

# SEX CHROMATIN IN SOME TISSUES OF RABBITS OF DIFFERENT SEXES

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Sex chromatin was detected in the following rabbit tissues: bone, cartilage, tendon, muscle, liver, lung, and skin. Despite considerable difficulties in identifying sex chromatin in the skin and liver, sexual dimorphism with respect to this criterion was clearly demonstrated in all the rabbit tissues investigated.

By means of the sex chromatin, the sex of an animal can be established by examination of its tissues. This test has been used in several branches of biology and medicine.

It can also be used to determine the fate of grafts when tissues are cross-grafted from female to male and vice versa. By determining the "sex" of the grafted tissue in such experiments, it is possible to discover by whose tissues (the donor's or recipient's) the graft is replaced.

Another matter of considerable importance is to identify animals on which such experiments can be carried out, and to determine in what species of animals and in what tissues sexual dimorphism can be clearly established. In particular, it was necessary to discover whether sexual dimorphism can be detected in tissues of the rabbit, a frequent object for these experiments.

Results of determination of sex chromatin in several species of rodents have recently been published. It has been found in the ameloblast of day-old female rats [5], in the nuclei of spinal neurons in female hamsters and rats [6], in rat liver cells [9], in cells of rabbit embryos in the early stages [1], in the cornea of rabbits and dogs [2], in neutrophils of the blood [7], and in the osteocytes of the bone tissue of these animals [3].

The object of the present investigation was to study whether the sex chromatin test can be used to investigate tissues of the locomotor system and skin, a matter of interest in orthopedic and traumatological surgery for tissue grafting.

## EXPERIMENTAL METHOD

Experiments were carried out on 16 adult rabbits (8 males and 8 females).

Sex chromatin was determined in the skin (concha auriculæ, dorsum), cartilage (concha auriculæ, rib, knee joint, larynx), bone tissue (rib, femur, cervical vertebra), muscle tissue (heart, tongue), and also in the ligamentum patellæ, the alveolar cells of the lung, and parenchymatous cells of the liver.

Bone tissue was decalcified by two methods: in 10% trilon B solution and in a mixture of equal volumes of 20% sodium citrate solution and 50% formic acid. The material was embedded in paraffin wax and sections, cut to a thickness of 8-10  $\mu$ , were stained with hematoxylin-eosin and by Feulgen's method. Sex chromatin was determined in 200-300 nuclei in sections stained by Feulgen's method.

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TABLE 1. Summarized Results for Numbers of Cells with Sex Chromatin in Various Rabbit Tissues

Type of tissue	Number of cells with sex chromatin	
	females	males
Skin (epithelial cells):		
ear . . . . .	28—35	0—10
abdomen . . . . .	32—52	0—8
dorsum . . . . .	26—56	0—7
Cartilage (chondrocytes):		
auricular . . . . .	18—56	0—4
costal . . . . .	28—58	0—1
laryngeal . . . . .	59—67	0—8
articular . . . . .	43—67	0—4
Bone (osteocytes):		
femoral . . . . .	43—62	0—6
from cervical vertebral body . .	28—56	0—1
Muscle tissue:		
heart . . . . .	48—60	0—4
tongue . . . . .	26—50	0—3
Lung (alveolar epithelium) . . .	69—71	0—12
Liver (hepatocytes) . . . . .	55—71	1—16

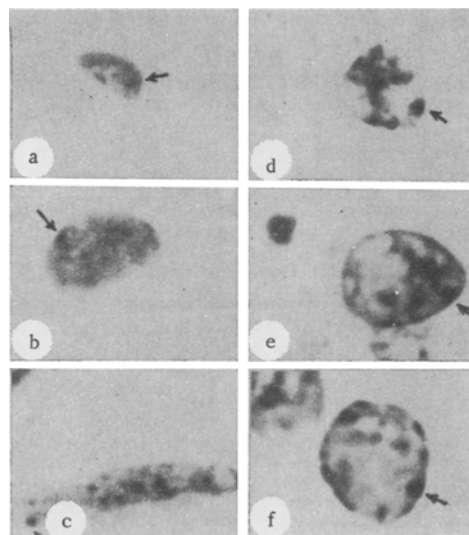


Fig. 1. Sex chromatin in nuclei of some rabbit tissues: a) osteocytes; b) cartilage; c) muscle; d) epidermis; e) alveolar cells of lung; f) liver. Feulgen's method, 630 $\times$ .

