PRECOCIOUS CENTROMERE SEPARATION IN FIVE GROUPS OF CHROMOSOMES IN CONTROL AND *PSEUDOMONAS* CULTURE-TREATED MICE AND THEIR PROGENY

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ABSTRACT

Metaphase spreads of bone marrow chromosomes in nutrient broth-injected control and log culture of the bacterium, *Pseudomonas aeruginosa* (17×10^7 cells/ml) treated mice (@ 1 ml/100 g b.w.) and that of liver cells of 15-day-old embryos and bone marrow cells of 3-month-old F_1 and F_2 control and treated line individuals of different periods showed high frequency of precocious separation of centromere specially in gr. V among 5 groups in the metaphase complements. The frequency was greater in the treated series than in the control. The overall genic involvement for the precocious centromeric separation, its higher frequency in treated than in the control series due to the mutagenic property of *Pseudomonas* and the inheritance of the genic factor controlling the precocious centromeric separation in successive generations have been suspected. Further, the higher incidence of the precocious separation of centromere in chromosomes of specially gr. V in mice might serve as an additional mutagenicity testing protocol.

INTRODUCTION

It was so far taken for granted that mitotic chromosomes separate synchronously during anaphase but recently the sequential separation of centromeres in diploid set of chromosomes of human lymphocyte, Chinese hamster, mouse, rat, frog etc has been reported[1,2] Studies from our laboratory showed that while scoring the data of chromosome aberrations induced by chemical and living mutagens, some chromosomes specially those belonging to gr V of treated mice were precociously separated in a number of metaphase plates of bone marrow cells (BMC)[3,4]. With a view to verifying this the present study was undertaken and it revealed that (i) the frequency of precocious centromeric separation in 5 groups of mouse chromosomes was differential[5,6], (ii) the frequency was greater in *P. aeruginosa* culture-treated series than that of control, and (iii) the frequency was also greater in treated lines than that of control lines in F_1 and F_2 generations. Incidentally the bacterium, *P. aeruginosa* mutagenic to experimental mice was studied earlier by various mutagenicity testing protocols[3,7,8] and the present method might serve as an additional one.

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MATERIALS AND METHODS

Different sets of Swiss albino mice, *Mus musculus* from inbred laboratory stock were used. The uncontaminated nutrient broth in control and log culture of the bacterium, *P. aeruginosa* (17×10^7 cells/ml) in treated specimens were intraperitoneally injected at the rate of 1 ml per 100 g body weight per individual. Materials used for the study were: (A) BMC of 3-month-old male and female control and treated specimens of 7 weeks after the injection, (B) BMC of each control and treated male parent used for mating to different sets of 4 normal virgin females after completing 7 weeks of consecutive mating programme, (C) liver cells of 15-day-old embryos of 2nd, 3rd, 4th and 7th week control lines and treated lines of F_1 generation, (D) BMC of 3-month-old of 1st to 4th week control and treated lines F_1 males and females, and (E) BMC of 3-month-old 2nd and 4th week control and treated line F_2 males and females obtained by F_1 brother-sister mating of each week line. The chromosome preparation of liver cells and BMC of control and treated series was made following the standard colchicine-sodium citrate-acetic alcohol-flame drying-Giemsa staining schedule. The frequency of precocious centromeric separation in 5 groups of 40 diploid acrocentric chromosomes from 100 metaphase spreads per individual was determined. The normal karyotype

of mouse contains 4 chromosomes in gr I, 7 (1X in ♂) or 8 (2X's in ♀) in gr II, 20 in gr III, 6 in gr IV and 3 (Y in ♂) or 2 (no Y in ♀)[5,6] (figure 1). The treatment of log culture of *P. aeruginosa* to male parents induced sterility for which the data of some weeks out of 7 weeks mating programme to cover the spermatogenesis and spermiogenesis cycle could not be scored in F_1 treated line. This was not so in the control line (table 1). This also curtailed the scoring of the data of F_2 generation.

