THE NORMAL KARYOTYPE IN MICE OF A HIGHLY LEUKEMIC AKR STRAIN

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The results of investigations in recent years have shown conclusively that the chromosomal apparatus of the cells of the hemopoietic system in man is affected in cases of leukemia. Hence, any study of the chromosomes in experimental animals with spontaneous or transplanted leukemia is of great interest.

The diploid number of chromosomes in the laboratory mouse is 40, the haploid is 20. The same number of chromosomes (n = 20 and 2n = 40) have been reported for the wild house mouse. In previous investigations, it has been found that such strains as CBA, RF, Strong A, DBA, C57, B1, C58, C3H, Swiss, etc. all have a chromosomal number 2n = 40.

The present work is concerned with an investigation into the normal karyotype of mice belonging to the highly leukemic strain AKR*.

EXPERIMENTAL METHODS

Chromosomes were investigated in the cells of bone marrow which was derived from mice previously (1 h, 15 min) given an intra-abdominal injection of colchicine (0.01 ml of 0.04% solution per g body weight). Bone marrow from both femoral bones was washed out with 1.12% solution of basic sodium citrate and this same material was placed in a thermostat at 37° for 20 min, after which the suspension was transferred to a refrigerator (0°) for 5 min. The citrate solution was removed by 10 min centrifugation at 400 r.p.m., and the cellular material transferred to chilled fixative (a mixture of 3 parts absolute methyl alcohol and 1 part glacial acetic acid). The fixative was added very carefully, drop by drop, down the side of the tube which was gently shaken to ensure thorough mixing and to maintain the cells in suspension. The bone marrow cells were fixed at 4° for 30 min. The fixative was then removed by repeated centrifugation, the cells were carefully suspended once again in 45% acetic acid, and a drop of suspension transferred to a previously prepared microscope slide, on which the cells were stained for 30 min in acetocarmine.

In general, we used a minimum volume of liquid ($\frac{1}{3}$-$\frac{1}{2}$ ml for bone marrow from two femora) at all stages of cytological investigation.

The described method which was used repeatedly, enabled us to obtain a sufficient number of mitoses in the cells of the bone marrow for successful investigation of metaphase plates.

The experiment was carried out on 25 mice of the highly leukemic strain AKR (15 males and 10 females).

EXPERIMENTAL RESULTS

Slightly curved chromatics of the acrocentric type were observed on the metaphase plates of bone marrow cells from the mice of the AKR strain.

*Synonyms: AK, AKm, Afb, RIL (H-2^k). Color white (aacc). Introduced into USSR in 1947. Frequency of spontaneous leukemias 64% in females, 48% in males. In recent years, as a result of selection, the frequency of leukemia in our substrain has increased to 80-85% [1,12].

