CHROMOSOMES OF THE GUINEA PIG

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The extensive development of immunogenetic research has brought up the question of analysis of the chromosomes of experimental animals used in immunologic practice. The authors have studied the karyotype of the guinea pig (Cavia cobaya).

EXPERIMENTAL METHOD

The chromosomes were analyzed in the bone marrow cells, taken from guinea pigs sacrificed 1 h after intraperitoneal injection of colchicine in a dose of 0.01 ml of a 0.04% solution per gram body weight. The marrow was washed out of the guinea pig's femur with 1.12% sodium citrate ($C_6H_5O_7Na_3 \cdot H_2O$) solution, and incubated in the same solution at 37° for 30 min, after which the suspension was kept for 5 min in a refrigerator (0°). The citrate was then removed by centrifugation for 10 min at 400 rpm and replaced with cold fixing mixture (three parts of absolute methyl alcohol and one part glacial acetic acid). The cells were fixed for 30 min at 4°. The fixing solution was removed by further centrifugation and the cells were carefully resuspended in 45% acetic acid and a drop of the suspension was allowed to run down a preliminarily heated glass slide, where the cells were stained after drying with acetoorcein for 30 min.

The method described, which the authors used earlier to analyze mouse chromosomes [3], provides an adequate number of mitoses in the animals' marrow cells for investigation of the metaphase plates.

Photomicrographs were taken of all the metaphases studied and the chromosomes were measured.

EXPERIMENTAL RESULTS

The karyotype of the somatic cells of guinea pigs consists [7, 11, 12, 14] of 64 chromosomes (Fig. 1), among which only the first three pairs have been reliably identified, two of them being autosomes with a subterminal

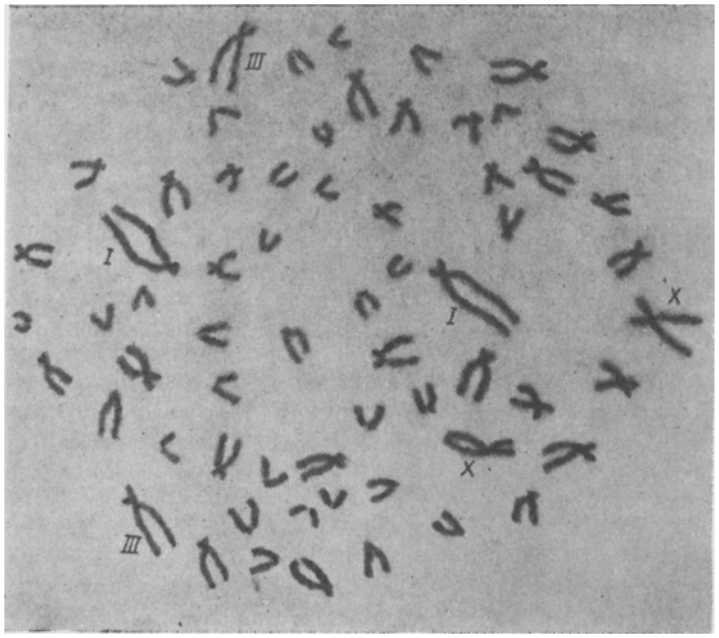

Fig. 1. Chromosomes of a female guinea pig.

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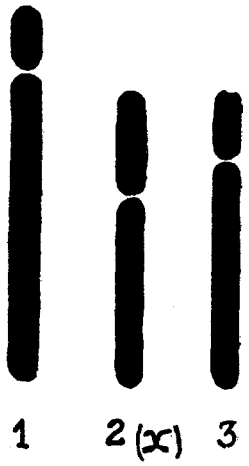

Fig. 2. Relative dimensions of the first three pairs of chromosomes in the karyotype of the guinea pig.

centromere while the other is a submetacentric X-chromosome. Because of the considerable heteromorphism, the dimensions of the latter vary. Some investigators therefore regard it as the third chromosome in order of size in the karyotype of the guinea pig, while others place it between the 1st and 2nd pairs of autosomes [11]. Measurements made by the authors support this latter view (Fig. 2), although the differences between the dimensions of the X-chromosomes and the chromosomes of the 3rd pair of the karyotype are very slight.

The X-chromosome in the karyotype of the guinea pig can be identified on a cursory examination of the metaphase plate, because it is the only large submetacentric among all the 64 chromosomes (index C = 35.4±0.49).

