USE OF CELLS OF GILL AND KIDNEY OF TILAPIA FISH IN MICRONUCLEUS TEST (MNT).

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ABSTRACT

The micronucleus test (MNT), one of the methods in bone marrow cells of mammals for screening mutagenic potentiality of odd agents has been deployed recently in some mutagen-treated fishes using peripheral blood smear. In this paper the newly developed method of detection of micronucleated cells (MNC) of gill and kidney in tilapia, *Oreochromis mossambicus* treated with x-rays and two chemicals against controls has been presented and the results compared with earlier results. The cells of gills and kideny of tilapia could be used for MNT. Cells of gill appeared more sensitive to x-rays and cadmium chloride than those of kidney and erythrocyte. The number of micronuclei was one or more in MNC of gills of X- irradiated and glucosamine-treated fishes while it was almost one per MNC in kidney of the same specimen treated with x-rays and chemicals. The MNT in cells of the three tissues revealed differential radio- and chemo-sensitivity.

INTRODUCTION

FISHES undoubtedly serve as the best model for detecting genotoxic agents in aquatic environment and several mammalian mutagenicity testing methods with modifications have been applied in recent years[1–4]. The MNT in bone marrow cells and mammlas has proved to be a simple, quick and dependable method for screening environment genotoxic agents [5,6]. In fish, MNT has been developed recently. Hooftman and Raat[7] reported the nuclear anomalies in peripheral erythrocytes of eastern mudminnow, *Umbra pygmaea* treated with EMS. Manna *et al*[8,9] reported similar observations in tilapia using x-rays and different chemical mutagens. With a view to finding out if cells other than peripheral erythrocytes of fishes could also be used for MNT, the present study was undertaken. The disadvantages of chromosome aberration analysis are that it is painstaking; karyotypes of all species are not ideal; mitotic frequency fluctuates considerably at different times and from tissue to tissue. The importance of MNT needs no emphasis since the data could be scored all the time and the above disadvantage would not affect this method. Moreover, the data on micronuclei might not only be useful as a supporting evidence to chromosome aberration frequencies induced by genotoxic agents but also for the study of the relative radiosensitivity and chemosensitivity of different tissues, mutagenic potentialities of different mutagens. Such investigations are lacking in fish genotoxicity testing and the present study is an attempt in this direction. The MNT technique employed for cells of gill and kidney has been described here.

MATERIALS AND METHODS

Exotically introduced freshwater species of tilapia, *Oreochromis mossambicus* females having characteristically mouth brooding habit[10], has been used as the experimental model for reasons specified earlier[2–4]. Two sets, each of the five adult specimens, of both sexes, individually weighing 15–20 g were employed. Immediately after taking them from water, they were exposed to whole body irradiation at doses of 200r and 800r respectively in surface moisture-free condition. The treatment was repeated a second time after an interval of 24 hr and at 6 hr after the second exposure, the gills and kidneys were taken out and put separately in 1 % sodium citrate solution. Every time after x-ray exposure, the specimens were put back in the aquarium which contained marked untreated normal individuals to serve as control. Further, a set of 5 specimens received 0.1 % solution of cadmium chloride, a metallic poison. It was injected intraperitoneally into each organism at the rate of 1 ml per 100 g body weight. The treatment was repeated 24 hr later. Six hours after the second injection of the chemical solution, they were sacrificed to remove the gill and kidney separately in 1 % sodium citrate solution. Another set of 5 specimens received 0.1 % solution of D-glucosamine, an antimetabolite chemosterilant at the same time intervals. Specimens which were given distilled water and handled in a similar way served as control 2. Both control and treated set of specimens were kept in the same aquarium to provide identical atmosphere.

Kidney and gill of each organism in sodium citrate solution were separately minced and centrifuged at

1000 rpm for 5 min. The precipitated cells were smeared on clean slides and allowed to dry overnight in air. Some slides were stained with Feulgen stain next day for 1 hr after hydrolyzing in 1 N HCl at 60°C for 10 min. Other slides were stained with Wright stain containing methylene blue and eosin Y as follows: The stain in excess was put on the smear and after 4 min was distilled and added drop by drop to make the dilution 1:3. After 12 min the stained slides were washed thoroughly with distilled water, dried in air and mounted in DPX. The staining intensity of the micronucleus and the main nucleus in cell was almost the same and that helped to eliminate artifact. The stained slides were examined under oil immersion and the scoring of MNC was made in 2000 cells per tissue and per individual (table 1).

RESULTS

Among the two types of controls, no MNC was found in 10,000 cells of gills or kidneys in control 1 but in distilled water injected control 2, the frequency of MNC was 0.1% in gills and 0.08% in kidneys (table 1).

