of the ventricle of amphibians has been reported by Johansen and Hansen[8].

Among reptiles, *Calotes* and *Geomyda* do not show significant variations in glycogen content of ventricular myocardium. Unlike *Lissemys*, which is a diving form, *Calotes* and *Geomyda* do not confront prolonged anoxia and may not be in need of large stores of glycogen. However, significant chamberwise variations are observed in the myocardium of *Lissemys*. In the present study, the highest glycogen values were obtained for the myocardium of *Lissemys*, followed by that of *Rana*, which are diving forms. High glycogen has also been reported for the myocardium of another diving turtle, *Chrysemys picta belli*[2]. Clark and Miller[3] attributed the anaerobic survival of the freshwater turtle *Pseudomys scripta elegans* to low metabolic requirement and capacity for sustained anaerobic glycolysis. Reeves[9] has shown that turtle hearts are capable of tolerating sustained anaerobiosis for prolonged periods, with glycogen the preferred substrate for myocardial energy supply.

In general, glycogen content of myocardium of birds and mammals are lower. No significant variations in glycogen level have been observed between *Gallus* and *Columba*. In fact, the myocardium of both species shows an oxidative type of metabolism, as evidenced by higher SDH activity with lipid as fuel[10]. Interestingly, the flying form *Pteropus* showed a much lower glycogen content than the less active and domesticated form *Capra*. High phosphorylase activity has been shown in the myocardium of *Pteropus*[11]. Probably, being a flying mammal, *Pteropus* needs greater glycogen turnover than *Capra*. Further, diurnal dormancy and subsequent bradycardia are generally observed in bats[12]. *Pteropus* also exhibits diurnal dormancy, and probably its myocardium depends on glycogen metabolism for energy as a result of physiological adaptation to daily torpor.

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MUTAGENIC POTENTIAL OF HUMAN KALA-AZAR HAEMOFLAGELLATE IN MOUSE

G. K. MANNA and A. K. SARKAR
Department of Zoology, University of Kalyani, Kalyani 741 235, India

WHILE the mutagenic potential of numerous chemical and physical agents in mice and other mammalian models is an established fact, the idea of microbes like viruses, mycoplasma, bacteria and fungi imperfecti acting as 'living mutagens' has also been proposed and examined[1-7]. Protozoans have not been similarly examined[8]. This communication extends the study to a protozoan parasite.

Leishmania donovani, the human kala-azar parasite, was cultured in diphasic Tobi's solid slant medium[9] and isolated in phosphate buffer, pH 7.2. One ml of a suspension containing approximately 5×10^7 parasites was injected intraperitoneally into Swiss albino mice (body weight about 20 g). Control mice, from the same stock, were given 1 ml of phosphate buffer. The parameters used to test the mutagenic potential of *L. donovani* in mouse were bone marrow chromosome aberrations, micronucleus test (MNT), frequency of sperm with abnormal head morphology and sperm depletion effect.

Bone marrow chromosome preparations were made 6 h and 24 h after injection following the standard colchicine–sodium citrate–acetic alcohol–flame drying–Giemsa staining schedule[10]. Two hundred metaphases from control and parasite-

injected mice were examined for chromosome aberrations.

For MNT, bone marrow was collected 24 h after the injection in 1% sodium citrate solution. Unfixed cell suspension from bone marrow was smeared on a clean grease-free slide, stained first in May–Grunwald stock solution for 3 min, immediately followed by staining again in distilled water-diluted May–Grunwald stain (1:1) for 2 min, and then finally stained further with diluted Giemsa stain (1:6, pH 6.8) for 10 min[10]. The stained slide was rinsed with water and air-dried. The frequency of micronucleated erythrocytes in 2000 poly- (bluish texture) and normochromatic (reddish texture) forms per mouse was assessed.

Sperm samples were prepared by squeezing out sperm from the tubules of epididymis of each side into 2 ml of distilled water. The sperm suspension was filtered through silk cloth to remove debris and the filtrate was mixed with more distilled water to make it up to 8 ml (ref. 10). The sperm suspension was smeared on a clean slide, air-dried, stained in distilled water-diluted Giemsa stain (1:20, pH 6.8), and finally air-dried[10]. The frequency of sperm with abnormal head morphology in 2000 sperm per male mouse was assessed. The same sperm suspension samples were used for sperm depletion test. Drops of the suspension were placed in five grooves of a Neubauer slide and the number of sperm in each groove was counted. The mean number was arrived at from five replicas of epididymis of each side.

