

## INDUCTION OF SIAMESE TWINS IN THE FISH, *OREOCHROMIS MOSSAMBICUS* AFTER THE TREATMENT OF AN INSECTICIDE, ROGOR 30E

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### ABSTRACT

Male tilapia, *Oreochromis mossambicus* intraperitoneally injected with 1.2 ppm Rogor 30E solution, at 1 ml per 100 g b w and mated to virgin females which yielded 30 Siamese type twins—28 attached at the skin of the belly, 1 at the belly and tail region and the remaining 1 from the trunk region to the end of the tail. Abnormal eye and body size were also found in some of them. It was envisaged that they originated due to impairment of genes controlling development.

### INTRODUCTION

THE genotoxic potentiality of x-rays<sup>1-3</sup>, various chemicals<sup>2-5</sup> and 'living mutagens'<sup>6</sup> is being tested mainly on tilapia<sup>7</sup> deploying various methods in our laboratory. While conducting the lethal test induced by different chemicals<sup>8</sup>, we came across 30 Siamese type twins of tilapia in connection with the injection of the organophosphate insecticide, Rogor 30E to male parents. The toxic effects of some insecticides on developmental stages leading to various abnormalities in the common carp, *Cyprinus*<sup>9</sup> and frog, *Microhyla ornata*<sup>10,11</sup> were reported earlier. The occurrence of some abnormal embryos of tilapia was also found when male parents were treated with Myleran and mated to normal females in connection with the dominant lethal test<sup>12</sup>. However, the occurrence of Siamese twins as encountered in the present study was not recorded earlier by other workers. The Siamese twins have been envisaged as due to the impairment of the genetic control of developmental process in tilapia and Rogor 30E seems to have both mutagenic and teratogenic potentialities.

### MATERIALS AND METHODS

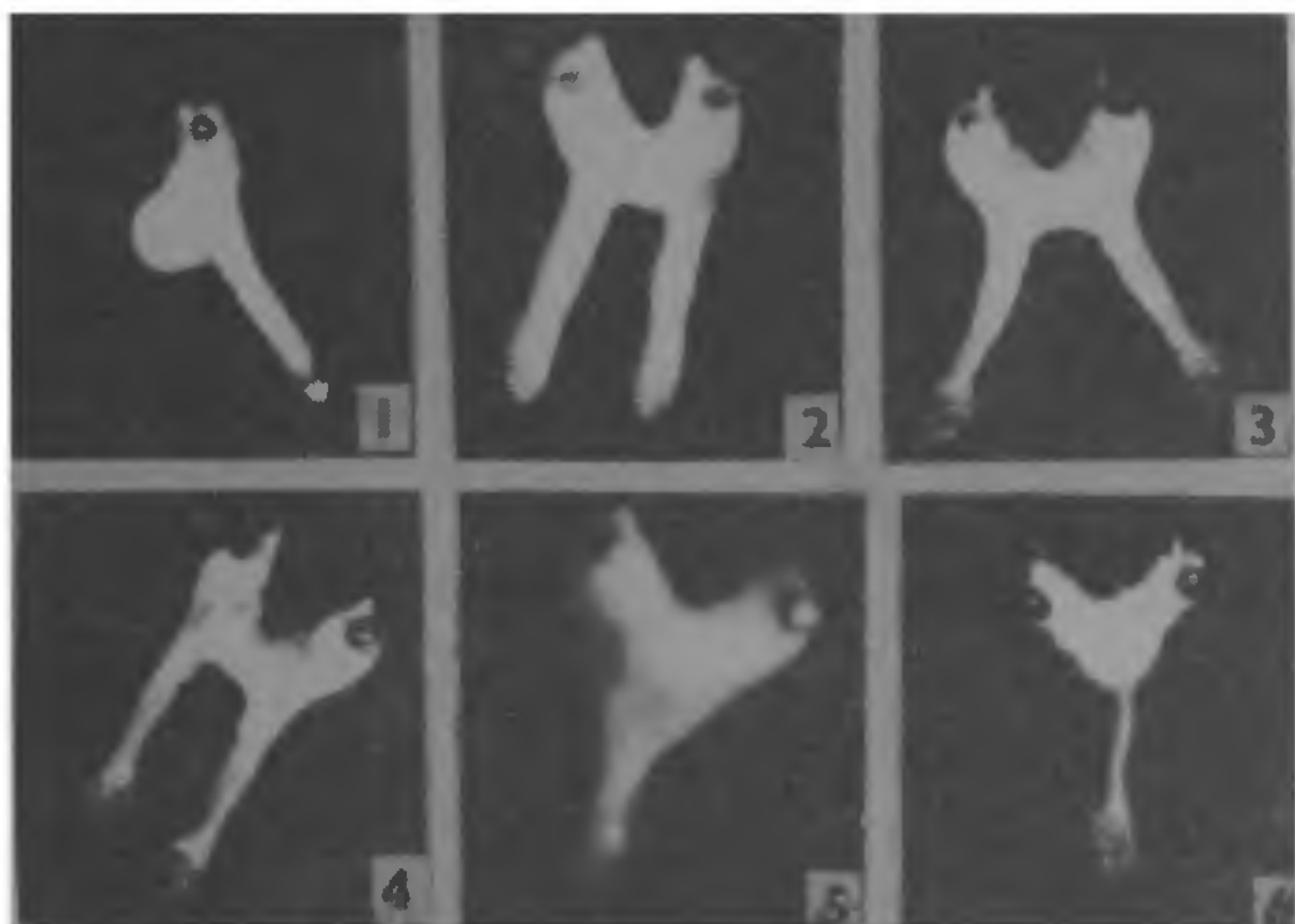
As detailed elsewhere<sup>8</sup>, adult males of tilapia, *Oreochromis mossambicus*, each weighing between 20 g and 25 g, were intraperitoneally injected with 1.2 ppm Rogor 30E solution at the rate of 1 ml per 100 g body weight (LC 50 dose). Four virgin females per treated male were allowed to mate in a cement vat containing about 100 litres of water having an average temperature of 28°C against the distilled