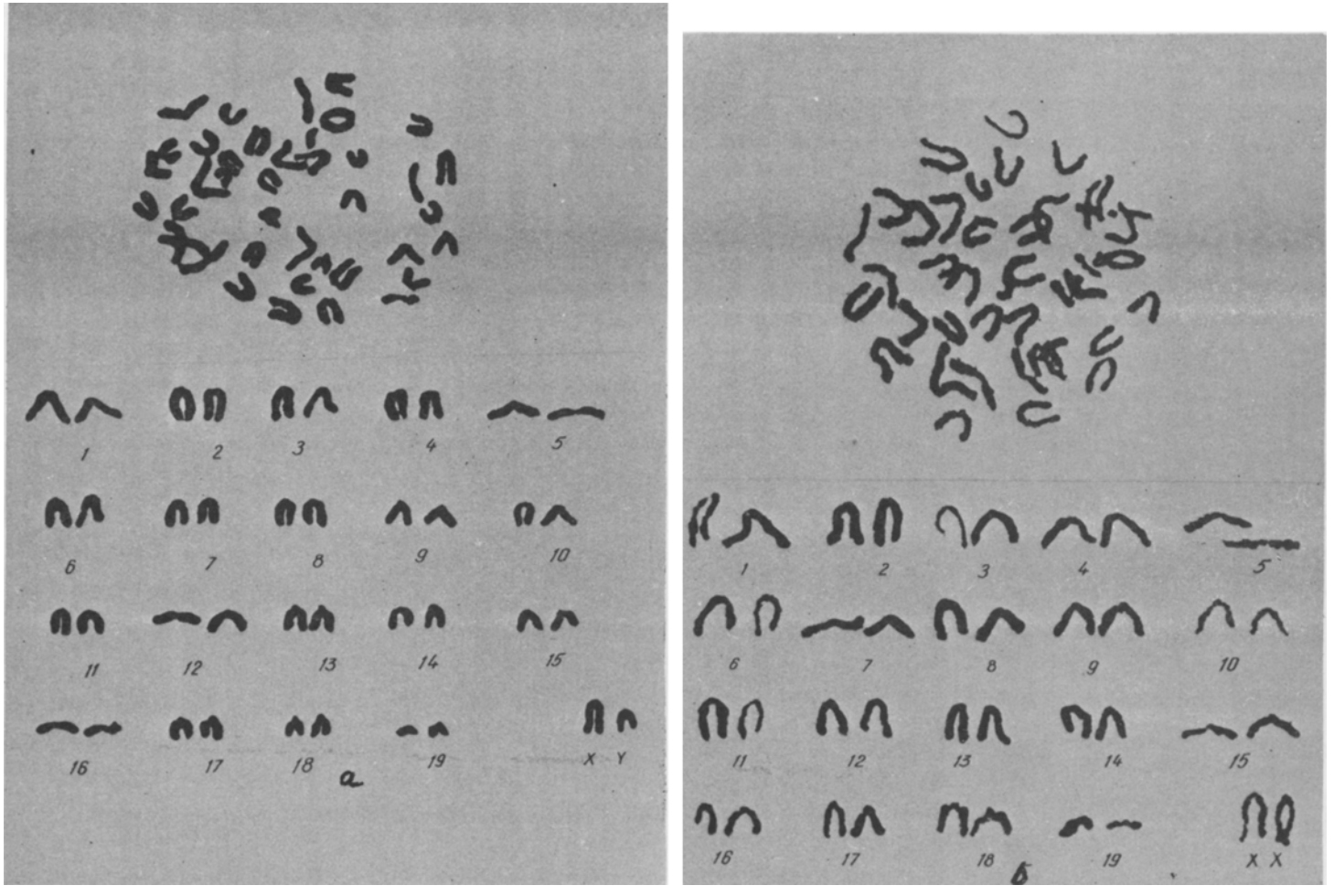

Fig. 1. Normal karyotype of mice belonging to the highly leukemic strain AKR: a) male; b) female.

Number of Chromosomes in Bone Marrow Cells of Mice Belonging to the Highly Leukemic Strain AKR and the Strain BALB/c

No. of animals	Strain and sex	Number of chromosomes															Total cells
		34	34	35	36	37	38	39	40	41	42	43	44	45	46	>46	
15	AK ♂		1					2	31	1						1*	35
19	AKR ♂							1	26								28
25	AKR ♀						1	1	33	2							37
Totals . . .			1				1	4	90	3						1	100
26	BALB/c ♀						3	1	64	1	1					2*	72
28	BALB/c ♂						1		25	1						1*	28
Totals . . .							4	1	89	2	1					3	100

*The plates consist of 160 chromosomes (8n). It is possible that they represent polynuclear megakaryocytes (Fig. 2).

The diploid number of chromosomes was found to be 40. On arranging the chromosomes in a decreasing line, it was possible to detect 19 pairs of autosomes and 2 sex chromosomes.

For convenience of karyological analysis, we propose to divide the chromosomes of mice up into 4 equal groups (Fig. 1) *

The most readily identifiable members of the karyotype are 5 pairs of large chromosomes which comprise the first group of the karyogram. Usually, the centromere occupies a terminal position in these chromosomes. In the large chromosomes, it is often possible to detect secondary threads which occur at the level of the middle third of the chromatid. The frequency with which they occur varies in different chromosomes and depends to a considerable extent on the method of preparation.

There is no particular difficulty in identifying the last pair of autosomes (No. 19); these are the smallest chromosomes in the mouse karyotype.

The absolute dimensions of the chromosomes vary considerably according to the degree of their spiralization and, therefore, in each particular case, we used the standard idiogram devised by Levan [8] to determine their position.

The majority of authors who have concerned themselves with the karyology of mice have stressed the difficulty of identifying chromosome pairs, because of the considerable individual variation in dimensions of each of the homologues. This difficulty can only be overcome by paying particular attention to the morphology and structure of the chromosomes. It is almost always possible to find some pairs of chromosomes with the centromere portion displaced to a subterminal position [6], giving a "rabbit's ear" configuration to them [8]. The chromosomes involved vary according to the particular strain.

Among mice of the highly leukemic strain AKR, the centromere portion of the chromosome may occupy a subterminal position in the 9th pair of the 2nd group, in the 11th, 13th, and 15th pairs of the 3rd group, and in the 17th, 18th, and particularly the 19th pair of chromosomes of the 4th group of the karyogram.

One important feature which facilitates the identification of individual chromosomes, is their distribution at metaphase. The probability of finding homologues adjacent to each other at this stage is very high in accurately prepared material.

The sex of the mouse can be determined by counting the number of short chromosomes in nuclei of somatic cells. The male sex possesses 3 small chromosomes, 2 of which constitute the last pair of autosomes, and the other

*Our investigations indicate that it is almost impossible to obtain a strict distributional representation of the karyotype of mice. However, it is possible to divide the chromosomes into 4 groups with a high degree of reliability and this assists the identification of abberant chromosomes and chromosome markers.

