nervous system, and from pigs, cows and horses were taken the non-nervous tissues. They are given in Tables 1 to 7.

Observation for the incidence and the position of the sex chromatin was made by the following method: 10 per cent neutral formalin was used as fixing fluid. The materials were embbeded in paraffin and cut at 10μ. The sections were stained with 0.5 per cent thionin which was prepared at pH 5

with acetic buffer. These staining sections were dehydrated by 95 per cent alcohol and absolute alcohol, and were cleared by xylene. By this method the sex chromatin was stained sharply. 200 nuclei containing a nucleolus were examined and they were classified into three kinds according to the position of the sex chromatin as lying adjacent to the nucleolus, adjacent to the nuclear membrane and free in the nucleoplasm.

For the morphological observation of the sex chromatin, the spinal cords and the spinal ganglions of the cats, Purkinje cells of the cows and the adrenal and pancreatic acini of the pigs were used as materials. They were fixed in a 10 per cent neutral formalin, zenker-formalin fluid and Davidson's formalin-acetic-alcohol mixture. The paraffin embedded tissues were cut at 5 to 7μ and stained with 0.5 per cent thionin (pH 5), 0.5 per cent cresyl echt violet (pH 4) and also by the Feulgen method.

Results and Discussion

A. Nervous tissues

1. Females

The charactristic feature of the female nuclei throughout the nervous system is the presence of a large mass of chromatin. Considering the various regions together, the sex chromatin is encountered in about 80 per cent of the cells which contains the nucleolus. Since a structure of comparable size and shape is rarely found in the male nuclei, this is clearly the sex chromatin. The main features of the sex chromatin in female cells are given in Tables 1 to 5.

a) Size. The sex chromatin is approximately $0.8\mu \times 1.0\mu$ to $1.1\mu \times 1.3\mu$. No variation in size is found in different kinds of animals, but it varies in different kinds of cells. In general, the sex chromatin is largest in the Purkinje cells ($1.1\mu \times 1.3\mu$) and smallest in the cerebral cortex ($0.8\mu \times 1.0\mu$).

Moore and Barr (8) studied the morphology of nerve cell nuclei in a series of mammals and reported that the sex chromatin is smallest in the goat and largest in the marten. In the present investigation, a different relation is obtained as mentioned above.

b) Position. The sex chromatin is divided into three types according to its position. In the first type, the sex chromatin lies adjacent to the nucleolus (Fig. 1), in the second type, adjacent to the nuclear membrane (Fig. 2) and in the third type, free in the nucleoplasm (Fig. 3).

Considering the various regions together, the sex chromatin of the first type can be identified in about 30 to 70 per cent of the female nuclei, that of the second type in about 20 to 50 per cent and that the third type in about 2 to 10 per cent. It is also noticed that the localization of the sex chromatin varies in different regions of the nervous system. For example, in such regions

as the cerebral cortex of the cat and goat, anterior horn of the cervical cord of the pig, spinal ganglion and stellate ganglion of the cow, the sex chromatin occurs more frequently at the nuclear membrane than in any other position.

Prince, Graham and Barr (11) studied the sex chromatin in *Macacus rhesus* and reported that it is more likely to be found at the nuclear membrane than in any other position with some variation from one type of neuron to another. Nakahara (10) studied the sex chromatin in the goat and reported almost the same relation as the above. Barr, Bertran and Lindsay (3) reported that the sex chromatin of the nervous tissues in the cat is situated adjacent to the nucleolus most frequently. Crouch and Barr (4) reported that the sex chromatin of many neuron of the female cat moves toward the nuclear membrane and enlarges slightly during axon reaction. Further, Graham and Barr (6) reported that the position of the sex chromatin of neurons changes during the development of the cat embryo. They claimed that metabolic factors influence the position of the sex chromatin within the nucleus.

In the present investigation, the sex chromatin is most likely to be found at the nucleolus than in any other portion, agreeing with the results obtained by Barr, Bertran and Lindsay. It is also noticed that the position of the sex chromatin varies in different types of neurons probably due to the metabolic

Table 1. Incidence and position of the sex chromatin in the nervous system of the cat.