RESULTS AND COMMENTS

The occurrence of chromosomes with precocious separation of centromere in 5 groups was not at all regular. A plate might contain one or more cases (figures 2–6). With a view to finding out if there was differential responses between two sexes, the data of all intervals in males and females were combined separately to have a large sample for comparison in all series (table 1). The data showed that on the whole the frequency was slightly lower in females than that of males in both control and *Pseudomonas*-treated series. Further, the *Pseudomonas*-treated series and the treated line F_1 and F_2 individuals of both sexes had markedly high frequency of centromeric separation than the respective control (table 1).

The data of the group-wise distribution of the precocious centromeric separation in chromosomes of control and treated series have been compared in greater detail (table 2). An analysis of the data of each week of nutrient broth injected control and *P. aeruginosa* culture injected individuals (table 2A), male parents deployed for mating with normal virgin females in connection with lethal test (table 2B), liver cells of F_1 embryos (table 2C) and BMC of F_1 adults (table 2D), and BMC of F_2 adults (table 2E) revealed that the frequency of early centromeric separation in all groups of chromosomes was higher in the treated series than the corresponding control series. Further, among five groups of chromosomes the frequency of precocious centromeric separation was highest in gr V (figures 2, 4–6), the next was gr IV (figure 4), then gr III (figure 4) while it was very low in gr I (figure 2) and gr II (figure 3). The number of chromosomes having precocious centromeric separation in different plates was variable. It was mostly with one and that too a chromosome of group V (figures 5, 6).

RESULTS

It could be inferred from the present precocious centromeric separation data that (a) the males show slightly higher frequency than that of females both in control and treated series (table 1) possibly due to the fact that the Y chromosome in males belonged to gr V in addition to one pair of autosomes while in females there is only one pair of autosomes and no Y in gr V. Thus males having 3 gr V chromosomes instead of 2 in females could be the reason for higher frequency of centromeric separation cases if sex factor was not responsible and this has not been critically studied. (b) The pretreatment of colchicine for chromosome preparation did not possibly have special impact on the incidence of a higher number

Table 1 *Combined data of precocious separation of centromere in males and females separately of different series*

	Male					Female				
Description	Week series comb/line	No. of individual	No. of metaphase	No. of centr. separ.	%	Week series comb/line	No. of individual	No. of metaphase	No. of centr. separ.	%
Nut Inj	7 Control	14	1400	32	2.28	7 Control	14	1400	21	1.50
Bact Inj	7 Treated	14	1400	266	19.00	7 Treated	14	1400	262	18 71
F_1 embryo	7 Con line	42	4200	76	1.80	7 Con line	49	4900	68	1.38
F_1 embryo	4 Tr line	24	2400	81	3.37	4 Tr line	22	2200	50	2.27
F_1 adult	4 Con line	15	1500	39	2.60	4 Con line	20	2000	40	2.00
F_1 adult	4 Tr line	9	900	150	16.66	4 Tr line	10	1000	131	13.10
F_2 embryo	4 Con line	14	1400	31	2.21	4 Con line	12	1200	31	2.58
F_2 adult	3 Con line	8	800	19	3.75	3 Con line	7	700	19	2.71
F_2 adult	2 Tr line	4	400	53	13.25	2 Tr line	4	400	49	12.25
Total	25 Control	93	9300	197	2.11	25 Control	102	10200	179	1.75
Total	17 Treated	51	5100	550	10.78	17 Treated	50	5000	492	9.84

