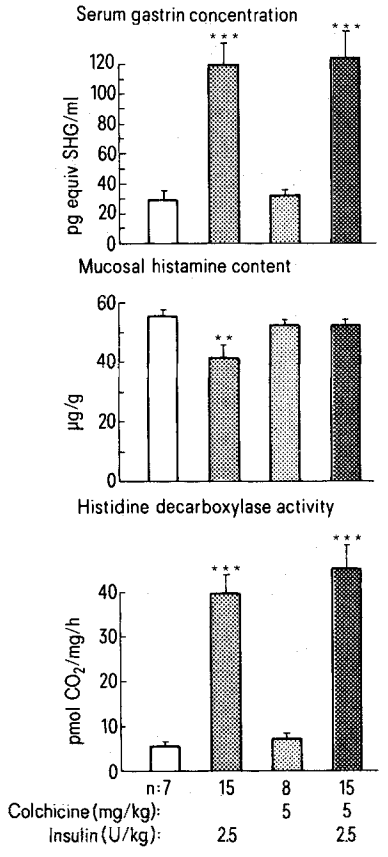

Effect of insulin on serum gastrin concentration, gastric mucosal histamine content and histidine decarboxylase activity after pretreatment with colchicine. Statistical analysis was made according to Student's t-test: Significant difference between untreated controls and drug-treated animals are indicated by **for $0.001 < p < 0.01$ and ***for $p < 0.001$. Colchicine alone or in combination with insulin had no significant effect on the mucosal histamine content, whereas insulin alone caused reduction of the histamine content ($0.001 < p < 0.01$). The difference in serum gastrin concentration and histidine decarboxylase activity between colchicine-treated and colchicine + insulin-treated rats was significant ($p < 0.001$).

blood was drawn from the aorta. Serum was lyophilized and stored at −25 °C until analysis. Gastrin was determined by radioimmunoassay, using antibodies raised in rabbits against synthetic human gastrin I. The assay technique, its accuracy and reliability on rat serum has been described elsewhere[6]. The oxyntic mucosa was scraped off the stomach wall and homogenized in 0.1 M phosphate buffer, pH 7.0, to a final concentration of 100 mg (wet weight) per ml. The histamine content of these extracts was measured fluorometrically[2] and the histidine decarboxylase activity was determined radiometrically as described in detail elsewhere[6].

Colchicine had no effect on serum gastrin concentration, gastric histamine content or histidine decarboxylase activity in fasted rats. However, colchicine prevented the reduction of gastric histamine following injection of insulin (figure) or pentagastrin (table), but did not prevent the activation of histidine decarboxylase. Insulin-stimulated gastrin release was not inhibited by colchicine (figure).

Microtubules are thought to be involved in the process of peptide hormone secretion, e.g., by promoting margination of cytoplasmic granules prior to exocytosis. Colchicine is known to prevent the assembly of subunits into microtubules[7] and has therefore been widely used in studies on the role of this organelle in hormone secretion. Our results seem to suggest that release of histamine from the endocrine cells in rat oxyntic mucosa is dependent upon an intact microtubular arrangement. Further, the results argue strongly against the contention that histidine decarboxylase is activated as a result of a reduced histamine content. On the contrary, it appears that gastrin-stimulated histamine release and gastrin-stimulated activation of histidine decarboxylase are 2 independent processes. Finally, the results give no indication that colchicine blocks gastrin release. This will be the subject of a separate study.

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Chromosome aberrations in mice by the antifungal antibiotic, nystatin

G. K. Manna and S. Bardhan

Department of Zoology, University of Kalyani, Kalyani (West Bengal, India), 23 June 1976

Summary. Nystatin, a fungicide of current medical use, was tasted in mice for its effect on chromosomes of bone marrow cells. A significant increase of aberrations, mostly of chromatid type, was observed over a period of from 15 min to 15 days following the application of the drug. Our data indicate a non-random distribution of the breaks.

The antifungal antibiotic nystatin, isolated as an intracellular product of Streptomyces nourscei[1] was found effective also in human monilial infections. The chromosome-breaking property of nystatin appeared not to have been studied before, which initiated the present investigation.

Material and method. 6-week-old random bred Swiss strain mice, Mus musculus, were injected with a 25% aqueous solution of nystatin[2] at a dose of 50 mg/kg b.w. (equivalent to a current human therapeutic dose), and another set with distilled water as controls. Their bone marrow cells were fixed at 8 different intervals (table 1) for the assessment of chromosome aberrations from slides prepared according to the colchicine-citrate-acetic alcohol-Giemsa-air-drying technique[3,4].

