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The bacterium, *Staphylococcus aureus* induced chromosome aberrations with their protection by penicillin, and mitotic inhibition in Syrian hamsters

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Abstract. The frequency of bone marrow chromosome aberrations in Syrian hamster, *Mesocricetus auratus* induced by the injection of log culture, saline suspension and culture filtrate of *S. aureus* was significantly high while the treatment of heat-killed bacterial suspension showed no difference with control data. In the first three treated series aberrations were of individual and gross types while others had only individual types. The chromatid breaks were nonrandom, the centromeric region being less vulnerable and the distal region was more susceptible. The aberration frequency was significantly reduced when log culture, saline suspension and culture filtrate treated specimens were also injected with penicillin 1 hr before, simultaneously and 1 hr after, of which the post-treatment rendered the maximum protection. Lastly, the treatment of isolated bacterial sample retarded the mitotic frequency significantly as compared to that of normal and controls.

Keywords. *Staphylococcus aureus*; induced chromosome aberrations; penicillin protection; mitotic inhibition; Syrian hamster.

1. Introduction

The chromosome aberrations in *in vitro* and *in vivo* cells of mainly man and mammals induced by pathogenic micro-organisms have been reviewed by some workers (Aula 1965; Bartsch 1970; Manna 1980). Manna (1973, 1980) classified them as 'living mutagens' in addition to physical and chemical mutagens. Since reported first by Hamper and Ellison (1961), studies on virus induced chromosome aberrations were carried out vigorously in different countries because of their carcinogenic and teratogenic implications (Aula 1965; Bartsch 1970; Manna 1980). On the other hand bacteria-induced chromosome aberrations have been studied almost exclusively by Manna and his collaborators (see Manna 1969, 1973, 1980), showing not only the same possibility of somatic mutation but also the hereditary implication in mice (Manna and Dey 1979; Manna 1980). The present study was undertaken with a view to finding out the effects of the human pathogenic bacterium, *Staphylococcus aureus*, on bone marrow cells of Syrian hamster, with regard to the frequency of chromosome aberrations at various

intervals, localization of breaks, if any, protective action of penicillin though some study was carried out earlier in mice (Manna and Chakraborty 1970, 1975) on frequency of aberrations and mitotic retardation against suitable controls.

2. Materials and methods

For the study of the aberration frequency, male Syrian hamsters, *Mesocricetus auratus* of about the same age and weight, were injected intraperitoneally at the rate of 1 ml/100 g body weight (1) in the control series with (a) freshly prepared nutrient broth, (b) incubated nutrient broth and (c) 0·85% normal saline and (2) in the treated series with (i) the culture at the log phase of growth (513 × 10^7 cells/1 ml), (ii) approximately the same number of isolated bacteria (513 × 10^7 cells/ml) suspended in normal saline, (iii) log culture filtrate after the complete removal of all bacterial cells and (iv) heat-killed bacterial suspension of *Staphylococcus aureus*. The bone marrow cells were fixed at various intervals (table 1) to assess the degree of chromosome aberrations from colchicine-citrate-acetic alcohol-flame drying-giemsa stained bone marrow chromosome preparations (Manna and Bardhan 1973). Standard bacteriological methods were followed to determine the log phase of growth, the approximate number grown per ml, their isolation and resuspension, etc.