The bone marrow cells of control mice had a very limited range of types of aberrations. True break types were practically absent. There were quite a number of cases of precocious centromeric separation and some grossly affected metaphases. The total aberration frequency from 400 metaphases (6 h and 24 h) was 8.2%, and only 1.5% (comprising 1 acentric fragment and 5 polyploids) had some genetic significance (table 1). On the other hand, in parasite-injected mice, the bone marrow chromosome aberrations were in the form of subchromatid (figure 1) and chromatid breaks (figure 2), acentric fragment (figure 2), translocations like ring (figure 1), centric fusion (figure 3), physiological gap and constriction, precocious centromeric separations (figure 2), etc. among individual chromosome anomalies, while the grossly affected metaphases included polyploidy (figure 4), aneuploidy, pycnosis, stickiness, etc. (table 1). Among individual chromosome anomalies, precocious centromeric separation was most common (table 1), and occurred overwhelmingly in chromosomes of group V, as found elsewhere[11]. Injection of *L. donovani* induced strikingly high increase in frequency of chromosome aberrations (table 1).

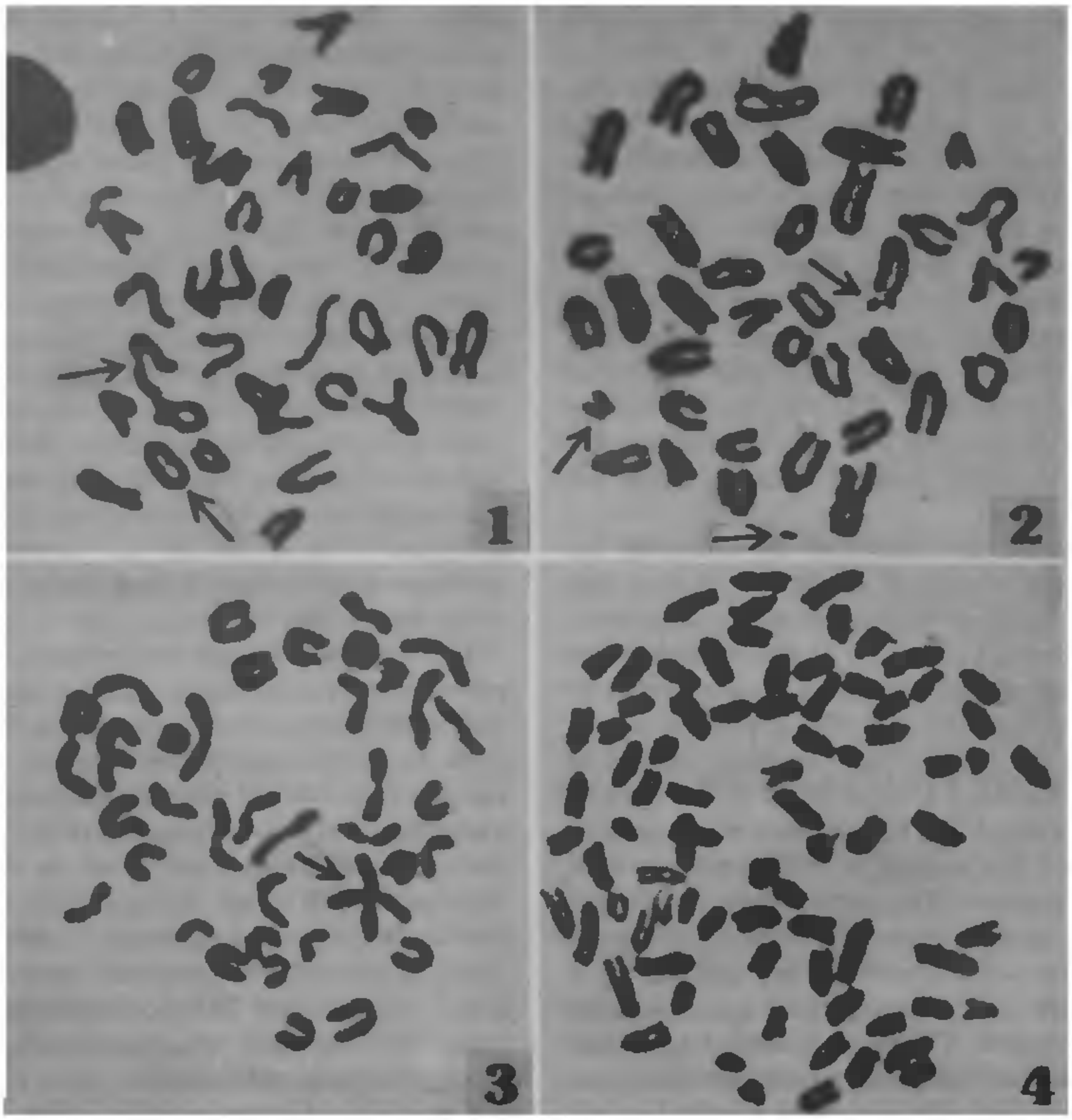
The micronucleus test showed one micronucleus per micronucleated bluish polychromatic (figure 5) and reddish normochromatic (figures 7, 8) erythrocytes; rarely two micronuclei were seen (figure 6). In samples from control mice, 4 out of 4000 polychromatic erythrocytes examined (0.1%) were micronucleated while there was none in 4000 normochromatic erythrocytes, giving a mean frequency of 0.05%. On the other hand, in *L. donovani*-treated mice, 32 out of 4000 polychromatic erythrocytes (0.8%) and 9 out of 4000 normochromatic erythrocytes (0.22%) were micronucleated, giving an average frequency of 0.51%.