water injected males of control series mated to normal virgin females in identical condition. There were 11 replicas in the experiment in treated and control series. Each male of the control and treated series was allowed to mate with different virgin females consecutively for 4 weeks. The vats were inspected every day. If any female showed the sign of her successful mating for keeping the laid eggs within her brooding buccal cavity, she was very tenderly transferred to another vat and the vacancy was filled up by putting another virgin female to the original vat. After 48 hr of incubation within the buccal cavity of the female, the eggs were made to release from her buccal cavity through her mouth to a petridish containing slightly alkaline water (pH 8) simply by holding her tail up and the mouth down into the petri-dish. The eggs thus collected from each mother of the control and treated series were examined separately to determine within 72 hr the number of fertilized and unfertilized eggs, the number of hatched out, dead and living fries etc<sup>8</sup>.

### RESULTS AND DISCUSSION

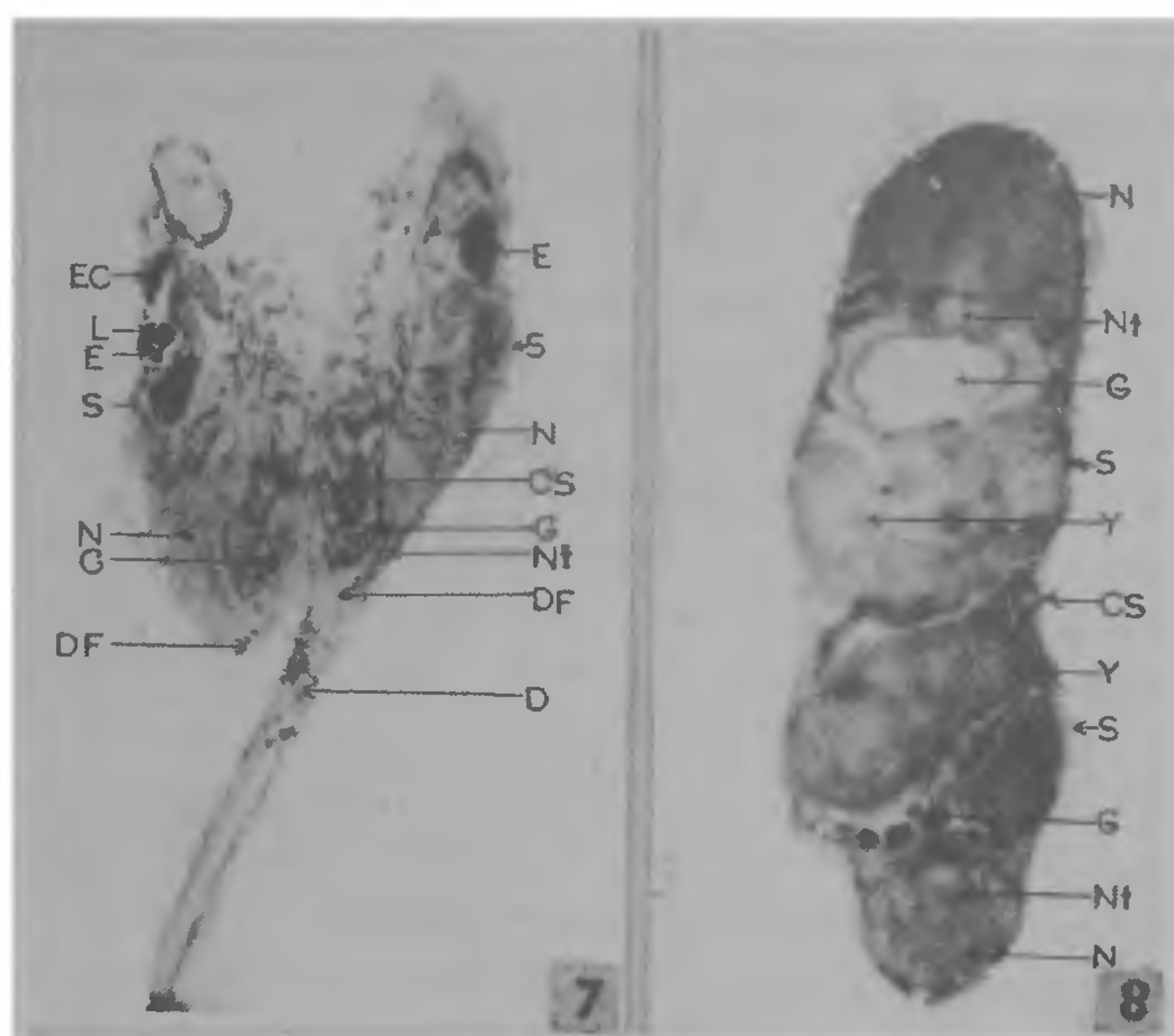
In a total of 4096 fries of 40 mothers which mated to 11 control males, there was neither a Siamese twin nor any apparently abnormal fry. On the other hand in the treated series, out of 3571 hatched out fries from 4189 eggs laid by 36 mothers after mating to Rogor injected 11 male parents, 30 Siamese twins were encountered from 8 mothers. Four out of these 8 mothers had mating with treated males in the second week after Rogor treatment. The remaining four mothers had mating with the Rogor treated male parents on the third week.





**Figures 1-6.** 1. A control normal fry. 2-4. A Siamese twin attached at the belly. 5. A Siamese twin attached ventrally at the belly and the tail. 6. A Siamese twin attached ventrally from trunk to the tip of the tail.