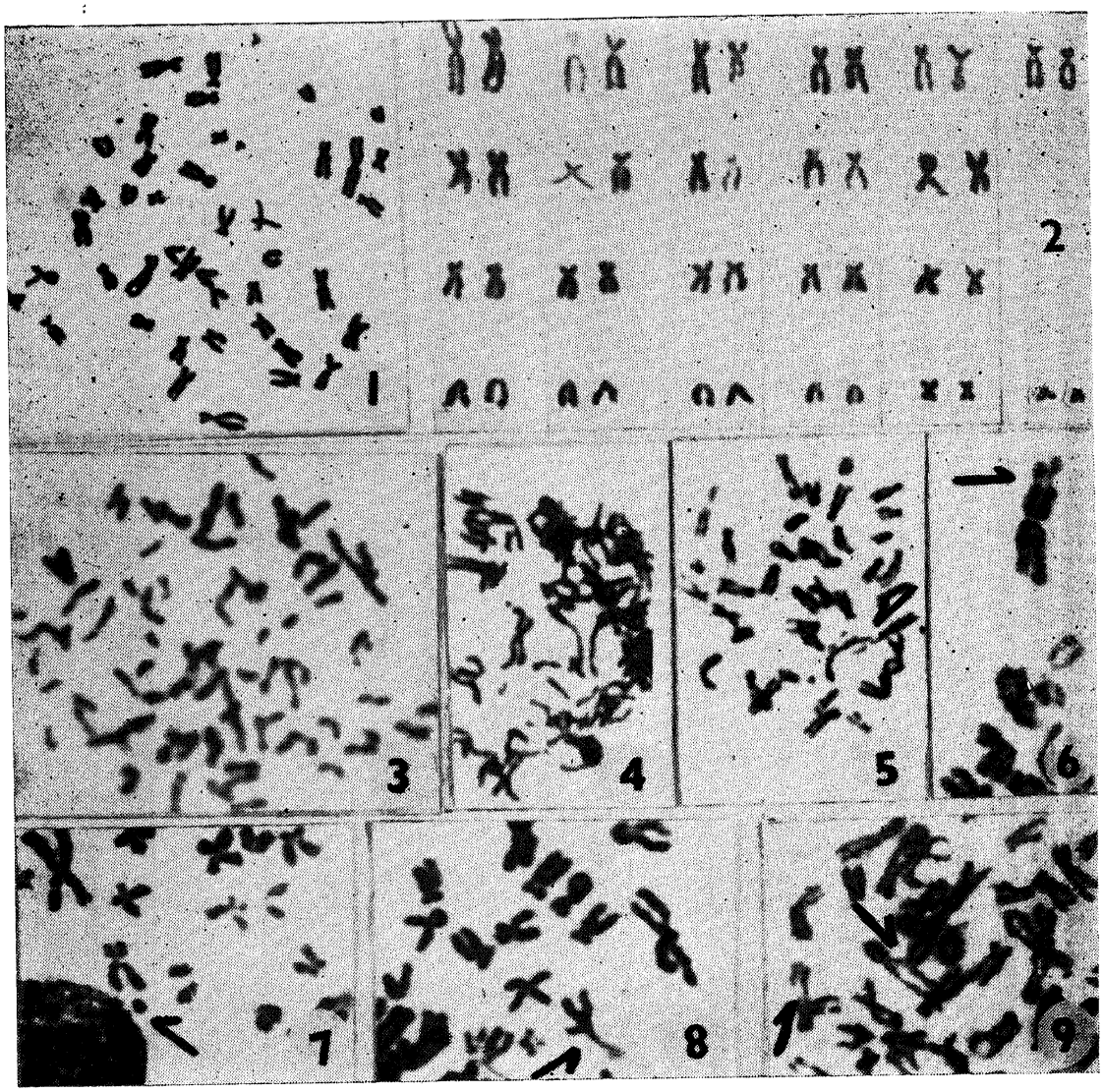
For the study of the protection of aberration frequency by antibiotic male hamsters were injected with a human dose-equivalent of penicillin solution one hour before, simultaneously and one hour after the injection of log culture, isolated bacteria in saline suspension and culture filtrate of *S. aureus* in separate sets. The hamsters were also separately injected with penicillin and bacterial samples for comparison of aberration frequencies in different series.

For the study of the mitotic frequency in normal, control and saline suspended isolated bacteria-treated hamsters, 2 male and 2 female specimens at each interval were used in which the actual number of dividing cells in 2000 cells in each sex was determined from fixed bone marrow samples stained temporarily with acetic-carmine.

3. Observations

3.1. *Aberration frequency*

The normal metaphase complement of $2n = 44$ in male (figure 1) contained 34 metacentric and submetacentric, and 8 acrocentric autosomes while the longest metacentric X and the longest submetacentric Y formed the distinct heteromorphic pair of marker sex chromosomes (figure 2). Four pairs of acrocentric autosomes could also be considered another group of markers. The karyotype prepared by us (figure 2) agreed in all respects with that of Hsu and Benirsche (1967) except that the X which they claimed to be a submetacentric type. The mean length in micra was 1·4 for X and 1·2 for Y and their relative percentage values were 4·3 and 3·8 respectively. Each of them constituted 1/44 part of the diploid number.