Sperm samples from male mice were examined at 6 and 24 h after injection. Thirty-two out of 2000 sperm (1.6%) were abnormal in head morphology (figures 10–12; compare with figure 9) in samples from control male mice and 248 out of 2000 (12.4%) in samples from parasite-injected mice 6 h after injection. At 24 h the frequency of abnormal sperm

Table 1 *Chromosome aberrations in bone marrow cells of normal and L. donovani-injected Swiss albino mice*

Time after injection	Group	No. of metaphase counted	No. of metaphase affected	Individual types						Gross types		Total aberrations (No.)	(%)
				Scb	Cb	Af	Tr	C&G	Pcs	Number	Other		
6 h	Control	200	17	—	—	—	—	—	14	2	1	17	8.5
	Treated	200	50	3	8	2	—	3	30	4	5	55	27.5
24 h	Control	200	15	—	—	1	—	—	9	3	3	16	8.0
	Treated	200	44	2	6	4	4	8	17	7	10	58	29.0
Total	Control	400	32	—	—	1	—	—	23	5	4	33	8.2
	Treated	400	94	5	14	6	4	11	47	11	15	113	28.2

Scb, Subchromatid; Cb, chromatid break; Af, acentric fragment; Tr, translocations and ring; C&G, physiological gap and constriction; Pcs, precocious centromeric separation.

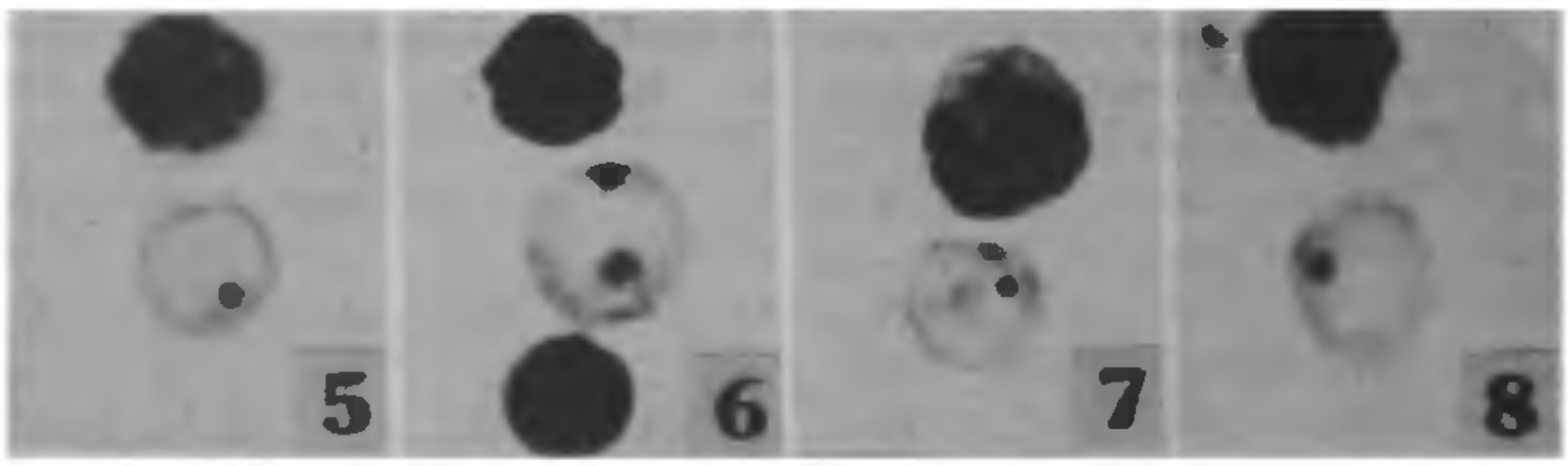


Figures 1–4. Bone marrow chromosome aberrations induced by *L. donovani* in Swiss albino mice: **1,** a ring and a subchromatid break; **2,** a chromatid break, an acentric fragment and precocious centromeric dissociation in a group V chromosome; **3,** a centric fusion; **4,** a polyploid metaphase.