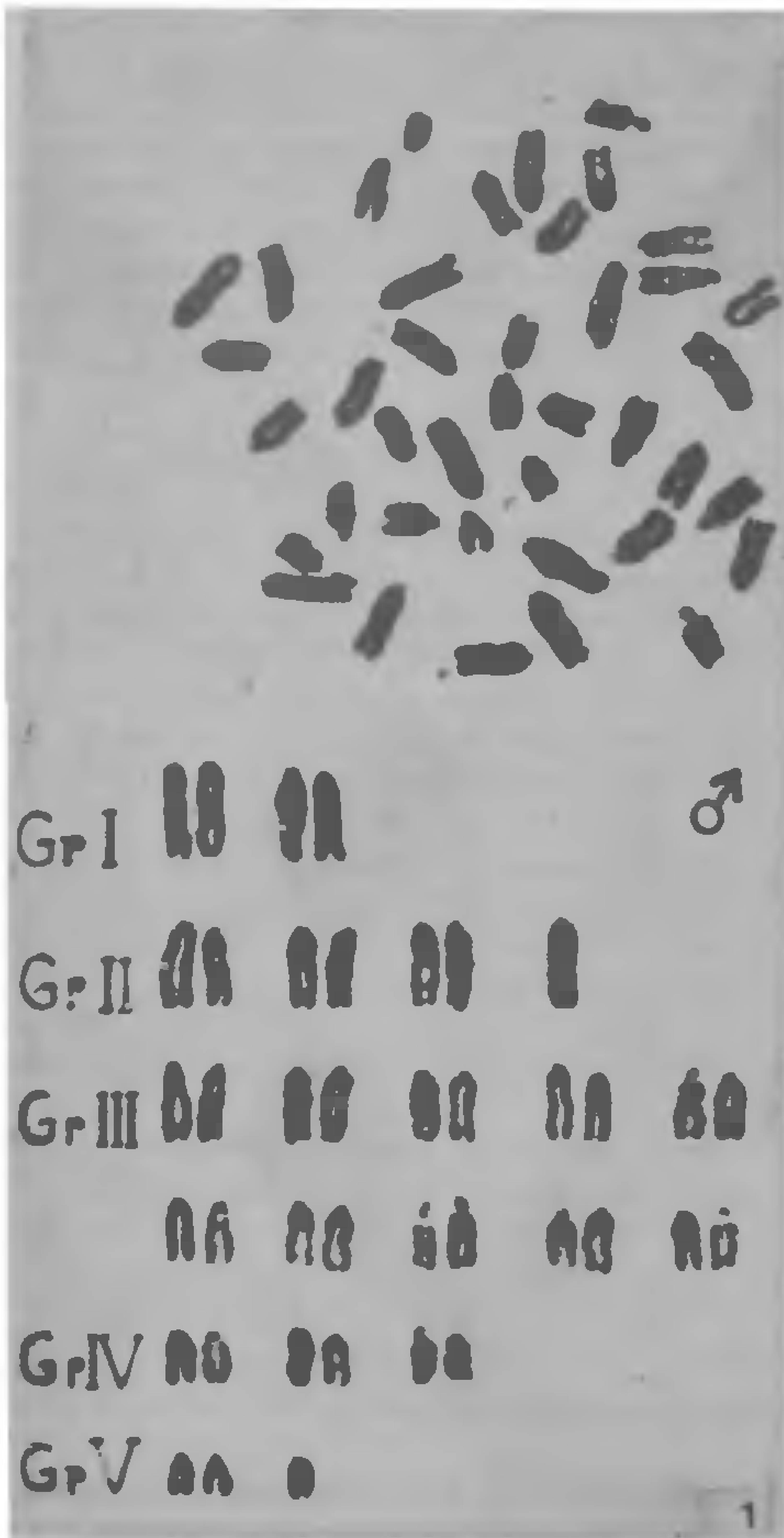
Table 2 *Frequency distribution of precocious centromeric separation in five groups of chromosomes in control and P. aeruginosa treated mice and in their progeny. Control data given in bracket.*