Figures 1–9. **1.** A normal metaphase complement of male hamster ($2n = 44$). **2.** Normal karyotype. **3.** A metaphase plate showing C-mitotic effect. **4.** A part of a metaphase plate showing stickiness. **5.** A part of a metaphase plate showing chromatid break. **6.** X-chromosome showing chromatid constriction and sub-chromatid break. **7.** A part of a metaphase showing a fragment of unknown origin. **8.** A part of a metaphase showing chromatid break. **9.** A part of a metaphase showing a chromatid break and a translocation.

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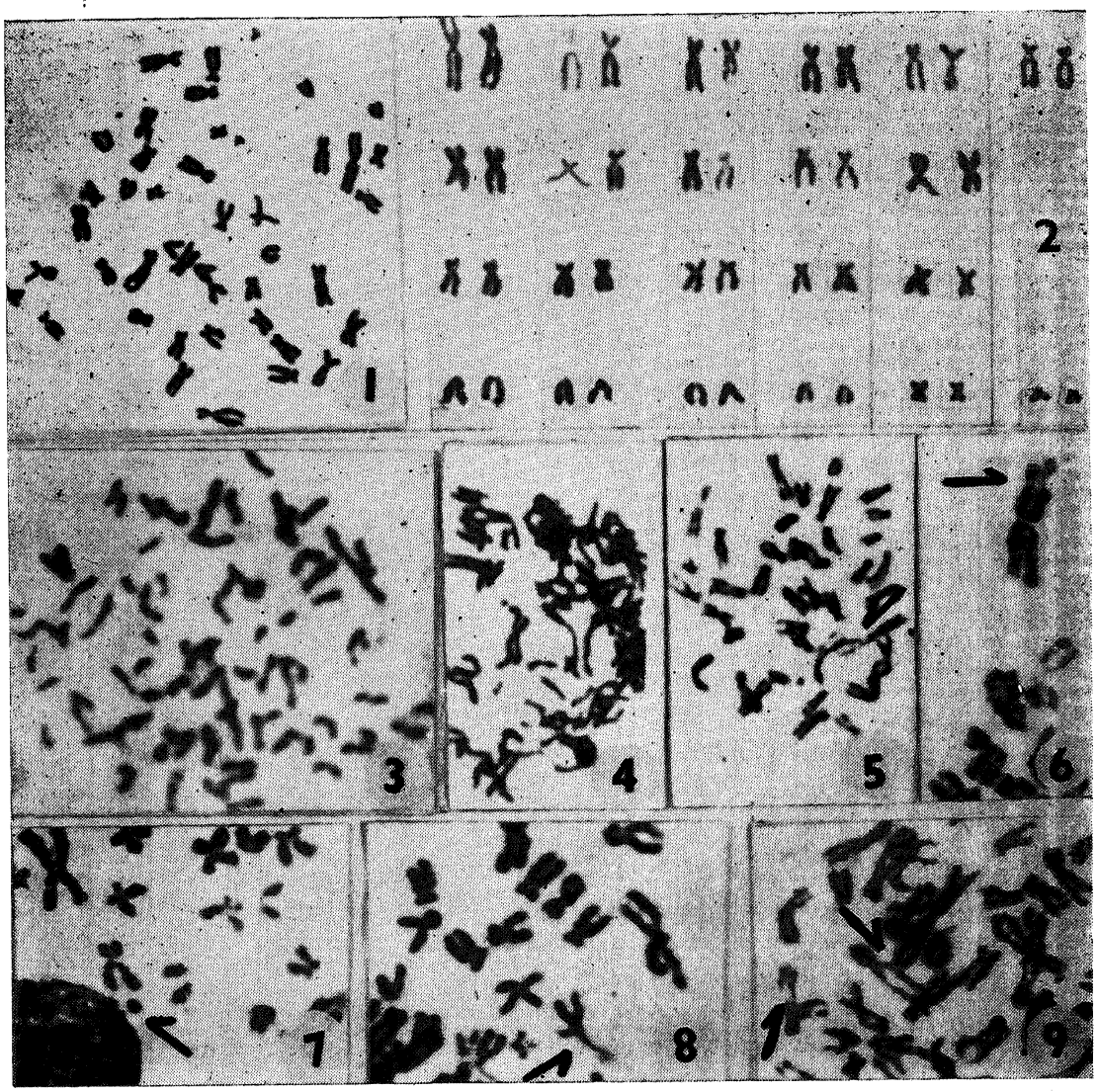