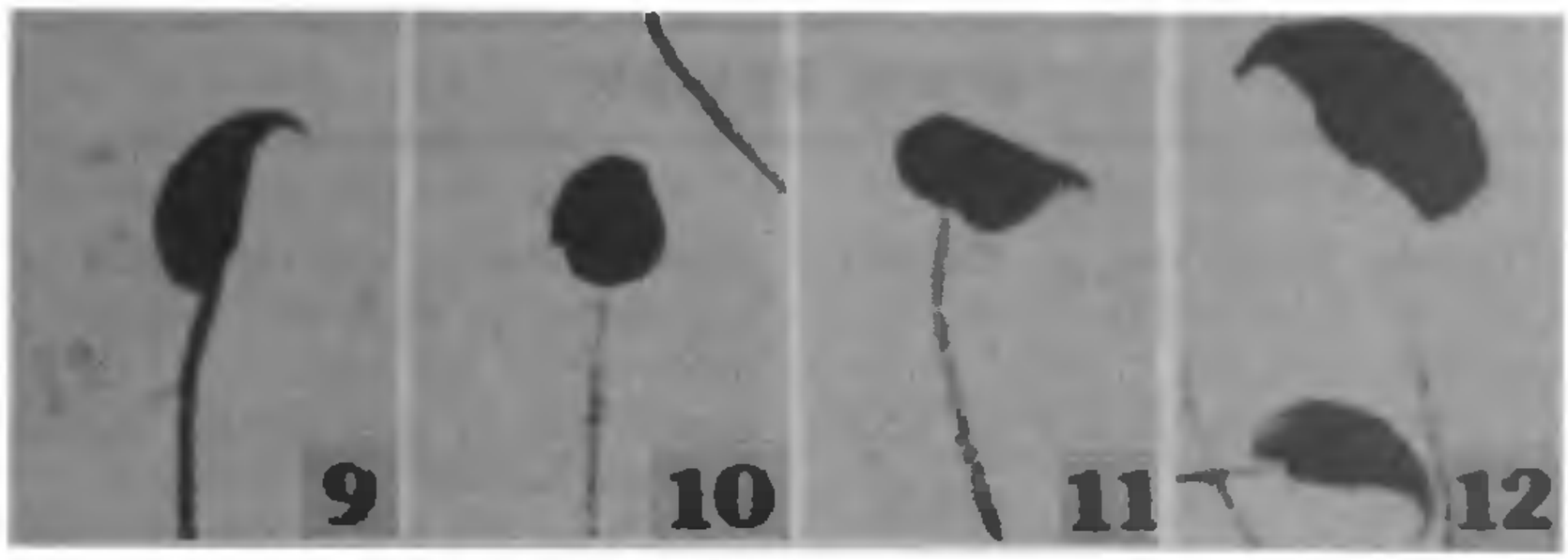
was 1.5% (30/2000) in control and 18.9% (378/2000) in parasite-injected mice.

The mean number of sperm was 1.2 and 1.1 at 6 h, and 1.2 and 1.6 at 24 h in right and left epididymes respectively of control males, but 0.84 and 0.84 at 6 h, and 0.96 and 0.84 at 24 h in the right and left epididymes of *L. donovani*-injected mice. This indicates some sperm depletion in the parasite-injected mice.

It has been recommended[12] that to consider an



Figures 5–8. Micronucleated erythrocytes from *L. donovani*-injected mice.



Figures 9–12. Normal sperm (**9**) and sperm with abnormal head (**10–12**) from *L. donovani*-injected male mice.

agent as mutagenic, positive result in more than one test is desired. In the present study results were positive with regard to bone marrow chromosome aberration frequency, MNT and frequency of abnormal sperm. Although injection of *L. donovani* did not bring about a marked sperm depletion effect, it is reasonable to suggest a mutagenic potential for *L. donovani* in mouse. Therefore the present study extends the concept of 'living mutagens', claimed earlier for viruses, bacteria and fungi, to a protozoan parasite. However, the precise mechanism by which *L. donovani* may act as a mutagenic agent is unknown. It has been shown[6,8] that toxins and vaccines can act as clastogenic agents. However, this must be carefully verified.

It is debated whether sperm head morphology is under genetic control but the idea is favoured by Wyrobek[13]. The effect was found in F_1 and F_2 generations of mice treated with chemicals, bacteria and fungi[6–8]. Use of sperm head morphology as a criterion in mutagenicity testing has been adopted by many workers. Chromosome aberrations have remained the most authentic indicator of mutagenicity[14]. MNT has also become a standard technique and is used widely.

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