In female guinea pigs two X-chromosomes are found (Fig. 1), but in males only one (Fig. 3). The second half of the chromosome in males (the Y-chromosome) has not been identified. It is evidently one of the smallest acrocentrics.

The X-chromosome of the guinea pig is thus the only marker of the karyotype and it can be used to determine the genetic sex of the bone marrow cells. In the peripheral blood the genetic sex of these animals is easily found by examining the interphase nuclei of the neutrophil leukocytes [9].

The large autosomes of the guinea pig are no less interesting. As is clear from Fig. 2, they differ clearly from each other in size, and in addition, compared with the first pair of autosomes, the chromosomes of the 3rd pair are more metacentric (index C for the 1st pair 11.3±0.62 and for 2nd pair 24.2±1.22). Their identification and arrangement in pairs has not been established beyond question.

In 1961, Onno and co-workers [12] noted the constant heteromorphism of the short arms of the acrocentric 1st pair of chromosomes. In their opinion this phenomenon was due to inactivation of the companion region in one of the homologues of this pair of autosomes. This phenomenon has been investigated in detail by one of the present authors (I. L. Gol'dman).

In the bone marrow of guinea pigs a heteromorphic chromosome was found in nearly all the metaphase plates investigated (Fig. 3). The behavior of the short and long arms of the 1st pair of chromosomes of the karyotype was observed to be definitely independent. The heteromorphism associated with the short arms of the chromosomes was apparently fixed, i.e., it was constant in degree (as confirmed by statistical calculations) in all the pairs, whereas the long arms of these chromosomes behaved more or less independently. In different cells, sometimes the chromosomes carrying the companion, sometimes its homologue in turn was slightly longer. The variation in the size of the long arms of the first pairs of chromosomes averaged 15.2%.

In the experiments of Onno and co-workers (as in those of the present authors) not all the experimental animals were related, and this ruled out the possibility of structural heterozygosis of the 1st pair of chromosomes in the guinea pig's karyotype. However, the alternative hypothesis must also be verified. It is not impossible that this phenomenon in fact is mutational in character. On this basis it may be assumed that a system of lethal genes arises in the first pair of chromosomes. The zygotes of only one type would be viable in these circumstances – those carrying these alleles in a heterozygous state, manifested by structural heterozygosis of the 1st pair of chromosomes.

It is important to know whether in this case the homologues of the 1st pair of chromosomes in the guinea pig are interchangeable or, in other words, whether we can distinguish between the homologues, considering that in each cell the same chromosome (coming from the male or from the female) is inactivated (or aberrant).

According to the hypothesis [4, 10] put forward for the X-chromosomes, in different types of somatic cells in females inactivation of either the paternal or the maternal X-chromosome takes place in accordance with the principle of randomness. On this basis there are fairly reliable grounds for asserting that the homologues of the 1st pair of chromosomes of the guinea pig can be distinguished in the cells of a hemapoietic tissue. The same conclusion is reached if the mutational nature of the origin of this chromosome is accepted.

It was mentioned above that the 3rd pair of chromosomes (autosomes) of the guinea pig has been reliably identified (Figs. 1-3). In the authors' material the heteromorphism of the homologues of this pair of chromosomes did not exceed a mean value of 14%.

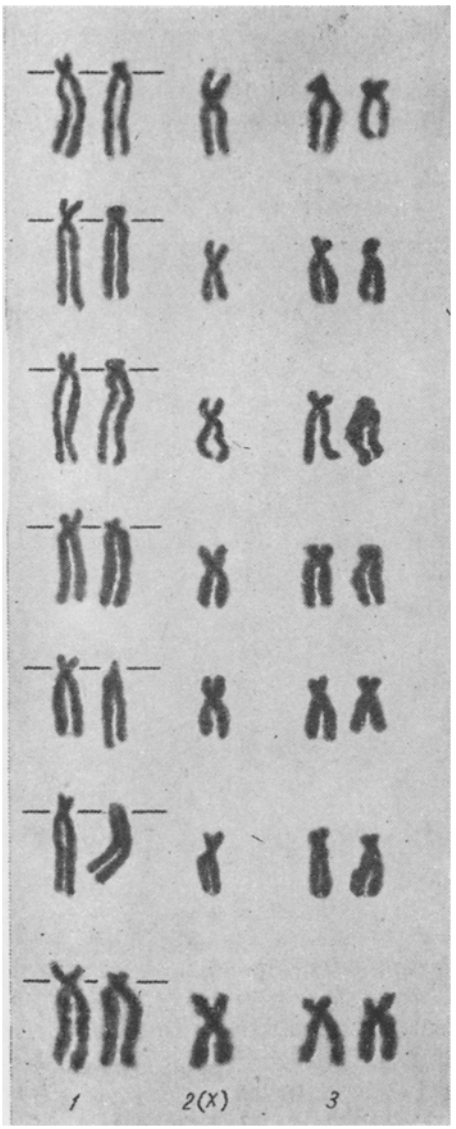

Fig. 3. Karyogram of the first three pairs of chromosomes of the male guinea pig.