In comparison to the normal fry (figure 1), 30 Siamese twins consisted of 28 with attachment at the belly (figures 2-4), 1 having the belly and tail region attached (figure 5) and the remaining one had the common hind part of the trunk to the tip of the tail (figure 6). Some of the twins also had other morphological abnormalities like unequal sizes of the eye (figure 3) and length of the body (figure 4). After hatching the twins did not survive beyond 3 or 4 days. Only 2 of them survived up to 17 days, by which time the area of the ventral attachment in each twin was gradually reduced and they were about to die. So they were preserved in 70% alcohol. The histological sections were cut 6  $\mu$  thick and the sections were stained with haematoxylin and eosin, dehydrated in upgrades of alcohol, cleared in xylol and mounted in DPX. The frontal sections (figure 7) and the transverse sections (figure 8) passing through the ventrally connected plane of the two twins showed separate eye (E), lens (L), eye cup (EC), neural tube (N), notochord (Nt), gut (G), yolk (Y), dorsal fin (DF), tail (T) independent of each other but their skin (S) was common (CS) in the attached portion of the belly. Histological sections of other forms of twins (figures 5, 6) could not be made for improper fixation. They had very early death.



**Figures 7 and 8.** 7. Frontal section, and 8. Transverse section of two Siamese twins, both showing common skin (CS) at the belly while all other organs are separate in two individuals of each twin indicated by arrows and explained in the text.

It might be speculated that the two individuals of each of the 28 Siamese twins connected by the skin of the belly could survive as two identical twins if they were made free surgically because all the vital organs were separate unless some lethal gene was involved for their premature death. On the other hand, each of the two other types of Siamese twins (figures 5, 6) having the common axial skeleton in the hinder region, muscles, tail etc would very likely be surgically inseparable. They would have relatively limited longevity if it was not further curtailed by some lethal gene (*vide infra*).

Since all the Siamese twins were produced by mating of the Rogor solution injected male parents with normal virgin females, it could be suggested that some genes which were responsible for normal embryonic development underwent mutational change. That might have led to the formation of different types of Siamese twins by interfering with the normal morphogenetic movements of the blastoderm cells. Moreover, a change in the adhesive property of the cells leading to a split of the blastoderm might be postulated for their origin. Pending further verification, it seemed that those twins in which two individuals were free except at the common skin of the belly (CS, figures 7, 8), might have originated at an early cleavage stage while

those individuals of other two types of Siamese twins (figures 5, 6), which had in common a part of the trunk and the tail region, might have resulted by splitting partly the 'tongue' of the primary organizer at a later stage indicating the genic control of the morphogenic process during epigenesis in tilapia. Further, the death of all the Siamese twins a few days after their emergence could be envisaged as due to the mutated gene controlling the developmental process and was also associated with lethal effect recalling the genes controlling 'taillessness' (lethal mutation in T locus) in house mouse having phenotypic and embryological effects<sup>13</sup>. Thus the death of all the twins was very likely not merely due to the mechanical obstruction created by the ventral attachment of two individuals of each twin but it was possibly due to the lethal mutation induced by Rogor impairing developmental process in tilapia. Incidentally it might be noted that the treatment of x-rays, zinc sulphate, Aldrex 30, Anisol etc to male tilapias and their mating to normal virgin females did not yield any Siamese twin. Anyhow Roger 30E was found to be potential mutagen by other tests<sup>4,7,8</sup> but it is also a potential teratogen as revealed in the present study. Lastly, the induction of Siamese twins was a rare mutation because they were not encountered in a few repetition of the experiments. Further studies are in progress.

#### ACKNOWLEDGEMENTS

Grateful thanks are due to Professor A. K. Bose, Department of Zoology, Kalyani University for

valuable discussion and to the UGC for financial support.

7 March 1986; Revised 13 June 1986

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## ANNOUNCEMENT

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### INDIAN SOCIETY OF DEVELOPMENTAL BIOLOGISTS, PUNE, BOMBAY

The Indian Society of Developmental Biologists has awarded to Dr K. K. Sharma, Department of Zoology, Rajasthan University, Jaipur, the Swami Pranavanand Science Award for young Scientist in Developmental Biology for his original contri-

butions on the role and mechanism of action of vitamin A on limb regeneration in amphibians. The award carries a cash prize of Rs. 3,000/- and a citation.