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The common types of aberrations found in control and treated series were mainly subchromatid breaks (figure 6), chromatid breaks (figures 5, 8, 9), fragment of unknown origin (figure 7), constrictions (figure 6) and gaps while the treated series had in addition to them rare occurrence of isochromatid constrictions, translocation (figure 9), etc. and the common occurrence of metaphases with some gross effects like C-mitosis (figure 3), polyploidy, stickiness (figure 4), etc. Quantitatively in the log culture-treated series out of 2700 metaphases examined in 9 intervals between 10 min and 96 hr, there were 8 subchromatid breaks, 43 chromatid breaks, 5 fragments of unknown origin, 6 gaps and 16 constrictions making a total of 78 individual types and there were also 84 metaphases with gross effects (table 1A). Thus the average frequency was 6%. Further, the frequency of aberrations at different intervals with a peak of 9·6% at 48 hr did not show a regular mode of rise and fall whether the individual types or gross types were considered. As controls the injection of the fresh nutrient broth yielded (table 1) in 2700 metaphases examined at the same 9 corresponding intervals, only 3 subchromatid and 6 chromatid breaks, 12 fragments of unknown origin, 10 gaps and 6 constrictions making a total of 37 aberrations with metaphases having no gross effect (table 1). The average frequency was 1·3% which was lower by 4·7% than that of treated series. The frequency was also higher at all fixation intervals in the treated series (table 1).

The injection of the same number of isolated bacterial cells suspended in normal saline yielded in the same 9 intervals out of 2700 metaphases 3 subchromatid breaks, 13 chromatid breaks, 14 fragments of unknown origin, and 3 constrictions making a total of 33 individual types and there were 253 metaphases with gross effects (table 1B). The average frequency was 10·5%. The frequency at different intervals did not show a regular mode of rise and fall though the peak effect of 27·6% was found at 24 hr (table 1B). The frequency of aberrations in saline injected control was relatively very low because it contained out of 2700 metaphases, 6 chromatid breaks, 2 fragments, 10 gaps and 9 constrictions making a total of 27 aberrations (table 1B). The average was 1% against 10·5% in the treated series which was lower by 9·5% (table 1B).

The treatment of log culture filtrate induced lower frequency of aberrations which was mainly due to the low number metaphases with gross effects because out of 2700 metaphases there were 4 subchromatid, 52 chromatid breaks, 13 fragments, 1 translocation, 16 gaps, 17 constrictions making a total of 103 individual types and 3 metaphases with gross effects. The average frequency was about 3·9%. The peak frequency (11·6%) was at 24 hour which showed no regular rise and fall on its either side (table 1C). In the incubated nutrient broth injected control, out of 1200 metaphases examined at 4 intervals (table 1C) there were 2 subchromatid and 4 chromatid breaks, 6 fragments, 3 gaps and 6 constrictions making a total of 21 individual types and 4 gross type aberrations. Thus the average frequency was about 2% which was almost half of the culture filtrate-treated series.

In the heat-killed bacterial suspension-treated series, the average frequency was 1·1% determined from samples fixed at 4 intervals in which out of total 1200 metaphases there were 3 chromatid breaks, 7 gaps and 4 constrictions making a total of 14 aberrations (table 1D). If the treated data were compared with that of incubated nutrient broth control referred to before it was lower by 0·9%

Table 1. Different types of chromosome aberrations in 300 metaphases at each interval in the bone marrow cells of bacteria-treated and control (figure in brackets) in male Syrian hamster.

