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SKELETAL MALFORMATIONS INDUCED BY MITOMYCIN C IN CHICK EMBRYOS

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One hundred and eighty chick embryos were studied for the presence of skeletal malformations after administration of mitomycin C at 72, 96 and 120 hours of incubation. Axial skeleton defects included those of ribs, vertebrae, beak and crania. Appendicular skeletal defects were mainly confined to the lower limbs e.g. absence of phalanges, bent and shortened femur, fibula, tibia and metatarsus, in that order. Absence of phalanges and curved scapulae were noted in the upper limbs. The length of femur, tibia and metatarsus was significantly reduced ($P < 0.001$) compared with the corresponding controls especially in the group treated at 96 h.

Key words: mitomycin C; chick embryos; skeletal defects; growth suppression

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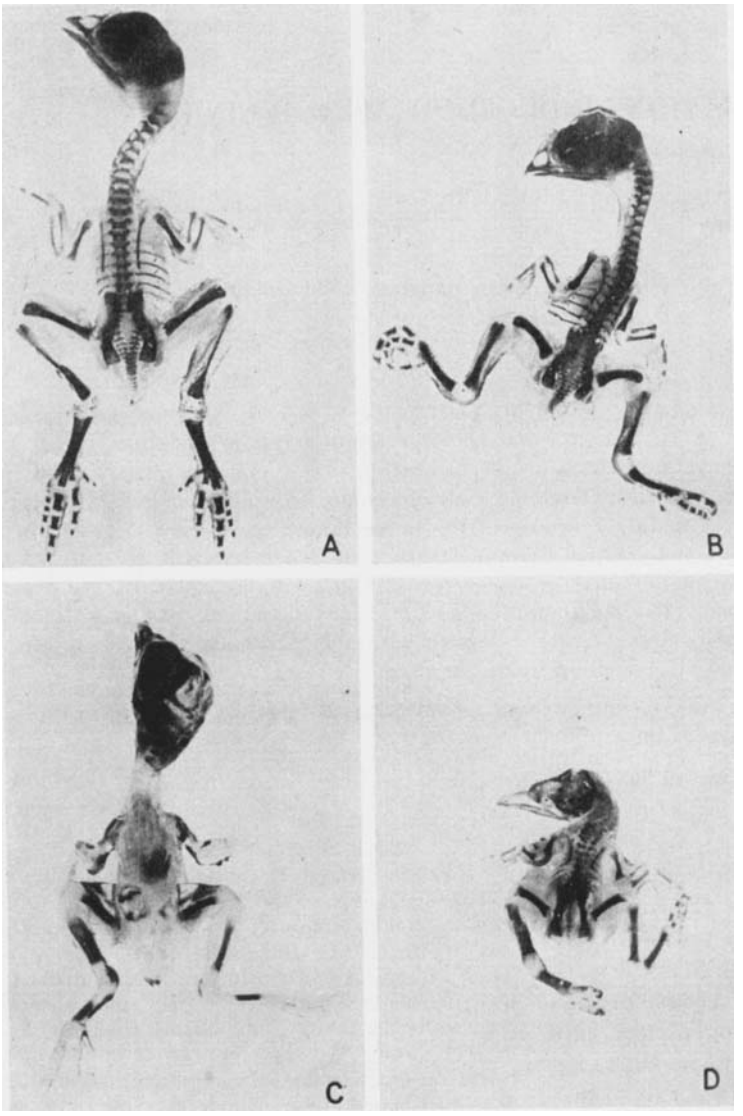
Mitomycin C, isolated from the broth of *Streptomyces caespitosus* (Hata et al. 1956), is used clinically in the palliative treatment of various malignancies (Frank & Osterberg 1960, Evans 1961). It has been reported to be teratogenic in rats (Takaya 1965), mice (Yamura 1961, Ito 1967) and chicks (Kury & Craig 1967, Singh & Singh 1975). However, the skeletal defects as such have not been studied. The present communication deals with observations made on mitomycin C induced skeletal malformations of chick embryos.

MATERIALS AND METHODS

Fertile White Leghorn chicken eggs, obtained locally from a Government poultry farm, from a stock known to be nutritionally healthy and free from genetic defects, were incubated at 37–38° C and at a relative humidity of 75–80 per cent. The eggs were turned twice a day by a mechanical device in the incubator. Mitomycin C (Kyowa Hakko Kokoya Co. Ltd., Tokyo, Japan) was obtained from the local dealers. For each batch of eggs, a fresh solution of mitomycin C was prepared in distilled water and injected aseptically into the yolk sac of the eggs, in various doses (20–24 μg/egg), at 72, 96 and 120 hours of incubation, using a simple technique described previously (Singh & Sinha 1973). Control eggs injected with distilled water were run with each batch of treated eggs. The volume of injection was kept to 0.04 ml in all cases (treated and controls). The embryos were collected on the 18th day of incubation.