Region of nervous system	Sex	Position of sex chromatin (%)			Total (%)
		Type 1	Type 2	Type 3	
Frontal cortex	♀	8.0	80.0	2.0	90.0
	♂	0.0	2.0	2.0	4.0
Parietal cortex	♀	8.5	53.5	4.5	66.5
	♂	0.0	2.0	1.0	3.0
Occipital cortex	♀	17.0	64.0	8.0	89.0
	♂	1.0	2.0	0.0	3.0
Purkinje cells of corebellum	♀	57.0	4.0	13.0	74.0
	♂	0.0	3.0	2.0	5.0
Nuclei pontis	♀	77.5	5.0	2.5	85.0
	♂	1.0	1.0	0.0	2.0
Inferior olivary nucleus	♀	70.0	12.5	7.5	90.0
	♂	2.0	4.0	3.0	9.0
Anterior horn of cervical cord	♀	61.5	24.0	2.5	88.0
	♂	2.0	0.5	2.0	4.5
Spinal ganglion of cervical region	♀	49.5	16.5	3.5	69.5
	♂	6.5	0.5	2.5	9.5
Average	♀	43.7	32.4	5.4	81.5
	♂	1.6	1.9	1.6	5.1

Type 1 ..... The sex chromatin lies adjacent to the nucleolus.
Type 2 ..... Adjacent to the nuclear membrane.
Type 3 ..... Free in the nucleoplasm.

Table 2. Incidence and position of the sex chromatin in the nervous system of the pig.

Region of nervous system	Sex	Position of sex chromatin (%)			Total (%)
		Type 1	Type 2	Type 3	
Frontal cortex	♀	57.0	26.5	7.0	90.5
	♂	1.5	0.0	0.0	1.5
Parietal cortex	♀	54.5	25.0	9.0	88.5
	♂	2.0	0.0	0.0	2.0
Occipital cortex	♀	66.5	16.5	5.5	88.5
	♂	0.5	0.0	0.0	0.5
Purkinje cells of cerebellum	♀	56.5	20.5	10.5	87.5
	♂	0.0	0.0	0.0	0.0
Lateral thalamic nucleus	♀	63.0	9.5	0.5	73.0
	♂	2.5	2.0	1.0	5.5
Nuclei pontis	♀	52.5	23.5	9.0	85.0
	♂	1.5	1.0	0.5	3.0
Inferior olivary nucleus	♀	61.5	17.0	6.0	84.5
	♂	4.0	0.0	0.0	4.0
Anterior horn of cervical cord	♀	33.5	43.0	12.0	88.5
	♂	4.5	0.5	0.0	5.0
Spinal ganglion of cervical region	♀	50.5	25.0	5.5	81.0
	♂	6.5	0.5	2.5	9.5
Average	♀	55.1	22.9	7.2	85.2
	♂	2.6	0.4	0.4	3.4

Table 3. Incidence and position of the sex chromatin in the nervous system of the goat.

Region of nervous system	Sex	Position of sex chromatin (%)			Total (%)
		Type 1	Type 2	Type 3	
Frontal cortex	♀	10.5	49.0	12.0	71.5
	♂	0.0	7.0	1.5	8.5
Parietal cortex	♀	11.5	63.5	3.5	78.5
	♂	0.0	5.0	4.5	9.5
Occipital cortex	♀	41.5	40.0	11.5	93.0
	♂	2.0	3.0	0.0	5.0
Purkinje cells of cerebellum	♀	44.5	27.5	5.5	77.5
	♂	1.5	0.0	0.0	1.5
Nuclei pontis	♀	54.5	28.5	10.5	93.5
	♂	4.0	0.5	0.0	4.5
Inferior olivary nucleus	♀	43.5	28.0	11.0	82.5
	♂	2.0	3.0	0.0	5.0
Anterior horn of cervical cord	♀	66.5	9.5	8.0	83.0
	♂	6.5	0.5	0.0	7.0
Spinal ganglion of cervical region	♀	62.5	7.5	4.0	74.0
	♂	5.0	0.0	0.0	5.0
Average	♀	41.9	31.7	8.3	81.9
	♂	2.6	2.4	0.8	5.8

Table 4. Incidence and position of the sex chromatin in the nervous system of the cow.