Treatment	Fix. hour	Individual type						Total Gross type	Combined %
		Sub. cht. brk.	Cht. brk.	Frag.	Cht. gap.	Cht. const.	Total		
A. *Log. culture*	10 min	1 (1)	1 (2)	– (1)	1 –	1 –	4 (4)	13 –	5·6 (1·3)
	30 min	1 –	9 (1)	1 (4)	– –	1 (1)	12 (6)	6 –	6·0 (2·0)
	1 hr	1 (1)	12 (1)	– (5)	1 (1)	2 (2)	16 (10)	5 –	7·0 (3·3)
	7 hr	1 –	3 (1)	2 –	1 (2)	3 (1)	10 (4)	9 –	6·3 (1·3)
	12 hr	– –	8 –	1 (1)	3 (2)	2 (1)	14 (4)	9 –	7·6 (1·3)
	24 hr	2 (1)	1 (1)	– –	– (1)	– –	3 (3)	10 –	4·3 (1·0)
	48 hr	1 –	8 –	1 (1)	– (2)	5 –	15 (3)	14 –	9·6 (1·0)
	72 hr	1 –	1 –	– –	– (2)	2 –	4 (2)	12 –	5·3 (0·6)
	96 hr	– –	– –	– –	– –	– (1)	– (1)	6 –	2·0 (0·3)
Total		8 (3)	43 (6)	5 (12)	6 (10)	16 (6)	78 (37)	84 –	6·0 (1·3)
B. *Saline suspension*	10 min	1 –	– –	– –	– (1)	– (2)	1 (3)	28 –	9·6 (1·0)
	30 min	– –	– –	1 –	– (1)	– (1)	1 (2)	22 –	7·6 (0·6)
	1 hr	– –	2 (1)	4 –	– (2)	1 (1)	7 (4)	23 –	10·0 (1·3)
	7 hr	1 –	7 (3)	2 (1)	– –	– –	10 (4)	1 –	6·0 (1·3)
	12 hr	– –	– –	– –	– (1)	1 (2)	1 (3)	48 –	16·3 (1·0)
	24 hr	1 –	3 (1)	7 –	– (1)	– (1)	11 (3)	72 –	27·6 (1·0)
	48 hr	– –	1 (1)	– (1)	– (2)	1 (1)	2 (5)	30 –	10·6 (1·6)
	72 hr	– –	– –	– –	– (2)	– –	– (2)	27 –	9·0 (0·6)
	96 hr	– –	– –	– –	– –	– (1)	– (1)	2 –	0·6 (0·3)
Total		3 –	13 (6)	14 (2)	– (10)	3 (9)	33 (27)	253 –	10·5 (1·0)

C. *Culture filtrate*	10 min	– (1)	3 (1)	2 (1)	2 –	1 (2)	8 (5)	1 (1)	3·0 (2·0)
	30 min	– –	3 –	– –	– –	– –	3 –	– –	1·0 –
	1 hr	1 –	5 (2)	3 (4)	1 (2)	3 (1)	13 (9)	1 (1)	4·6 (3·3)
	7 hr	2 (1)	9 (1)	1 (1)	3 (1)	3 (1)	18 (5)	– (2)	6·0 (2·3)
	12 hr	– –	3 –	1 –	– –	2 –	6 –	– –	2·0 –
	24 hr	1 –	21 –	3 –	6 –	3 –	34+1 –	– –	11·6 –
	48 hr	– –	4 –	3 –	1 –	3 –	11 –	1 –	4·0 –
	72 hr	– –	1 –	– –	3 –	1 –	5 –	– –	1·6 –
	96 hr	– –	3 –	– –	– –	1 (2)	4 (2)	– –	1·3 (0·6)
Total		4 (2)	52 (4)	13 (6)	16 (3)	17 (6)	103 (21)	3 (4)	3·9 (0·9)
D. *Heat killed*	10 min	– –	1 –	– –	1 (1)	2 (2)	4 (3)	– –	1·3 (1·0)
	1 hr	– –	– (1)	– –	2 (2)	1 (1)	3 (4)	– –	1·0 (1·3)
	7 hr	– –	2 (3)	– (1)	3 –	– –	5 (4)	– –	1·6 (1·3)
	96 hr	– –	– –	– –	1 –	1 (1)	2 (1)	– –	0·6 (0·3)
Total		– –	3 (4)	– (1)	7 (3)	4 (4)	14 (12)	– –	1·1 (1·3)

indicating that the treatment of heat-killed bacterial suspension had no additional effects.