**Ligamentum Patellae.** Nuclear chromatin of the fibrocytes of the ligamentum patellae has a delicate reticular pattern, and sometimes consists of small granules of different sizes. The sex chromatin was a small, oval-shaped structure situated on the nuclear membrane or it appeared as a thickening of the membrane.

In fibrocytes of the ligamentum patellae of females, 65–67% of the nuclei contained sex chromatin, compared with 0–7% in males.

**Skin.** Besides sex chromatin, many large granules of nuclear chromatin appeared in the cells of the epidermis. This made investigation of the sex chromatin much more difficult, for its granules could not always be clearly differentiated from nuclear chromatin. In addition, the detection of sex chromatin was hindered by the presence of pigment in the epidermal cells.

## EXPERIMENTAL RESULTS

The experimental results are given in Table 1.

**Bone Tissue.** Sex chromatin was found in the nuclei of osteocytes. As a rule these contain a finely granular nuclear chromatin. Some osteocytes had dense chromatin material, not forming separate granules (pycnosis). Sex chromatin could not be detected in such nuclei. In the osteocytes of females sex chromatin appeared as circular vesicles or granules lying close to the nuclear membrane (Fig. 1a). Sex chromatin was found in 43–62% of femoral osteocytes, and in 28–56% of osteocytes from the body of the cervical vertebra. As a rule no sex chromatin was present in the osteocytes of males. It was found only in a few nuclei as a small granule on the nuclear membrane. The largest number of granules of the sex chromatin type (6%) was found in the osteocytes from the male femur.

Determination of sex chromatin in bone tissue is rendered more difficult by the presence of pycnotic nuclei or of empty bony cavities not containing osteocytes. For this reason, it was necessary to examine many fields of vision.

**Cartilage Tissue.** Nuclear chromatin of the chondrocytes has a delicate structure, so that it was not difficult to detect sex chromatin in this tissue. In females, granules of sex chromatin were most frequently triangular or oval in shape. They were in contact with the nuclear membrane (Fig. 1b) or close to it. Sometimes the granules were of large size, almost as big as the nucleolus. In the cartilage cells of males, granules of the sex chromatin type also were found on the nuclear membrane, which appeared to be thickened.

In females, the largest quantity of sex chromatin was found in the laryngeal cartilage (59–65%), the smallest in the cartilage of the ear (18–56%). In males, the proportion of nuclei with sex chromatin was 0–18%.

**Muscle Tissue.** Sex chromatin was found in the muscle tissue of the tongue and heart at one pole of the nuclei, in the form of a compact granule (Fig. 1c). Sex chromatin was found distinctly in the muscle tissue of 40–60% of nuclei in females and in 1–4% of nuclei in males.

It was easier to test for sex chromatin in areas of skin (abdomen and medial surface of the ear) almost free from pigment. In the epithelial cells of females, the sex chromatin may be of 3 types: thickening of the nuclear membrane, or a circular or triangular granule located on the membrane (Fig. 1d).

Granules of sex chromatin were found very rarely in males. They were much smaller than in females. The quantity of sex chromatin in the skin of females varied between 26 and 56% of nuclei, and in males from 0 to 13%.

Lung. The nuclear chromatin in the alveolar epithelium was present as large granules, making detection of the sex chromatin more difficult. In most nuclei the sex chromatin of the alveolar cells was in the form of a triangular particle located on the nuclear membrane (Fig. 1e). In males, the sex chromatin was compact in structure and small in size. The percentage of sex chromatin in the lung tissue varied from 69 to 71, and in males from 0 to 7.

Liver. In the nuclei of the hepatocytes the nuclear chromatin consisted of numerous granules of different sizes, so that it was difficult to identify the sex chromatin in these cells. However, definite sexual dimorphism (Table 1) detected in the epithelial cells of the liver in males and females in blind tests (when the sex of the tissue was unknown) shows that this difficulty is no obstacle to the identification of sex chromatin.

The sex chromatin of the hepatocytes appeared as a thickening of the nuclear membrane or as an oval shaped particle (Fig. 1f) in 55-71% of nuclei.

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