Meanwhile, it has been reported in the literature [11] that structural heterozygosis at the 3rd pair of chromosomes is widespread in a population of Indian guinea pigs (Cavia porcellus).

When summarizing the results of the analysis of the morphological structure of the large autosomes of the guinea pig it must be recognized that the unusual combination of two types of structural heterozygosis of the autosomes encountered in these animals (associated with inactivation of the nucleolus-forming region of one of the 1st pair of chromosomes and heterozygosis at the 3rd pair of chromosomes – not due to inactivation – which is widespread at certain populations), makes individual identification possible within the homologues of these pairs of chromosomes. This cannot be found in any of the species of laboratory animals widely used for experimental purposes. In mice, for instance, among the 40 chromosomes composing its karyotype, generally speaking not one pair of chromosomes can be reliably identified [2, 8].

The central factor is undoubtedly the phenomenon of inactivation of the companion region in one of the first chromosomes of the guinea pig's karyotype. The genetic nature of this phenomenon will be investigated by the authors in the future.*

No structural aberrations of the chromosomes could be found (at least in the large chromosomes) in the bone marrow cells of healthy guinea pigs.

Considerable difficulty is being experienced at the present time in determining the true significance of the aneuploidy of mammalian somatic cells. The reason for this is that in badly scattered metaphase plates it is difficult to determine the number of chromosomes, and if they are well scattered, the possibility remains that one or several chromosomes may have been lost. It is assumed that this may be the mechanism responsible for the predominance of, for example, hemopoietic cells of the hypodiploid class in a population. Since the same method was used to analyze the bone marrow cells of different mammals, it was interesting to compare two species of laboratory animals differing in their chromosome number. In these circumstances it was natural to expect that if the main cause of the appearance of hypodiploids is mechanical loss of one or several chromosomes during cytologic treatment of the cells (centrifugation, treatment with hypotonic solutions, fixation), the greater the total number of chromosomes in the animal's karyotype the greater the total number of hypodiploids would be. The authors investigated aneuploidia in the mouse (40 chromosomes) and the guinea pig (64 chromosomes). In this experiment they compared healthy sexually mature individuals, because it has been shown in their laboratory that animals of different ages may possess a different background of aneuploid cells [1]. The experimental results confirmed the suggestion made above. In the bone marrow of the mice, in which the total number of aneuploid cells was 10%, there were 6% of hypodiploid cells [2], while in the guinea pig, in which the total number of aneuploid cells exceed 30%, $^4/_5$ of them were hypodiploid, as would be expected on the basis of theoretical calculation, although, of course, this could be pure coincidence. In an investigation by Tuscany [14], who analyzed aneuploidy in the bone marrow cells of guinea pigs, an even higher percentage of aneuploid cells was described.

*Additional investigations undertaken by the authors on animals obtained from various nurseries of the Academy of Sciences and Academy of Medical Sciences of the USSR confirmed the hypothesis that the structural heteromorphism observed in the 1st pair of chromosomes of the guinea pig is mutuational in character. In a population of guinea pigs, individuals were found with identical chromosomes in the first pair and a new type of structural heterozygosis associated with the deletion of the short arm of one of these chromosomes.

It is interesting that no disturbances in the sets of large chromosomes [1st 2nd (X), and 3rd pairs] were observed in any of the aneuploids (both hypo- and hyperdiploid cells) in the bone marrow of the guinea pigs.

On the average, for every 100 diploid cells in the bone marrow of the healthy animals one or two polydiploid cells were found, and the degree of polyploidy could be determined extremely easily after counting the X-chromosomes.

The results of this investigation and an analysis of data in the literature thus show that the guinea pig's chromosomes possess a number of specific features, the study of which may be a valuable addition to the genetic experiments performed on these animals [5, 6, 13, 15].

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