3.2. *Localisation of breaks*

With a view to finding out if the breaks induced by the treatment of bacterial samples were nonrandomly distributed or not, the observed number of chromatid breaks was compared with the expected number calculated for autosomal and sex chromosomal groups (table 2). The difference between the observed and the expected number calculated on the basis of proportional number and relative percentage length (table 2) for two groups of autosomes, the X and Y was statistically insignificant ($P > 0{\cdot}05$). On the other hand the difference between the observed and the expected number of breaks in autosomes and all combined along the length of chromosomes was statistically significant ($P < 0{\cdot}05$) if the affected chromosomes was artificially demarcated into the regions from the centromere. Thus in biarmed autosomes and sex chromosomes, the breaking point if any was determined in the arm concerned. The data showed that the proximal or the region near to the centromere was less vulnarable while the distal region was more vulnerable to break. As the data were not extensive, we refrained from making definite claim of non-random distribution for the present.

3.3. *Protective action of penicillin on aberration frequency*

Since penicillin is known to be the specific drug against staphylococcal infection, it was injected 1 hr before (pre-treated series), simultaneously and 1 hour after (post-treated series) into hamsters which were in separate sets treated with log culture, isolated bacteria suspended in saline and log culture filtrate. The frequency of chromosome aberrations in 300 metaphases from their bone marrow cells was studied at the interval which was the peak period for individual type of aberrations determined elsewhere. The frequency of aberrations induced by the penicillin solution individually and also that of specific bacterial sample was taken checks against the conjoint treatment of both (table 3). The treatment of penicillin did not cause any gross effect but induced more or less the same individual types of aberrations as that of individual bacterial samples (table 3). On the other hand the conjoint treatment of penicillin and the bacterial sample induced few simple chromatid breaks and constrictions and rarely some fragment and gap (table 3). In the conjoint treatment of log culture of *S. aureus* and penicillin the maximum protection was shown by the post-treatment of penicillin as the frequency of aberrations was 1·6% while pre-treatment had 2% and simultaneous treatment of penicillin and log culture had 3% aberrations (table 3A). However, the conjoint treatment in all cases reduced the aberration frequency because it was 5·3% in only penicillin and 7% in only log culture-treated series. In post penicillin-treated series the frequency was reduced by 3·7% in comparison with only penicillin treated series and by 5·4% from only log culture treated series (table 3A). The frequency in post penicillin treated series was same as that of nutrient broth control (table 1).

The conjoint treatment of bacteria suspended in saline and penicillin showed also the post-treatment of penicillin had the maximum protective action, the next

Table 2. Group-wise and region-wise distribution of chromatid breaks induced by different bacterial samples in bone marrow cells of treated hamster.

Sample treated	Types of Autosomes						Types of sex chromosome						Total		
	Meta and submeta			Acrocentric			X			Y					
	Pr.	Mi.	Di.	Pr.	Mi.	Di	Pr.	Mi.	Di.	Pr.	Mi.	Di.	Pr.	Mi.	Di.
Log culture	7	14	14	1	1	2	..	..	2	..	..	1	8	15	19
Saline suspension	2	3	5	..	2	1	..	..	..	..	..	..	2	5	6
Culture filtrate	9	16	16	1	1	3	1	..	1	1	1	1	12	18	21
Total observed	18	33	35	2	4	6	1	..	3	1	1	2	22	38	46
Expected number :															
(1) Per region	28·6	28·6	28·6	4	4	4	1·3	1·3	1·3	1·3	1·3	1·3	35·3	35·3	35·3
Group total		=86			=12			=4			=4			=106	
(2) Per prop. number		82·0			19·2			2·4			2·4			106	
(3) Per relative % length		81·3			10·6			4·3			3·8			106	

Table 3. Effects of conjoint and separate treatments of penicillin and different bacterial samples on the frequency of chromosome aberrations in hamster.