Region of nervous system	Sex	Position of sex chromatin (%)			Total (%)
		Type 1	Type 2	Type 3	
Anterior horn of cervical cord	♀	62.0	7.0	3.0	72.0
	♂	1.0	2.0	0.5	3.5
Spinal ganglion of cervical region	♀	20.5	67.5	5.0	93.0
	♂	3.0	0.0	0.0	3.0
Stelleate ganglion	♀	18.5	65.0	3.5	87.0
	♂	0.0	1.0	0.0	1.0
Average	♀	33.7	46.5	3.8	84.0
	♂	1.3	1.0	0.2	2.5

Table 5. Incidence and position of the sex chromatin in the nervous system of the horse.

Region of nervous system	Sex	Position of sex chromatin (%)			Total (%)
		Type 1	Type 2	Type 3	
Anterior horn of cervical cord	♀	58.0	3.0	4.0	65.0
	♂	4.0	1.0	1.0	6.0
Spinal ganglion of cervical region	♀	62.5	6.0	1.5	70.0
	♂	3.0	2.0	2.0	7.0
Stellate ganglion	♀	78.5	5.0	1.5	85.0
	♂	2.5	1.0	0.0	3.5
Average	♀	66.3	4.7	2.3	73.3
	♂	3.2	1.3	1.0	5.5

changes within the neurons as has been suggested by Graham and Barr.

c) *Number.* The incidence of visible sex chromatin in various regions of the nervous system is shown in Tables 1 to 5. The average incidence of the sex chromatin in various animals is as follows: 81.5 per cent in the cat, 85.2 per cent in the pig, 81.9 per cent in the goat, 84.0 per cent in the cow and 73.3 per cent in the horse.

d) *Shape.* The feature of the sex chromatin stained with thionin and cresyl echt violet is as follows: The sex chromatin is spherical or slightly irregular in profile outline when it lies free in the nucleoplasm. When it lies adjacent to the nucleolus and the nuclear membrane it is triangular or hemispherical in outline and may be flattened on the nucleolar and nuclear membrane. A narrow interval between the nucleolus and adjacent sex chromatin is often visible, and the surface of the nucleolus may be flattened.

e) *Internal structure.* The fine internal structure of the sex chromatin is clearly determined in the section stained by the Feulgen method. The sex chromatin is composed of two masses, each of which is rod shape in form and

$0.5\mu \times 0.8\mu$ in size (Fig. 5). These two masses are separated from each other by a distance of about 0.2μ. The total size of the two masses is $0.8\mu \times 1.2\mu$, approximately coinciding with the size of the sex chromatin stained with thionin or cresyl echt violet. The reaction of the two masses for the Feulgen method differs from each other; the one shows less affinity than the other. When the sex chromatin is located adjacent to the nucleolus or nuclear membrane its larger dimension takes mostly a position vertical to the nucleolus or nuclear membrane, and rarely lies at the parallel position (Fig. 5). It is also found that the two masses often appear as two dot forms probably according to the orientation of the rod shaped sex chromatin to the optical axis.

Moor and Barr (8) reported that a pale area is present in the sex chromatin of many cells and this may represent an interval between two components of a double structure. Uchida (12) studied the sex chromatin of the ganglion cells of the vermiform appendix in man and reported that the external region of the sex chromatin reacts strongly and the intenal region reacts weakly by the Feulgen method. Klinger (7) studied the fine structure of the sex chromatin and reported that the most common form of the sex chromatin is the bipartite form, and sometimes the two small masses show a spiral like form of the letters O, S or W. Adachi (1) recently studied the fine structure of the sex chromatin of the human foetus with electron microscopy and stated that this body is of double structure.