One hundred and eighty treated and 50 control embryos were preserved in rectified spirit, cleared and stained with alizarin red S (Staples & Shnell 1963) for the study of skeletal elements. The length of femur, tibia and metatarsus of each embryo treated with 20 μg of mitomycin C in all three experimental groups was measured and statistically analysed to see the degree of suppression of growth in long bones as compared with their corresponding controls.



ure 1. Skeletal lformations in chick bryos induced by omycin C = control).

RESULTS

Of the 180 chick embryos treated with mitomycin C, 99 (55 per cent) showed bony defects (Figure 1). The frequency of bony defects was highest in the series treated at 96 h of incubation with both doses (20 μg and 24 μg) used (Table 1). Defects of the axial skeleton included those of the beak, skull cap, vertebrae and ribs. Rib anomalies were the most common (20 per cent) and included extra or absent ribs. Defects in the beak (16 per cent) included parrot beak, cross beak or short (either upper or lower) beak while defects in the cranium included absence of skull cap, which was maximum (22 per cent) in the group treated at 96 h (Table 1). Vertebrae (14 per cent) were absent in variable numbers mostly in the group treated at 96 h of incubation.

Table 1. Malformations of the axial skeleton of chick embryos induced by 20–24 μg of mitomycin C.

Time of treatment	Embryos examined	Embryos abnormal	Beak	Vertebrae	Ribs	Crania
72 h	60	28	8 (29%)	2 (7%)	6 (22%)	4 (14%)
96 h	60	38	5 (13%)	9 (24%)	12 (32%)	8 (22%)
120 h	60	33	3 (9%)	3 (9%)	2 (6%)	1 (3%)
Total	180	99	16 (16%)	14 (14%)	20 (20%)	13 (13%)
Control	50	–	–	–	–	–

Amongst the defects of the appendicular skeleton, those of hindlimbs were far more frequent. The bones affected in order of frequency were phalanges (81 per cent), femur (41 per cent), fibula (28 per cent), hip bone (27 per cent), tibia (13 per cent) and metatarsus (6 per cent) (Tables 2 and 3). The phalanges showed absence of ossification and in the hip bone there was absence of its pubic parts. The femur and tibia were mostly curved and shortened while the fibula was either shortened or absent. Most of the defects were bilateral. In the forelimbs, only scapulae (30 per cent) and phalanges (40 per cent) were involved (Tables 2 and 3) but in one embryo all the bones of the forelimbs along with the other bones of the body were defective. The scapulae showed increased curvature and shortening while in the phalanges there was absence of ossification.

The degree of suppression of growth of the femur, tibia and metatarsus was highly significant ($P < 0.001$) when com-

Table 2. Malformations of the limb bones of chick embryos induced by 20–24 μg of mitomycin C.

Time of treatment	Embryos examined	Embryos abnormal	Scapula	Hip bone	Femur	Tibia	Fibula
72 h	60	28	11 (39%)	6 (22%)	16 (57%)	6 (22%)	11 (39%)
96 h	60	38	13 (36%)	16 (43%)	17 (45%)	5 (14%)	14 (37%)
120 h	60	33	6 (18%)	5 (15%)	8 (24%)	2 (6%)	3 (9%)
Total	180	99	30 (30%)	27 (27%)	41 (41%)	13 (13%)	28 (28%)
Control	50	–	–	–	–	–	–

Table 3. Skeletal malformations of the digits of chick embryos induced by 20–24 μg of mitomycin C.

Time of treatment	Embryos examined	Embryos abnormal	Hand		Foot	
			Metacarpus	Phalanges	Metatarsus	Phalanges
72 h	60	28	–	3 (11%)	2 (7%)	18 (64%)
96 h	60	38	–	17 (45%)	2 (6%)	33 (90%)
120 h	60	33	–	20 (61%)	2 (6%)	30 (91%)
Total	180	99	–	40 (40%)	6 (6%)	81 (81%)
Control	50	–	–	–	–	–

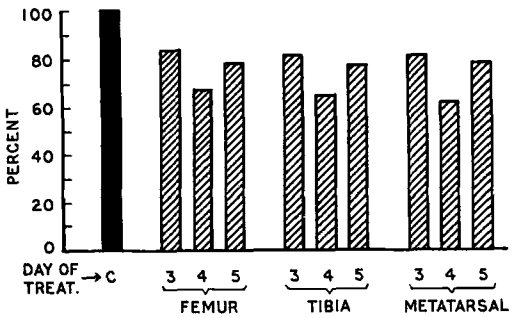


Figure 2. Suppressive effect of mitomycin C on growth of chick embryos.

pared with the corresponding controls (Figure 2) in all the treated groups (Table 4). The group treated at 96 h showed the greatest degree of growth suppression ($P < 0.001$ when compared with the 72 h group and $P < 0.01$ when compared with the 120 h group, Table 4).