Results. As found with the use of other antibiotics[4–9], the aberrations induced by nystatin were mainly chromatid constrictions and gaps, subchromatid and chromatid breaks, and to a minor extent fragments of unknown origin and translocations. In the control series, the last 2 types were not encountered, while the frequencies of other types were very low (table 1). The average was

Table 1. Data of chromosome aberrations in 300 metaphases at each fixation time determined from 4 (2♂ + 2♀) nystatin treated and 4 (2♂ + 2♀) distilled water control specimens (control data are in brackets)

Fixation time	Types of aberrations Chromatid break	Subchromatid break	Constriction	Gaps	Fragment	Translocation	aberrations	Net per cent aberrations
15 min	7	2(2)	1	–	–	–	10(2)	2.6
4 h	12	3(1)	–(1)	–	–	–	15(2)	4.3
12 h	13	1	2(2)	2(1)	2	1	21(3)	6.0
24 h	13	2	–	–(1)	–	1	16(1)	5.0
72 h	9	1	2(2)	–	–	1	13(2)	3.6
5 days	13(1)	3	1(1)	1(1)	–	–	18(3)	5.0
9 days	3	–	1	1(2)	–	–	5(2)	1.0
15 days	1	1(1)	2(1)	–	–	–	4(2)	0.6
Total	71(1)	13(4)	9(7)	4(5)	2	3	102(17)	3.5

0.7%, of which 0.2% constituted the true break-type aberrations which would perhaps fall within the limit of spontaneous incidence.

In the treated series, the affected metaphases contained generally one aberration, sometimes two, but there was no gross effect on the entire complement. No isochromatid breaks were encountered but very rarely a chromosome had 2 types of aberrations in 2 chromatids at the identical position. Among different types of aberrations, the breaks in chromatids were found in different groups and regions of chromosomes with fragments lying at various distances. Their frequency was relatively high from 15 min up to the 5th day, but with no definite peak (table 1). After deducting the control data, the net frequency of total aberrations at different intervals up to the 5th day was quite high, with peak at 12 h. The average frequency was 3.5%.

With a view to finding out if the breaks in chromatids were randomly distributed or not, 71 breaks were analysed according to the method described elsewhere[3,4,13]. A comparison of the observed and expected number of chromatid breaks (table 2) revealed that chromosomes belonging to group II were more vulnerable, while those of groups III–V were relatively resistant to the action of nystatin. On the whole, the breaks were nonrandomly distributed in 5 groups of chromosomes ($p < 0.001$)[10]. Further, if the affected chromosomes were arbitrarily demarcated into 3 equal regions, the distribution of chromatid breaks would indicate that the distal region was more vulnerable while the proximal region was quite resistant in chromosomes of groups I–III ($p < 0.001$). Therefore, the analyses made above led us to conclude that the breaks in chromatids were nonrandom and somewhat localized.

Comments. The present study has revealed chromosome-breaking properties of the antifungal drug nystatin which are even higher than those of antibacterial antibiotics[4–9]. In connection with its mode of action on lower organisms, it was suggested[11] that a sterol was required for its binding to cell membranes and that it affects cell permeability. It was thus put among the polyene group of antibiotics[12]. However, this mode of action would not help to account for the chromosome aberrations in mice. Nystatin appears to have acted on chromosomes as and when they entered into division, because the chromatid type aberrations were encountered at 15 min but also as late as on the 15th day. As suggested elsewhere[5,13,14],

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Table 2. Group-wise and region-wise distribution of breaks in chromatids induced by nystatin (* for female karyotype)

Region of break	No. of breaks in chromosome groups I	II	III	IV	V	Total observed	Expected
Proximal	1	2	2	1	–	6	23.3
Middle	2	12	9	1	–	24	23.3
Distal	6	22	12	1	–	41	23.3
Total observed	9	36	23	3	–	71	
*Exp. as per No. of chromosome	7.1	14.2	35.5	10.65	3.55	71	
*Exp. as per cent length	9.23	16.33	33.79	8.52	2.13	71	

nystatin very likely inflicts damage on dividing chromosomes due to some physico-chemical stress and not through the synthetic phase of DNA.

Kihlman[15], reviewing work on chemically induced chromosome aberrations, stated that the breaks were generally of chromatid type and nonrandomly distributed within and between chromosomes. He[15] suggested some probable mechanisms involved for the localized breaks induced by MH, EOC, CA, BUdR, etc. However, the analysis of our data of chromatid breaks induced by nystatin and other antibiotics and mutagens lead us to conclude that the breaks are mostly due to some stress on inherently weaker regions in chromosomes[13, 14].