In the present investigaation, it is found that the sex chromatin is clearly composed of two small masses of rod form as has been stated by Klinger, but no spiral like form of the leters O, S or W which has been described by Klinger is demonstrated. Since the sex chromatin is Feulgen-positive it is found to be the DNA. It is also found that the two masses of the sex chromatin differ in stainability with the Feulgen reagent, the one being stained less densely than the other, and accordingly the amount of the DNA also differs in each mass of the sex chromatin. It is highly probable that the two components of the sex chromatin represent heterochromatic portions of two X chromosomes as has been suggested by Barr and his co-workers, Klinger and Adachi.

2. Males

In the Feulgen-stained sections, a mass of chromatin comparable in size, position and internal structure with the sex chromatin of the female nuclei is seldom encountered in the male nuclei (Fig. 4). However, a special chromatin mass which is slightly larger than the particulate chromatin is contained in the male nuclei. As shown in Tables 1 to 5, its occurrence is less than 9 per cent of the nerve cells. It is a single, half sized structure that coincides with one side body of the two components of the sex chromatin in the female nucleus.

B. Non-nervous tissues

1. Pigs

The incidence of the sex chromatin in the cells of the non-nervous tissues of the female pig is shown in Table 6.

The sex chromatin in the ovarian stromal cells is identified in about 68 per cent of the total nuclei counted and that in the adrenal cortex in about 64 per cent. The nuclei of the ovarian follicular cells contain a large number of mutiple, coarse chromatin, and therefore the sex chromatin in this nuclei is not recognizable. In the nuclei of small cells such as the surface epithelium of the duodenum mucosa or the pancreatic acini, it is difficult to identify the sex chromatin. When the cells contain the sex chromatin it is most likely to be located at the nuclear membrane (Type 2) and the average size of it is $0.8\mu \times 1.1\mu$, showing almost the same size as that of sex chromatin in the nervous tissues. In the female nuclei of the non-nervous tissues, the sex chromatin shows the same internal structure as that of the sex chromatin in the female nuclei of the neuron (Fig. 6).

A structure of comparable size is not seen in the nuclei of male pigs, although a mass of chromatin which is slightly larger than the ganeral particulate chromatin is recorded in 1 to 10 per cent of the nuclei (Table 6).

Table 6. Incidence and position of the sex chromatin in the non-nervous tissues of the pig.

Tissue	Sex	Position of sex chromatin (%)			Total (%)
		Type 1	Type 2	Type 3	
Adrenal cortex	♀	13.5	46.5	4.0	64.0
	♂	1.0	0.0	0.0	1.0
Adrenal medulla	♀	9.0	38.5	1.0	48.5
	♂	0.0	1.0	0.0	1.0
Pancreatic acini	♀	5.5	33.5	1.0	40.5
	♂	0.5	9.0	0.5	10.0
Liver	♀	7.0	20.0	3.0	30.0
	♂	1.0	2.0	1.0	4.0
Renal convoluted tubules	♀	12.0	42.0	7.0	61.0
	♂	1.5	2.0	0.0	3.5
Epithelial cells of gastric gland	♀	5.5	43.5	5.0	54.0
	♂	0.0	1.5	0.5	2.0
Epithelial cells of duodenum	♀	1.0	30.5	2.5	34.0
	♂	0.0	2.0	0.0	2.0
Brunner's glands	♀	4.0	30.5	1.0	35.5
	♂	0.5	0.5	0.0	1.0
Smooth muscle of duodenum	♀	0.5	34.5	2.0	37.0
	♂	0.0	1.0	0.0	1.0
Ovarian stromal cells	♀	0.0	68.0	0.0	68.0
Ovarian follicular cells	♀	not found			
Uterine glands	♀	0.0	57.5	0.5	58.0

There remains a question as to whether this mass represents a special mass of chromatin derived from the sex chromosomes or whether it is a mass of autosomal origin.

2. Cows and Horses

In horses and cows, the nuclei in most non-nervous tissues contain multiple, coarse chromatin masses and therefore, a sex difference can not be established. However, as shown Table 7, the nuclei of the epithelial cells and the smooth muscle cells of the duodenum in horses contain the sex chromatin in about 85 and 77 per cent of the total cells counted (Figs. 9, 11).