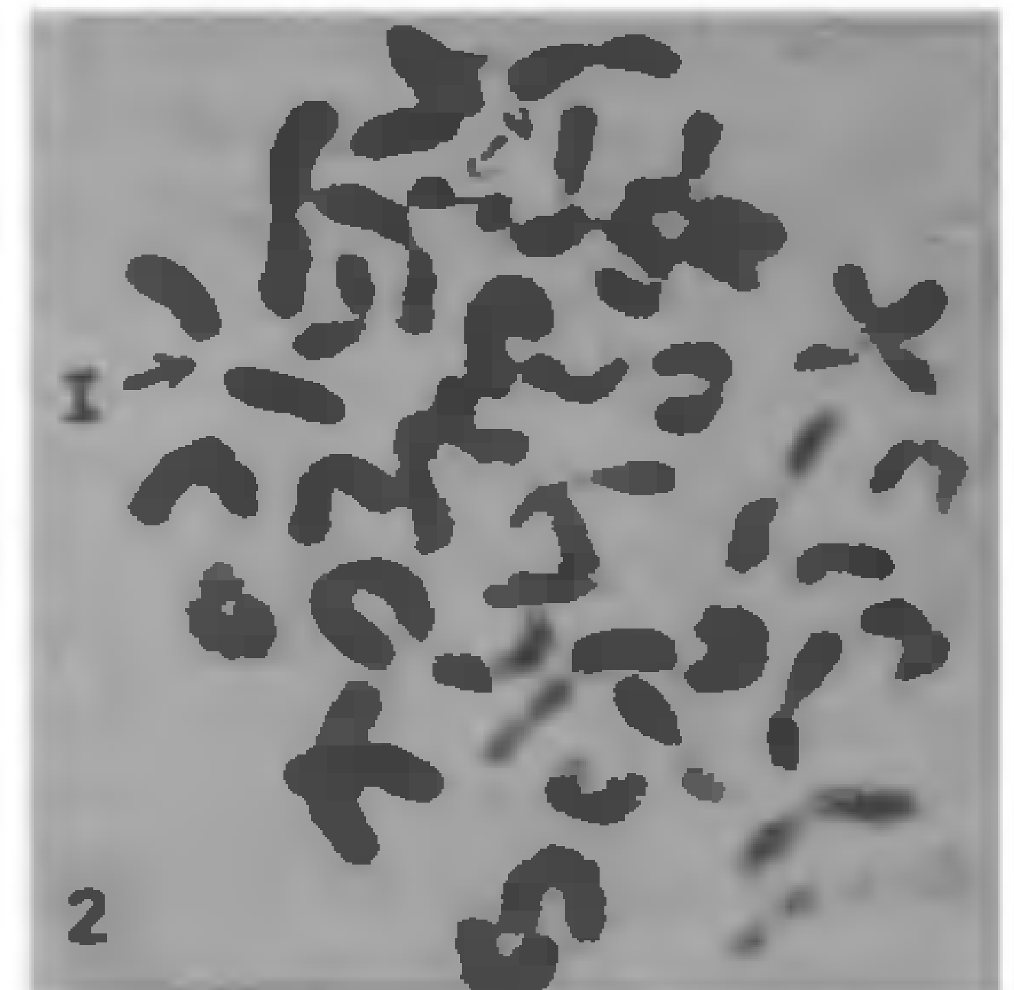
Set	Week/ line	No. of individual	No. of metaphases	No. of chrom. with precocious centromeric separation				
				Group I	Group II	Group III	Group IV	Group V
A. BMC of control and P. aeruginosa treated mice:								
	1st	4(4)	400(400)	1(0)	1(0)	19(0)	16(3)	34(5)
	2nd	4(4)	400(400)	1(0)	1(0)	13(0)	15(0)	20(5)
	3rd	4(4)	400(400)	0(0)	1(0)	32(0)	20(4)	37(9)
	4th	4(4)	400(400)	0(0)	1(0)	6(0)	8(0)	37(6)
	5th	4(4)	400(400)	0(1)	1(0)	11(1)	26(1)	70(9)
	6th	4(4)	400(400)	2(0)	0(0)	18(2)	35(1)	68(6)
	7th	4(4)	400(400)	1(0)	1(0)	19(0)	20(0)	34(0)
	Total	28(28)	2800(2800)	5(1)	6(0)	118(3)	140(3)	300(40)
B. BMC of control and treated male parents after 7 weeks of mating:								
	7th	4(4)	400(400)	0(0)	0(0)	3(0)	13(0)	29(4)
C. Liver cells of 15-day-old F_1 embryos:								
	2wk li	10(10)	1000(1000)	0(1)	0(0)	7(4)	0(3)	14(11)
	3wk li	13(11)	1300(1100)	0(0)	0(0)	1(4)	4(2)	9(18)
	4wk li	15(15)	1500(1500)	0(0)	2(3)	5(2)	8(4)	57(15)
	7wk li	8(15)	800(1500)	0(0)	0(0)	4(0)	8(2)	12(7)
	Total	46(51)	4600(5100)	0(1)	2(3)	17(10)	20(11)	92(51)
D. BMC of 3-month-old adults:								
	1wk li	3(5)	300(500)	0(0)	0(0)	6(0)	11(1)	17(12)
	2wk li	8(13)	800(1300)	0(1)	0(1)	5(7)	27(4)	51(20)
	3wk li	3(5)	300(500)	2(0)	1(0)	14(0)	14(0)	30(9)
	4wk li	5(12)	500(1200)	0(0)	1(2)	21(3)	35(4)	46(15)
	Total	19(35)	1900(3500)	2(1)	2(3)	46(10)	87(9)	144(56)
E. BMC of 3-month-old F_2 adults:								
	2wk li	4(4)	400(400)	0(0)	1(0)	2(1)	8(6)	34(7)
	4wk li	4(6)	400(600)	1(1)	0(0)	3(2)	9(9)	44(14)
	Total	8(12)	800(1000)	1(1)	1(0)	5(3)	17(15)	78(21)