Sample treated	Fix. time	No. of meta.	Individual type					Gross types	Total	%
			Sub. cht. brk.	Cht. brk.	Frag.	Gaps	Const.			
A. *Conjoint treatment of log culture and penicillin* :										
Penicillin only	1 hr	300	1	8	..	4	3	..	16	5·3
Log culture only	1 hr	300	1	12	..	1	2	5	21	7·0
Pre-penicillin and log culture	1 hr	300	..	2	..	..	4	..	6	2·0
Simultaneous penicillin and log culture	1 hr	300	..	6	..	..	3	..	9	3·0
Log culture and post-penicillin	1 hr	300	..	3	..	..	2	..	5	1·6
B. *Conjoint treatment of saline suspended bacteria and penicillin* :										
Penicillin only	7 hr	300	1	9	..	3	2	..	15	5·0
Saline suspension only	7 hr	300	1	4	2	4	3	1	15	5·0
Pre-penicillin and saline suspension	7 hr	300	..	3	..	..	2	..	5	1·6
Simultaneous saline suspension and penicillin	7 hr	300	..	6	..	1	1	..	8	2·6
Saline suspension and post-penicillin	7 hr	300	..	2	..	1	1	..	4	1·3

C. *Conjoint treatment of culture filtrate and penicillin* :

Penicillin only	24 hr	300	..	7	..	2	2	..	11	3·6
Culture filtrate only	24 hr	300	1	21	3	6	3	..	34 + 1	11·6
Pre-penicillin and culture filtrate	24 hr	300	..	3	..	..	1	..	4	1·3
Simultaneous culture filtrate and penicillin	24 hr	300	..	7	2	..	5	..	14	4·6
Culture filtrate and post-penicillin	24 hr	300	..	1	..	..	2	..	3	1·0

one was pre-treated and then simultaneous treated series as these data were compared with the frequency of only penicillin and only saline suspension-treated series, both having the same frequency (5%). The frequency was reduced by 3·7% in post-penicillin, 3·4% in pre-penicillin and 2·4% in the simultaneous penicillin treated series (table 3B).

The conjoint treatment of culture filtrate and penicillin also revealed the same trend (table 3C). In comparison to the frequency of penicillin induced one (3·6%) and that of culture filtrate-treated series (11·6%), the maximum protection was also shown by the post-treatment of pencillin which was 2·6% and 10·6% respectively. The next one was pre-penicillin-treated series which was 2·3% and 10·3% respectively while in simultaneous treated series no protection was given as the frequency was 4·6% against 3·6% in penicillin-treated series but it gave 7% protection as compared to the frequency (11·6%) of only culture filtrate-treated series (table 3C). Therefore, conjoint treatment of penicillin and three different bacterial samples on chromosomes of hamsters revealed in common that the post-treatment had the maximum protective action and the next one was pretreated series, the difference being very little (table 3). The simultaneous treatment of penicillin and bacterial sample had relatively less protective action. Among three post-penicillin-treated results, the maximum protection was shown in culture filtrate-treated series, the next was the log culture series (table 3). The same trend was also shown by the three pre-penicillin treated series.

3.4. *The effect on mitotic frequency*

The frequency of dividing cells in bone marrow cells of normal Syrian hamster was 2·8% in male and 2·9% in female in the first set and 2·7% in male and 2·8% in females of the second set. As difference in mitotic frequency in two sexes was negligible, the data were combined and the average frequency was 2·8%.

The frequency of dividing cells in the saline injected control was in the average 2·5% in both sexes and therefore in combined data of both sexes it was also 2·5%. Further the average frequency of division in control was 2·5% at 10 min, 2·4% in 1 hr, 2·5% in 7 hr and 2·6% at 96 hr indicating that the frequency did not fluctuate considerably between 10 min and 96 hr. Therefore, the time factor like the sex factor did not have any influence on the mitotic frequency of the control series. On the other hand a comparison of the mitotic frequency between the normal and control specimens revealed that the difference was significant ($P < 0·001$).

In bacteria-treated series as the data of the mitotic frequency of the male and female individuals did not differ significantly, they were combined and the average frequency thus obtained was 1·02% each at 10 min, 1 hr and 7 hr and 0·9% at 96 hr while the mean frequency was 1%. Statistical analysis of the data between the normal and treated and between control and treated revealed that the difference was highly significant ($P < 0·001$) in both cases indicating that the treatment of the bacterial sample caused significant decrease in the mitotic frequency. Further in the treated series the time factor did not show any significant role indicating that the retardation effect was being continued from the beginning to the end of the experimental period.