Table 7. Incidence and position of the sex chromatin in the non-nervous tissues of the horse.

Tissue	Sex	Position of the sex chromatin (%)			Total (%)
		Type 1	Type 2	Type 3	
Epithelial cells of duodenum	♀	9.0	70.0	6.0	85.0
	♂	1.0	9.0	0.0	10.0
Smooth muscle of duodenum	♀	0.0	75.0	2.0	77.0
	♂	0.0	4.0	0.0	4.0
Brunner's glands		not found			
Liver		not found			
Renal convoluted tubules		not found			
Pancreatic acini		not found			
Adrenal cortex		not found			
Adrenal medulla		not found			

Moore, Graham and Barr (9) reported that in the cells other than neurons, in the cow, the nuclei contain multiple, coarse chromatin clumps and a sex difference can not be established. Graham and Barr (6) stated that a sex difference in the nuclear structure is demonstrable in most tissues and organs of the cat. Prince *et al.* (11) also, using the monkey, stated almost a simillar relation. Nakahara (10) stated that a sex difference according to the nuclear morphology can be established in the non-nervous tissues of the goat.

In the present investigation, a sex difference in the nuclear morphology is found in most tissues of the pig and in the epithelium and smooth muscle tissue of the duodenum of the horse, mainly or partially agreeing with the results obtained by Graham and Barr in the cat, by Prince *et al.* in the monkey and by Nakahara in the goat. In the cow, however, a sex difference is not demonstrable in any tissue and organ, showing a good agreement with the results found by Moore, Graham and Barr.

Summary

The sex difference according to the nuclear morphology was examined in cats, pigs, goats, cows and horses. The results are summarized as follows:

1. In the nerve cells of the female, the sex chromatin appeared in about 80 per cent of the total cells. In the cells of the male, however, it appeared rarely.

2. The sex chromatin was divided into three types according to its position. In the first type, the sex chromatin lies adjacent to the nucleolus, in the second type, adjacent to the nuclear membrane and in the third type, free in the nucleoplasm.

3. The sex chromatin was composed of two small, rod shaped masses, each of which was estimated at about $0.5\mu \times 0.8\mu$ in diameter, and was separated from each other by a distance of about 0.2μ. It was also noticed that the reaction of the two masses for the Feulgen reagent differed from each other; the one showed less affinity than the other.

4. In the female pig, the sex chromatin was found in most cells of the non-nervous tissues, with the exception of ovarian follicular cells. In the female horse, it was detected only in the epithelial cells and the smooth muscle cells in the duodenum, and in the cow, none any where.

5. It was suggested that the sex chromatin of the female was composed of heterochromatic portions of the XX chromosomes.

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Plate 1

Explanation of Figures

Figs. 1 to 4 are stained with thionin, ×1500. Figs. 5 and 6 are stained according to the Feulgen method and are reproduced at a magnification of ×3000.

Fig. 1. Spinal ganglion cell, female horse. The sex chromatin is situated adjacent to the nucleolus.

Fig. 2. Spinal ganglion cell, female horse. The sex chromatin is located at the nuclear membrane.

Fig. 3. Spinal ganglion cell, female horse. The sex chromatin is free in the nucleoplasm.

Fig. 4. Spinal ganglion cell, male horse. There is no visible sex chromatin.

Fig. 5. Purkinje cell, female pig. The sex chromatin (indicated by arrow) is composed of two masses. The one shows less affinity for the Feulgen reagent than the other.

Fig. 6. Adrenal cortex cell, female pig. The sex chromatin (marked by arrow) is the same as the above.