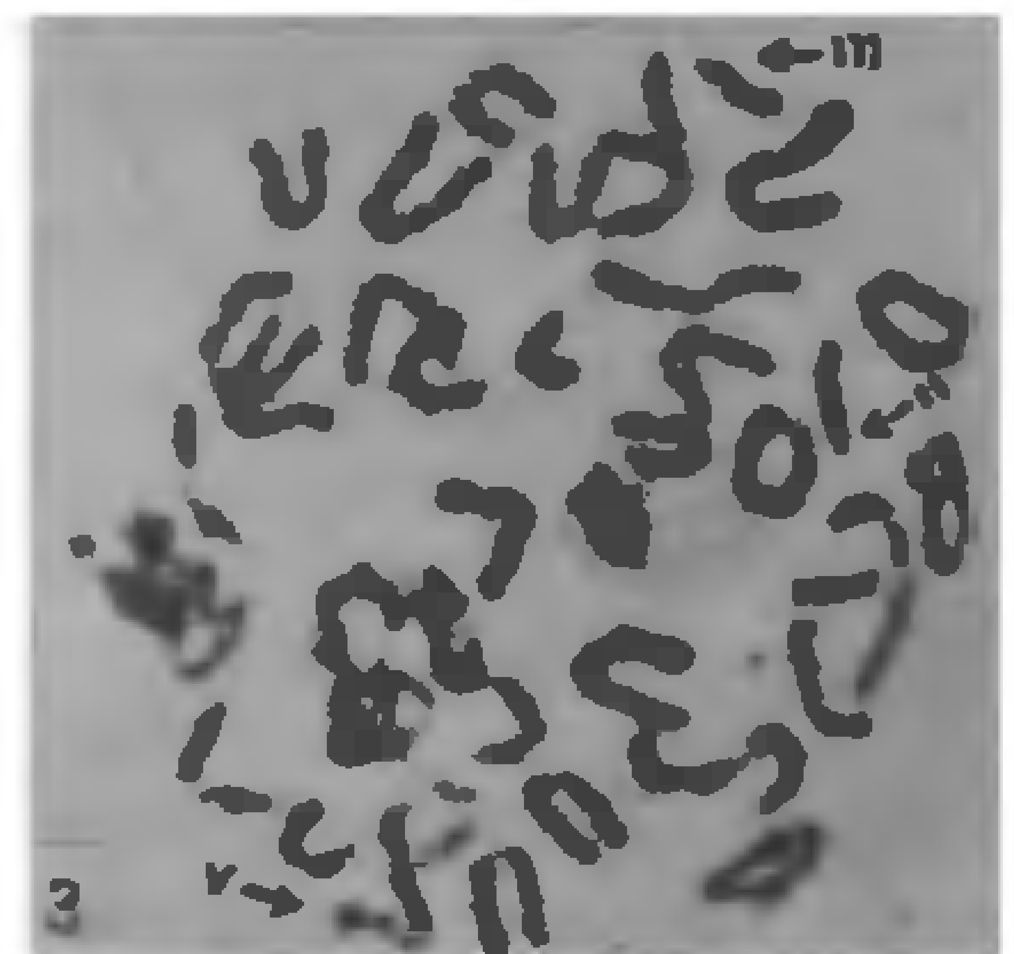
of centromeric separation in treated series than control since it was used in both cases. Moreover, the effect was not expected to be differential, (c) there was a size-frequency relationship, the shortest ones had the maximum and the longest chromosomes had the minimum frequency of centromeric separation, (d) more or less the same trend in centromeric separation frequency was shown by chromosomes either in control or in treated series from parent to progeny.