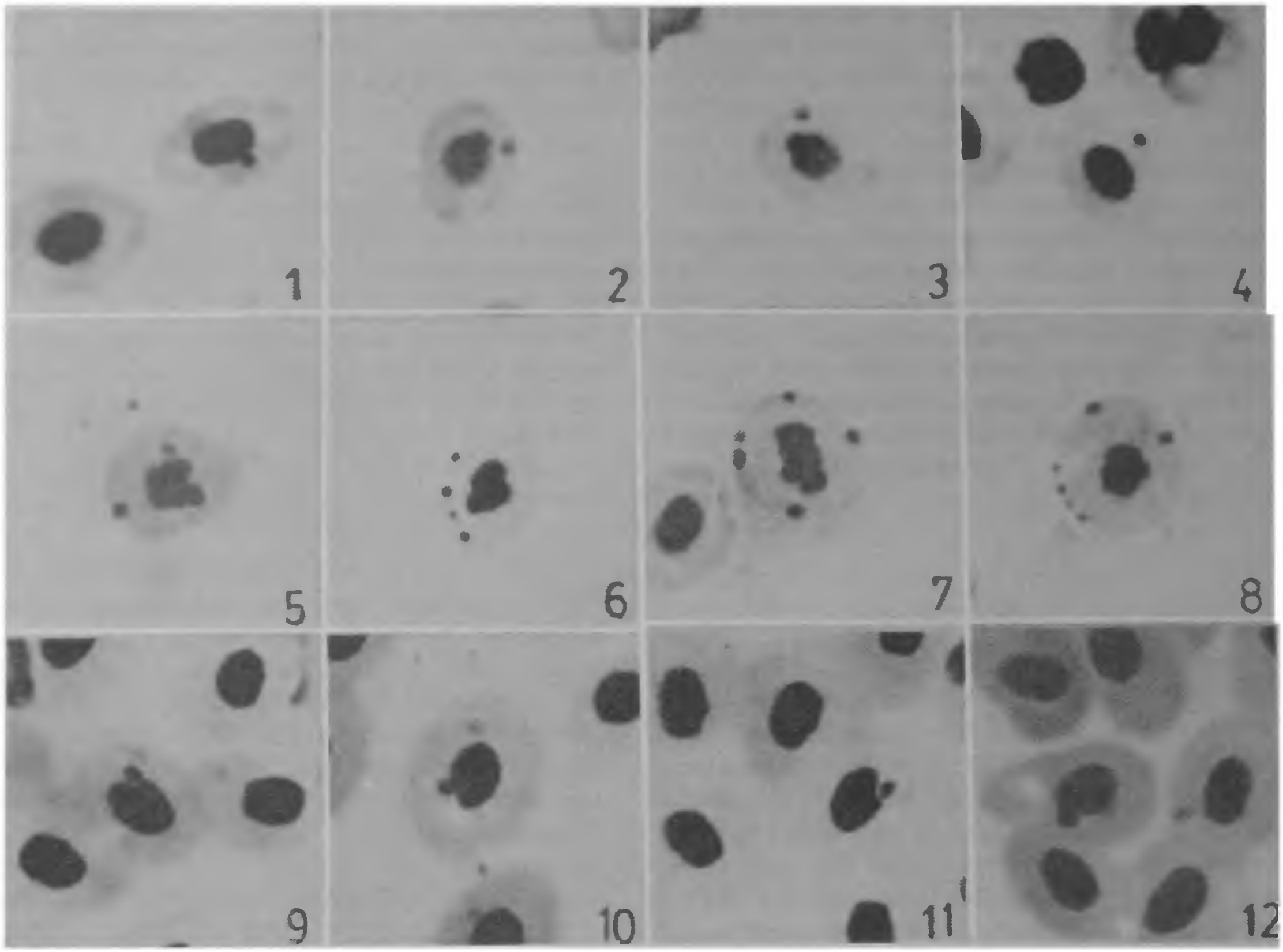
In treated series, the size and number of micronuclei were quite variable in MNC of gills of X-irradiated fishes specially in dose of 800r. The number of micronuclei in different cells varied between 1 and 6 (figures 1–8). The more prevalent number was 1 (figures 1–4). It seemed that as the number of micronuclei increased, their size diminished slightly but it needed to be very critically evaluated with adequate data for arriving at a definite conclusion. Exposure of kidney cells either to 200r or 800r of x-rays did not result in induction of more than one micronucleus per MNC. Similarly treated cells of gill epithelia, however, were found to possess higher number of micronuclei per MNC and this was more pronounced in those fishes irradiated with 800r, compared with those treated with 200r (table 1).

Cells of gill and kideny in cadmium and glucosamine injected tilapia had almost always one micronucleus per MNC lying at variable distances from the main nucleus. The size of the micronucleus in two chemically treated cells was not specifically variable. It appeared to be 20–25 times smaller than the main nucleus in the MNC.

The frequency of MN and MNC in gills of 200r and 800r X-irradiated specimens showed clear dose-dependent effect while it was not so evident in the kidneys (table 1). Further, the increased frequencies of MN and MNC observed in gills as compared with that of kidneys suggest the differential radiosensitivity of two tissues. While a similar response was observed on treatment with cadmium chloride, those treated with D-glucosamine did not exhibit such a differential sensitivity. It was specially apparent, when the frequency of MNC was compared with that of MN (table 1). The frequency of MN or MNC was remarkably high in organisms treated with radiation or chemicals.