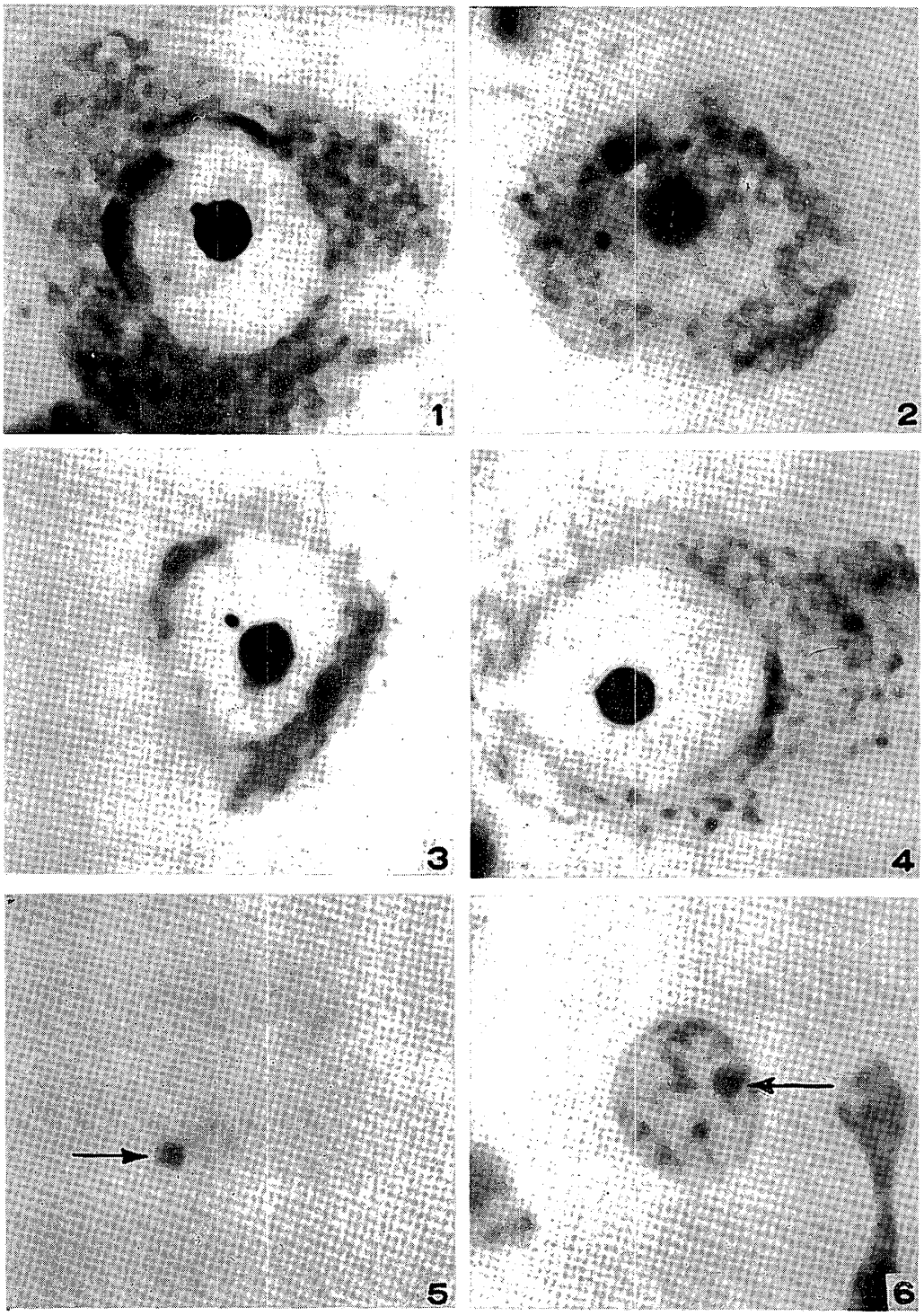
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Plate 2

Explanation of Figures

Figs. 7 to 11 are stained with thionin, ×2000.

Fig. 7. Renal convoluted tubules cells, female pig. The sex chromatin (indicated by arrow) is situated adjacent to the nuclear membrane.