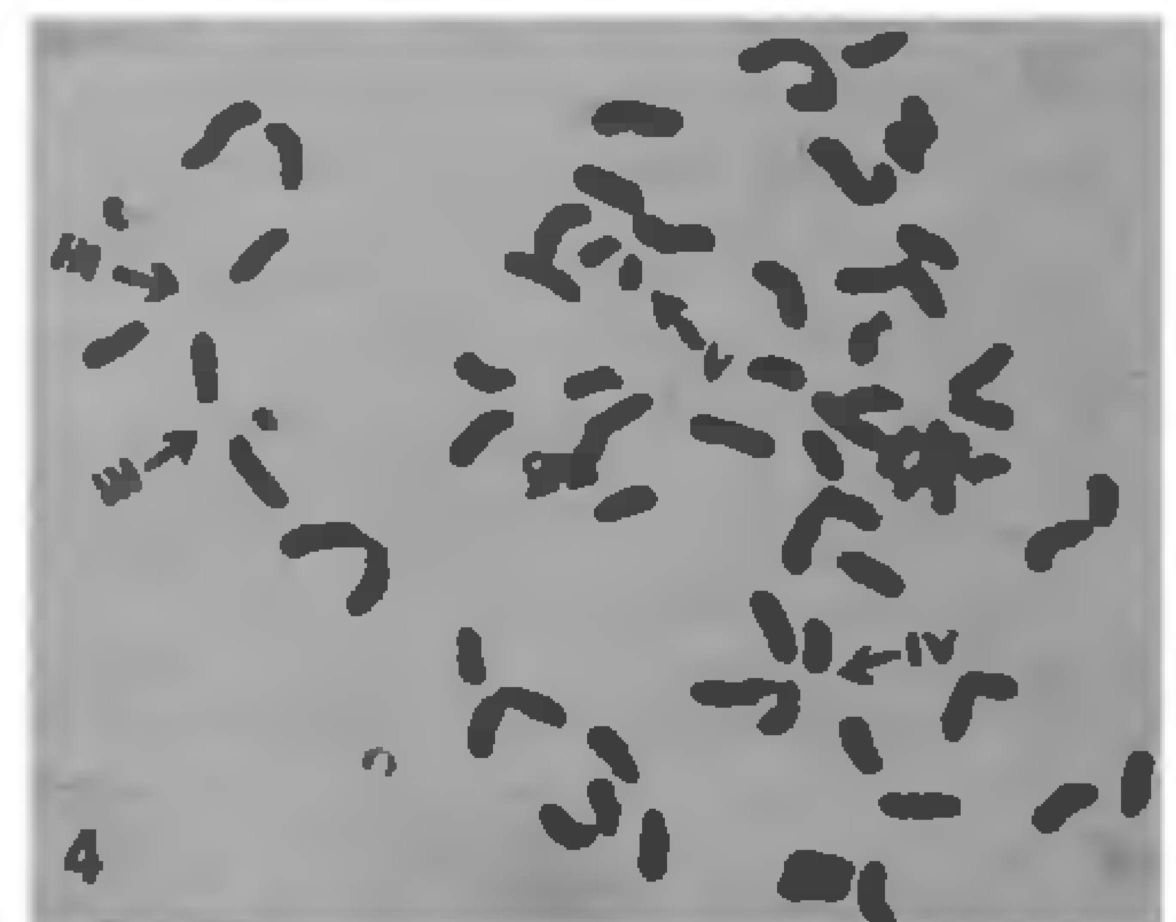
The present data show the genic involvement in precocious centromeric separation because (i) in *P aeruginosa*-treated specimens the frequency of precocious centromeric separation in chromosomes was markedly high than in control, (ii) the trend of higher frequency was maintained in F_1 and F_2 generation and (iii) the variations in the data in F_1 and F_2 generations of the treated line in different weeks could be due to the non-random inheritance of the genic factor producing them. The occurrence of the somatic chromosome aberrations in higher frequency in F_1 and F_2 treated lines using chemical and living mutagens than that of controls has been explained on the same presumption[4,9].