4. Discussion

The aberration frequency data between hamster and mice (Manna and Chakraborty 1975), if compared, would show that the average frequency was 6% against 4·3% in log culture, 10·5% against 7·2% in saline suspension and 4% against 5·5% in culture filtrate-treated hamster and mice respectively indicating that the response was generally less in mice. In the two mammals on the whole the trend was the same as saline suspension was most effective. The higher frequency of aberrations induced by the saline suspended bacteria-treated series was also recorded by Manna and Gupta (1979) for Meningococcus treatment. Possibly the isolated bacteria introduced after washing to the animal body had more metabolic activity liberating higher amount of toxins which caused higher frequency of aberrations. The frequency of aberrations which was higher in hamsters than that of mice was mainly due to the occurrence of higher frequency of gross-type aberrations. This was insignificant in number although this was not recorded in mice (Manna and Chakraborty 1975). Experimental induction of chromatid breaks by the treatment of odd mutagens was found to be nonrandomly distributed between and within the chromosomes of mice. Nonrandom occurrence of breaks has also been recorded in other materials treating with x-rays (Manna and Majumder 1962; Manna and Dey 1980) chemicals (Kihlman 1966); viruses (Huang 1971; Makino and Aya 1968). In the present study the breaks were nonrandomly distributed along the length of the chromosomes starting from the centromeric region. The distal half was most vulnerable while proximal half was less susceptible. The present data though limited would suggest some inherent weaker region in chromosomes postulated by Manna (1971, 1975, 1978). The possible mechanism of induction of chromosome aberrations by treatment of *S. aureus* has been discussed earlier (Manna and Chakraborty 1970, 1975) where it was suspected that the bacterial toxins was primarily responsible for the induction of aberrations. The aberrations, data of hamsters also supported the same especially since the aberration frequency in heat-killed bacteria-treated series was almost similar to the control series. While the bacterial toxins could be the primary mutagenic factor there could be some other agents. How the toxin or any other mutagenic factor of the bacteria induced chromosome aberration is yet to be solved. In case of virus-induced chromosome aberrations in man and mammals (Bartsch 1970) two main possibilities were suggested as their effect on lysosomes and on cellular metabolism. However we opined a third possibility as physico-chemical stress (Manna 1975; Manna and Bardhan 1973) because similar breaks were induced by odd mutagens. The present study had the same impact.

The antibiotic penicillin as a protective agent against x-ray and bacteria-induced chromosome aberrations in mice and insects has already been reported (Manna 1980; Manna and Dey 1980; Manna and Gupta 1977, 1978, 1979; Debkirtaniya and Manna 1976). In our present study on the conjoint treatment of penicillin with log culture, isolated bacteria in saline suspension and culture filtrate showed the same trend of effect. It was generalised that the maximum protective action was obtained for the post-treatment and the next one was the pre-treatment while the simultaneous treatment had little less effect than the other

two. The difference between the pre- and post-treatment data was very negligible. If the treatment of the penicillin had reduced the frequency of aberrations for the conjoint treatment with the log culture or bacteria in saline suspension only and not for culture filtrate, one could suggest that the bactericidal effect of penicillin was responsible. However as the conjoint treatment of penicillin with culture filtrate also reduced the frequency, we had no other alternative but to think that the penicillin had the neutralising effect in common in addition to its bactericidal effect. Further if the frequency in pre-, and post-treatment of penicillin with log culture, bacteria in saline suspension and culture filtrate were compared with that of only penicillin-induced ones, the protective action of penicillin was also very striking suggesting that in conjoint-treated series penicillin not only had the neutralising effect but there was antagonistic effect too.

The problem of mitotic inhibition has been reviewed by different workers (Biesele 1958; Gelfant 1963; Kihlman 1966) but the precise mechanism has not been clearly understood. All kinds of mutagens have been found to have some amount of mitotic retardation effect. Similarly the present data were also found to have a similar effect. The mechanism of mitotic retardation caused by the injection of saline solution could not be explained. The frequency was lower than that of the normal individual and the result was significant. On the other hand the treatment of bacterial sample had highly significant retardation effect in the bone marrow cells of hamster. It would perhaps suggest that the bacterial toxins were responsible indicating that the same mutagens were effective in causing chromosome aberrations as well as mitotic retardation as the dual properties of chemical mutagens have also been shown (Kihlman 1966).

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