Table 1 *Frequency of MNC and micronuclei (MN) in gills and kidneys of control and treated O. mossambicus*

Series	Dose	No of indiv.	Tissue	Total cells	Total MN	Total MNC	Percentage MN	Percentage MNC
Control 1 (norm. and x-rayed kept in same aquarium)	—	5	Gills	10000	—	—	—	—
			Kidneys	10000	—	—	—	—
Control 2 (i.p. inj. dist. water)	1 ml/100g b.w.	5	Gills	10000	10	10	0.1	0.1
			Kidneys	10000	8	8	0.8	0.8
X-rays	200r	5	Gills	10000	64	48	0.64	0.48
			Kidneys	10000	38	38	0.38	0.38
	800r	5	Gills	10000	375	205	3.75	2.05
			Kidneys	10000	49	48	0.49	0.48
0.1% cadmium chloride	1 ml/100g b.w.	5	Gills	10000	127	127	1.27	1.27
			Kidneys	10000	91	91	0.91	0.91
0.1% D-glucosamine hydrochloride	1 ml/100g b.w.	5	Gills	10000	69	49	0.69	0.49
			Kidneys	10000	77	71	0.77	0.71



Figures 1–8. Micronucleated cells of gills and kidney of tilapias treated with x-ryas, cadmium chloride and D-glucosamine hydrochloride.

DISCUSSION

As reported earlier the frequency of micronucleated erythrocytes in peripheral blood smear of X-irradiated tilapias with 200r was 0.23%[9] and with 800r it was 0.50%[8] while MNC in kidney was 0.38% and in gills it was 0.48% for 200r and 0.48% in kidney and 2.05% in gills for 800r which indicated that in all the three tissues MNC frequency was dose-dependent but the response was differential. It was more pronounced in gills than in kidney. Further among the three tissues gill was the most sensitive but the difference in the frequency was not so evident between kidney and erythrocytes for 800r unlike 200r. On the whole the MNT in three tissues of irradiated tilapias revealed that the effect was more pronounced in these groups than in control.

The frequency of MNC for the treatment of 0.1% cadmium chloride was 0.72% in peripheral erythrocytes[9], 0.91% in kidney and 1.27% in gills which also showed the diffrential response and the gill was the most sensitive among the three tissues examined. On the other hand the frequency of MNC for the treatment of 0.1% D-glucosamine was 0.70% in erythrocyte[8], 0.71% in kidney and 0.49% in gill showing that unlike the effect of x-rays and cadmium chloride, gills were the least sensitive while kidney and erythrocyte were almost equally sensitive to D-glucosamine hydrochloride.

The MNT technique which was developed for the first time for cells of gill and kidney and that of peripheral erythrocytes earlier[7–9] has rendered the possibility of assaying not only the genotoxic potentialities of x-rays and the two chemicals but also probing into other problems related to differential sensitivites. Some of

these aspects have been studied using chromosome aberration as a parameter, in different materials[11,12] but MNT technique which is new in the field of fish genotoxicology has advantages over chromosome aberration study. However, the frequency of chromosome aberrations was higher than that of MNC when the same dose of x-rays was used[8,13] because MN is formed due to limited types of anaphase chromosome aberrations like laggards and asymmetrical exchanges and its durability is time-bound[7] while many types of chromosomal aberrations can be directly observed cytologically. In spite of some limitations, the MNT technique has other advantages mentioned before and as more than one procedure is recommended for mutagenicity testing[14], MNT can be applied more easily.

25 June 1984

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NEWS

EXTENDING USE OF ULTRASOUND TO DIAGNOSE BONE DISEASE

More than a year ago, researchers at Hull (Northern England) announced the development of a machine which uses ultrasound to detect the presence of osteoporosis, a bone disease which affects 25 per cent of women over 55.

Since then, the machine has gone into limited production. Devices are now installed in hospitals in different parts of Britain for use in diagnosing osteoporosis and for undertaking further research. The new machine is quicker, easier, cheaper and safer to use than present techniques using x-rays or radioactive tracers.

Dr Stuart Palmer, Reader in the Department of Applied Physics at Hull University, developed the machine with Mr. Richard Porter, consultant orthopaedic surgeon at Doncaster Royal Infirmary and Dr. Christian Langton, a research assistant at the same hospital.

The ultrasound machine involves the patient immersing her foot in a tank of warm water. Ultrasound waves are passed through the heel and the machine measures the extent to which the heel bone absorbs the ultrasound. The process takes just a few seconds. Readings from the heel give a clear indication of the presence of the disease at other points of the body. Present diagnostic techniques rely on X-rays or gamma rays, which do not give very accurate results, and radioactive tracers, with their consequent dangers, the need for expensive equipment and prolonged discomfort for the patient. *Spectrum* – British Science News, 1986, No. 196/12; British Information Services, British High Command, New Delhi 110028).