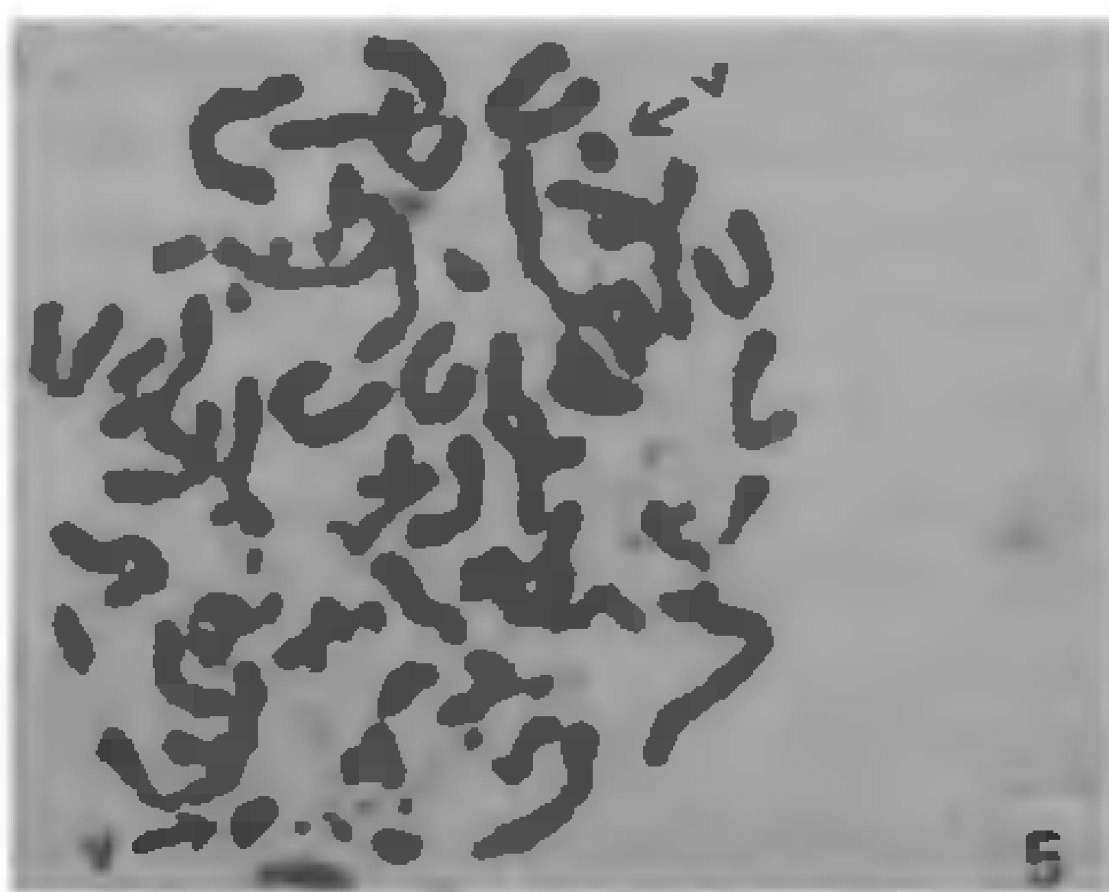
The differential responses of precocious separation of centromere in chromosomes belonging to different groups as well as between control and treated series could not be precisely explained. One might suspect that it was due to colchicine pretreatment, flame-drying etc used for chromosome prepa-