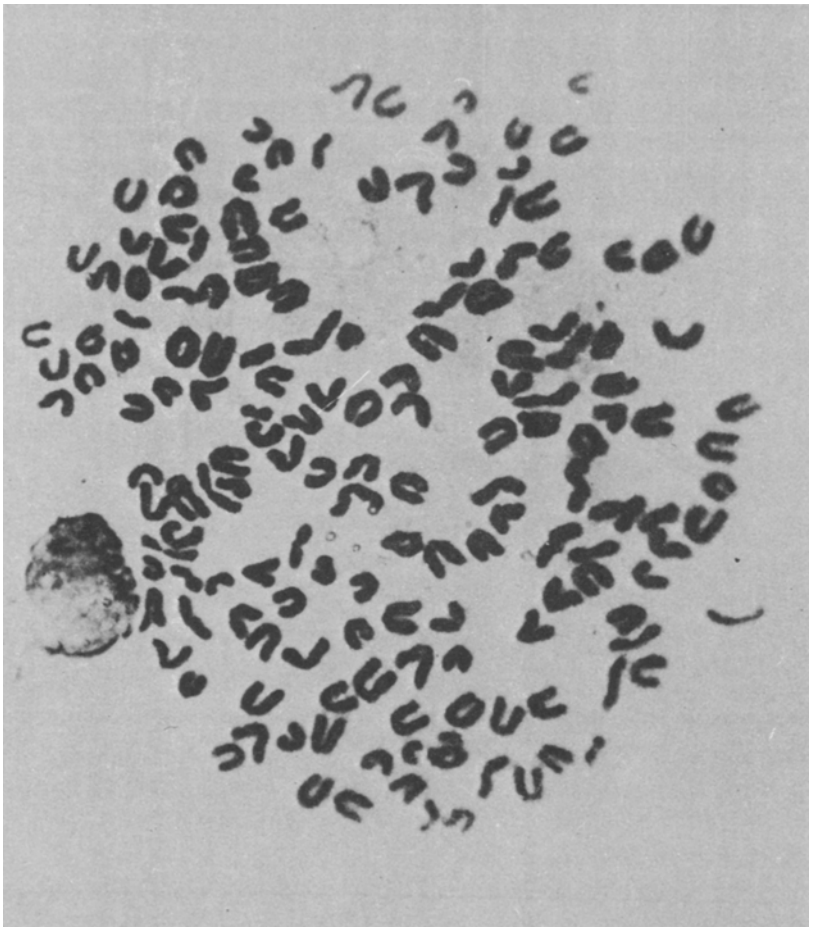

Fig. 2. Polyploid cell of mouse bone marrow in metaphase of mitosis. Microphotograph. Stained acetoorcein. Ob. × 90, oc. × 15.

being the Y-chromosome (Fig. 1a). The dimensions of the latter vary considerably in mice of different strains; thus, the Y-chromosome in mice of the C57 B1 strain is only ¼ as long as that of mice belonging to the Swiss and CZN strains [8]. In mice of the highly leukemic strain AKR, the Y-chromosome falls in between chromosomes of the 18th and 19th pairs of the 4th group in terms of its size.

The differentiation of the X-chromosomes is a matter of some difficulty. According to our observations, these are large chromosomes, next in size after the 2nd pair. One distinguishing feature of their morphology in mice of the highly leukemic strain AKR is the subterminal position of the centrome and their heterochromatism. The bone marrow cells of female mice are characterized by their possession of 2 small chromosomes and the sex chromosomes are represented by 2 large X-chromosomes (Fig. 1b).

Several authors [2,3,10,11] describe the variability exhibited by the chromosomal complement of somatic cells in mice. Thus, it has been found [14,15] that the number of chromosomes in cells of mouse uterine mucosa varies from 4 to 104, and in extreme cases 80% of examples exhibit hypodiploidy and only 10% are normal diploid cells. Similar findings have been mentioned in review articles [2] and in separate works describing the chromosomal numbers for liver cells [4], cells of the decidua of the uterus [9], and blood cells [7]. All these articles stress the so called variability of the chromosomal complement in somatic cells of mice [3,6]. However, in contrast to them, there are other writers who draw attention to the absolute constancy of the chromosomal complement in the somatic cells of mice [3,6]. The differences between the results obtained by various authors can be explained in terms of the very imperfect techniques used in karyological investigations [16].

We calculated the number of chromosomes in 100 metaphase plates of bone marrow cells from 3 mice of the

AKR strain (c.f. table); for this analysis, we selected rounded cells. Mice belonging to the BALB/c* strain were used as karyological controls.

It is evident from the table, that healthy, sexually mature mice of the highly leukemic strain AKR and leukemia resistant mice of the strain BALB/c have a similar background (10%) of aneuploid cells to the hemopoietic system†. We were unable to detect any structural changes in the chromosomes among the control group of mice belonging to the AKR strain.

Our findings are in agreement with the karyological results of foreign authors who have investigated one of the AKR substrains [13,16].

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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*Synonyms: Balb C, C. [H-2^d]. Color white (BBCC). This strain was introduced into the USSR in 1958. Resistant to leukemia [12].

†One of the authors in special investigations has established that the frequency of aneuploid cells in mouse bone marrow varies with age in a manner corresponding to a parabolic curve. From a high value at birth, the percentage of aneuploid cells falls until the period of sexual maturity and then increases again in old animals. Change in the population of aneuploid cells is characterized by a shift from hyperdiploidy at birth to hypodiploidy in old animals.