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Telocentric chromosomes in pearl millet, Pennisetum typhoides

P. S. R. L. NARASINGA RAO, M. V. SUBBA RAO and I. NARAYANA RAO[1]

Department of Botany, Andhra University, Waltair–530 003, (India), 22 September 1975

Summary. In *Pennisetum typhoides* S. and H., a new karyotype was found with 13 normal chromosomes and 2 stable telocentric chromosomes, which represent 1 arm each of a submetacentric chromosome.

Pennisetum typhoides Stapf and Hubb., has a diploid number of $2n=14$ chromosomes. In the normal complement, 5 pairs of chromosomes with near median to submedian centromeres and 2 pairs of chromosomes with subterminal centromeres are present[2]. In 4 out of 97 seedlings raised from a $3n \times 2n$ cross, a different karyotype with $2n=15$ chromosomes was observed. Seeds from the $3n \times 2n$ cross were germinated in petri dishes on moist filter paper root-tips from germinating seedlings were pretreated in 0.003 *M* 8-hydroxy quinoline for 4 h at 14–16 °C, fixed in glacial acetic acid for 12 h and stained by Feulgen technique. The new karyotype differed from the normal one in the presence of 2 small chromosomes, in which primary constrictions were not detected. The 2 small chromosomes were unequal in size and roughly corresponded to the 2 arms of one of the submetacentric chromosomes (Figure 1), and thus were apparently telocentrics.

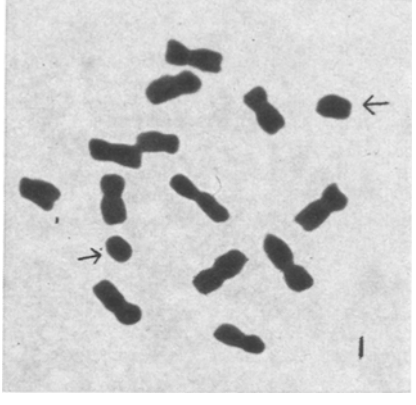

Fig. 1. Chromosome preparation from root tips showing 2 telocentrics (arrows) and 13 normal chromosomes.

Acetocarmine squashes of PMCs from these plants also revealed 15 chromosomes. In all the 200 PMCs observed at diakinesis, the 15 chromosomes formed 6 bivalents and a trivalent. The trivalent was a heteromorphic chain of 3, with 2 small chromosomes associated one on each end of a longer middle chromosome (Figure 2). The 2 small chromosomes were not observed to pair with each other directly. In more than 60% of the PMCs at metaphase-I, the trivalent was oriented with the normal chromosome towards one pole and the 2 smaller chromosomes towards the other pole (Figure 3). This orientation would lead to disjunction of the normal chromosome from the 2 smaller chromosomes, and thus daughter nuclei with 2 small plus 6 normal chromosomes and 7 normal chromosomes would result. Such types of disjunction were observed at ana-

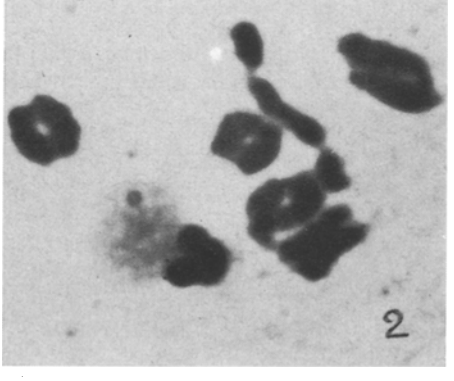

Fig. 2. PMC at diakinesis showing a trivalent (centre) with the telocentrics, one on each end of a longer middle chromosome.

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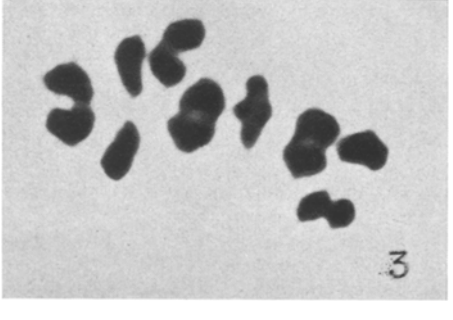

Fig. 3. PMC at the end of metaphase-I showing orientation and disjunction of the trivalent (extreme right), where the telocentrics separate from the normal chromosome.