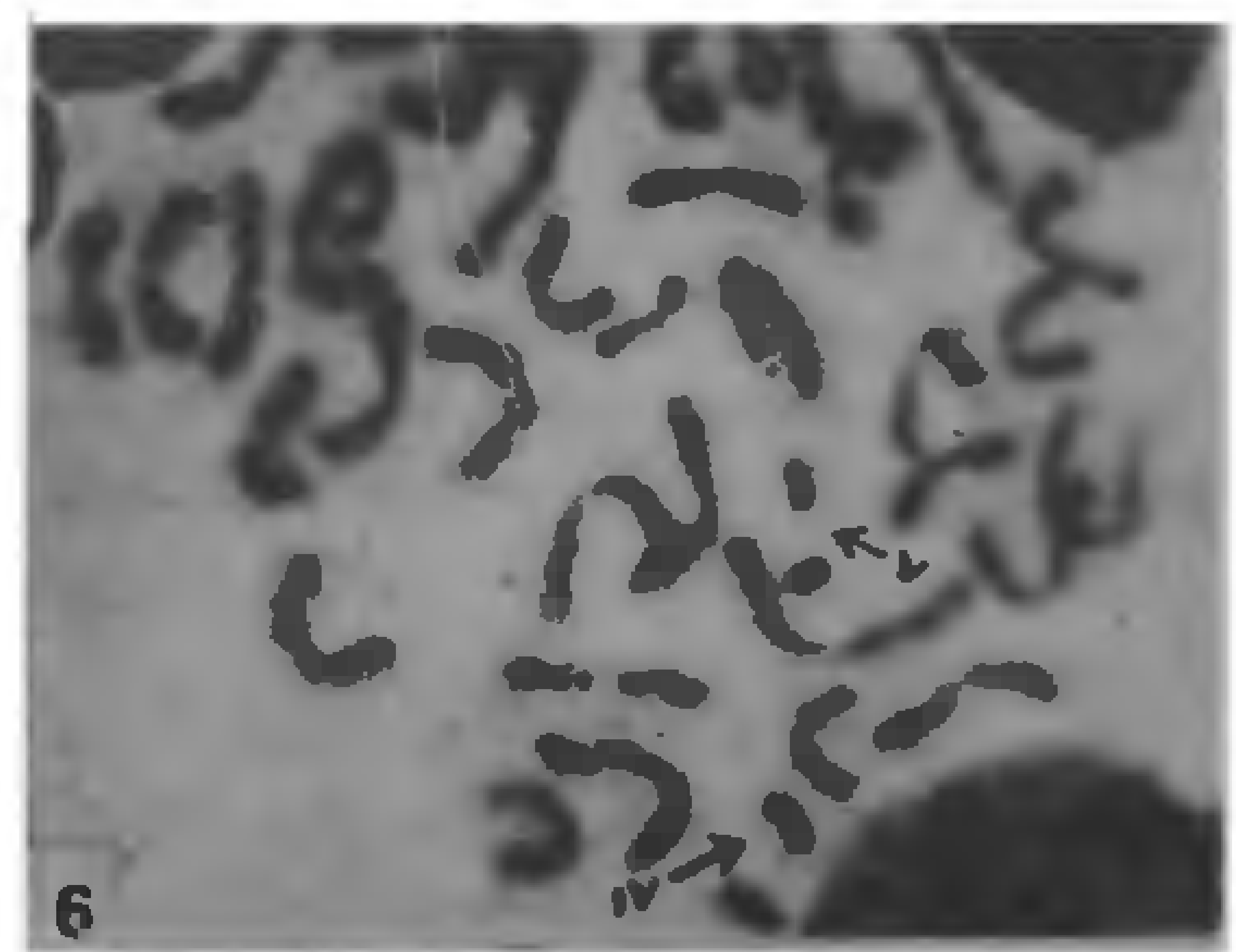


Figure 1. Karyotype of male mouse arranged in five groups. **2–6.** Metaphase plates showing precocious centromeric separation in different groups of chromosomes of bone marrow cells.

ration but the possibility was remote as explained before or even it could be found without colchicine pretreatment. On the other hand, the behaviour of chromosomes appeared to be genetically controlled. The sex chromosomes during meiosis in insects are known to behave asynchronously[10-12]. It has now been seen in somatic chromosomes of many mammals normally[1,2] and at present under experimental condition as well. The occurrence of a 'Barr' body in buccal epithelia of normal female is another example.

Incidentally it might be mentioned that the exact nature of the mutagenic agent produced by the *P. aeruginosa* is not known because the treatment to separate sets of mice with log culture, log culture filtrate after the complete removal of bacteria and the isolated bacteria in saline suspension induced much higher frequency of bone marrow chromosome aberrations than that of heat-killed bacterial sample while all the treated samples had higher effect as compared to controls[13]. The same situation was also found with treatment of log culture, culture filtrate and isolated bacteria of *Staphylococcus aureus*[14] and others[13,15] for bone marrow chromosome aberrations in experimental mice. It was suspected that the bacterial toxin could be the mutagenic factor[13,15] though the effect of the component parts of bacterial cells is yet to be undertaken.

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