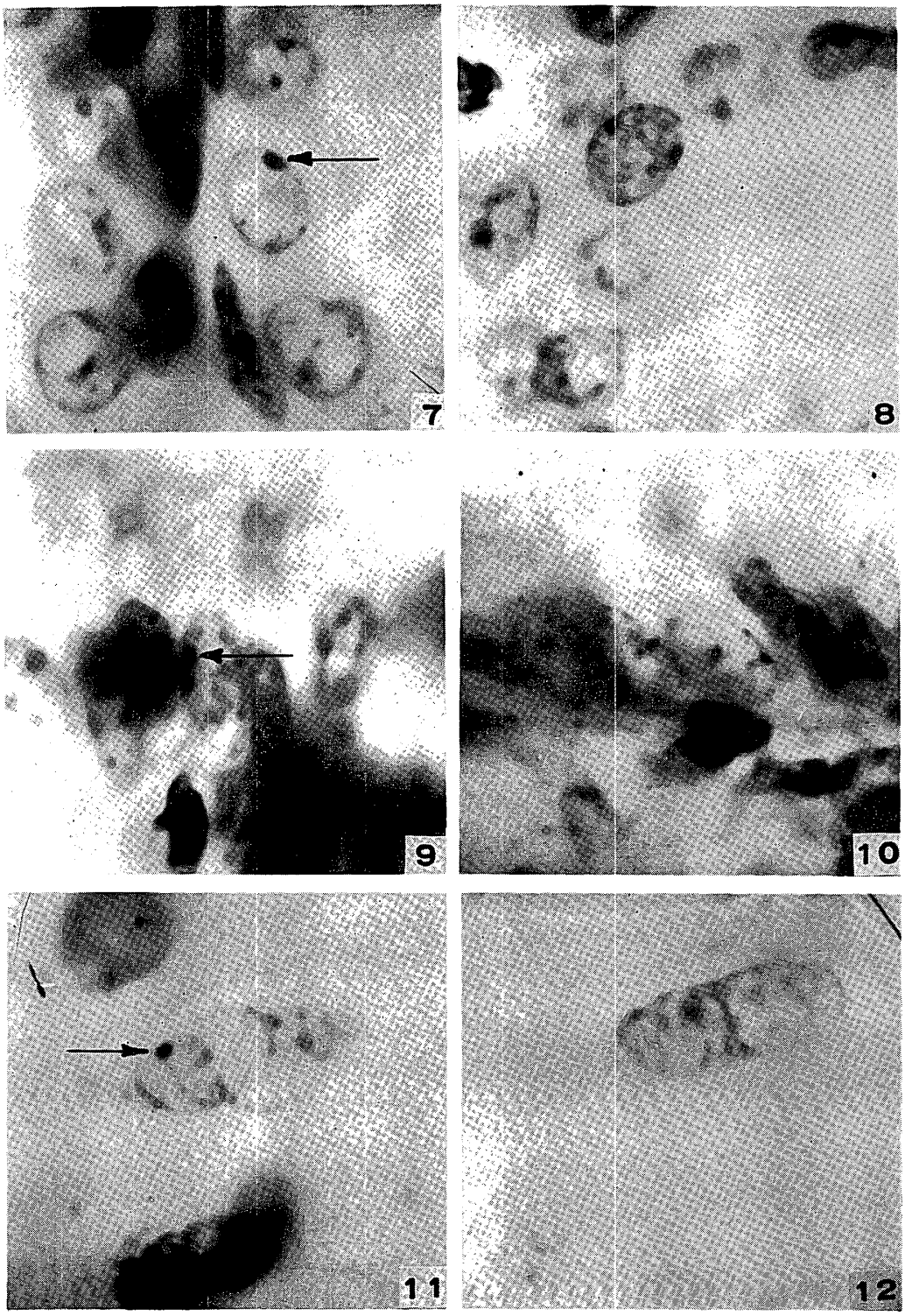
Fig. 8. Renal convoluted tubules cells, male pig. No sexchromatin is found.

Fig. 9. Epithelial cells of duodenum, female horse. The sex chromatin (indicated by arrow) is located at the nuclear membrane.

Fig. 10. Epithelial cells of duodenum, male horse. The sex chromatin is not found.

Fig. 11. Smooth muscle cells of duodenum, female horse. The sex chromatin (marked by arrow) is located at the nuclear membrane.

Fig. 12. Smooth muscle cells of duodenum, male horse. There is no visible sex chromatin.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