DISCUSSION

In a previous study mitomycin C proved lethal to 50 per cent of chick embryos treated on the 3rd through the 6th day of incubation besides inducing malformations in 59 per cent of the surviving embryos (Singh & Singh 1975). Limb anomalies including those of digits were most frequently encountered in the groups treated on the 4th and 5th days in that study. Accordingly, the present study of skeletal defects was confined to this critical peroid of chick susceptibility to mitomycin treatment. Treatment on the 4th day (96 h) was found to induce the maximum skeletal anomalies which is in agreement with the observations of Karnofsky (1955). Hindlimb bones were more frequently involved and the most affected part was the distal end of the extremities, i.e., the phalanges. Since the skeletal differentiation in the limbs, i.e., mesodermal condensation, chondrification and ossification, proceeds in a proximodistal sequence in general (Zwilling & Hansborough 1956, Amprino 1965, Milaire 1965), the suppressive effect of mitomycin on the proliferating and differentiating mesenchyme (Singh et al. 1974, 1975) is likely to depend on the stage of differentiation of the limb buds at the time of the administration of the drug. The skeletal elements which differentiate at an early stage will be more resistant than those which differentiate later. Hence the long bones were less often affected. While studying the effect of mitomycin C on developing chick embryos Kury & Craig (1967) found, in contrast to this study, that the tibia was the most commonly affected bone and the vertebrae and ribs were the least involved. However, these discrepancies can be explained on the basis of the different species of chicks used since the teratogenic response in various species and strains is known to differ (Cahen 1964, Nogami 1964). Similarly skeletal anomalies in mice induced by mitomycin (Ito 1967) vary somewhat according to the species.

The hindlimb bones were more involved than the forelimb bones this being related to the difference in time of initial

Table 4. Suppressive effect of 20 μg of mitomycin C on growth of bones of chick embryos.

Time of treatment		72 h *	96 h *	120 h *	Control *
Femur	Mean	1.14 ± 0.22	0.92 ± 0.20	1.07 ± 0.17	1.36 ± 0.10
Tibia	Mean	1.55 ± 0.26	1.25 ± 0.24	1.49 ± 0.30	1.90 ± 0.17
Metatarsus	Mean	1.11 ± 0.22	0.86 ± 0.30	1.07 ± 0.26	1.36 ± 0.14

* $P < 0.001$.

development and rate of growth of the limbs. Such a stage dependent sequence of bone involvement is known to occur (Singh & Sanyal 1974).

As regards the mechanism of action of mitomycin, it primarily inhibits DNA synthesis and protein synthesis from their precursors in the living cells (Orstavik 1972). Its antimitotic activity has been observed to suppress cell growth *in vitro* (Singh et al. 1974) and cartilage cells in particular are suppressed, as seen by their uptake of S^{35} (Singh et al. 1975), and the marked shortening of treated bones ($P < 0.001$) may be explained on the basis of this suppression of growth.

Inhibition of DNA synthesis in rapidly proliferating embryonic tissue, when prolonged, probably leads to localised cell death (Ritter et al. 1971) which *per se* has also been associated with the process of normal development, e.g., removal of excess cells in the shaping of organs (Glucksmann 1965, Saunders1966) and may accompany or precede congenital malformations (Menkes et al. 1970). Cell death disrupts the normal mitotic pattern of tissues and destroys cell contacts and cell movements vital to normal tissue interactions and inductive events. However, dead cells may be removed and replaced by mitotically active cells which subsequently attempt to reconstitute the tissue or organ. Such repair and regeneration is known to occur in fetuses after radiation damage (Hicks et al. 1957, Sakurai 1968) and after radiomimetic drugs (Kreybig & Schmidt 1967, Chaube et al. 1967, Singh & Sanyal 1974). The capacity for such regeneration and the ultimate outcome is dependent on the number of mitotically active cells that survive and the tissue remaining for such organogenesis. This may account for the variable expression of the malformations induced by such drugs.

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