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POSSIBLE RELATIONSHIP BETWEEN TETRACYCLINE STABILITY AND EFFECT ON FOETAL SKELETON

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A possible relationship between stability of a tetracycline and persistence in the foetal skeleton is discussed; it seems that the greater the stability of a tetracycline, the more it may interfere with bone-forming processes. Therefore, assuming that limited instability does not impair antibiotic potency *in vivo*, the use of a low-stability tetracycline seems to be a safer form of treatment in order to avoid undesired effects on the foetal skeleton.

In recent years the occurrence of skeletal malformations in animal and human foetuses after administration of tetracycline has frequently been reported (Bevelander, Nakahara & Rolle, 1959; Bevelander & Goss, 1962; Wallman & Hilton, 1962; Hurley & Tuchmann-Duplessis, 1963; Smith & Chapman, 1963); this occurrence is probably due to the typical avidity of tetracycline for metal cations acting as a prosthetic group in bone-forming enzymes (Eagle & Saz, 1955; Shils, 1962).

All the investigations on teratogenicity or abnormal bone calcifications have until now been carried out with tetracycline itself and not with other members of this group; moreover, in the case of tetracycline, only the relationship between abnormal effect and dosage or length of treatment has been investigated. In the course of work on the monosodium salt of *N*-methylol-chlortetracycline (chlormethylenecycline), experiments on developing chick embryos were carried out with the object of evaluating possible variations of its chelating effect compared with the actions of other tetracyclines.

METHODS

Chick embryos of an inbred hybrid strain were used 8 days after the onset of hatching; single 1 mg doses of demethylchlortetracycline, tetracycline, chlortetracycline and the monosodium salt of chlormethylenecycline were dissolved in 0.1 ml. and were injected under sterile conditions into the yolk sac of the embryo. Demethylchlortetracycline, tetracycline and chlortetracycline were used as the hydrochlorides. All doses refer to the bases.

Eggs were sealed with sterile cellulose tape and placed in an incubator at 38° C; embryos and their sagittal sections (prepared by the same method as for rabbits; see below) were examined on the 16th day after the onset of hatching and were viewed under ultra-violet light.

Groups of five female rabbits were injected intravenously from the 10th to 20th day of pregnancy with 10 mg/kg/day of the tetracyclines. The neonates were then rapidly frozen with a dry-ice mixture of solid carbon dioxide and acetone and whole-body sagittal sections were cut using a 1213 Leitz freezing microtome equipped with a 90 mm Jung type C knife.

Groups of five Wistar female albino rats were similarly treated and their newborn were examined with the same method. Distribution in the various organs was studied by means of fluorescence activated by a 52202 E/70-HPW 125/W Philips ultra-violet lamp. Photographs were taken with a Leica apparatus equipped with an Elmar objective (f=5 cm) and Wratten gelatin filter 2 B Kodak with the object at about 40 cm from both the light source and the camera. A Kodak Panatomic X, F×135, film was used.

RESULTS

The relationship between various tetracyclines and the weight of the embryos is shown in Table 1. The analysis of variance of the experimental results given in Table 1 is shown in Table 2. Several malformations were noted and are compared in Table 3.

TABLE 1

EFFECT OF TETRACYCLINES ON WEIGHT OF 16-DAY CHICK EMBRYOS INJECTED INTO THE YOLK SAC ON THE EIGHTH DAY AFTER THE ONSET OF HATCHING

1 mg of each tetracycline was injected

Drug	No. of embryos	Average weight (g)
Demethylchlortetracycline	12	9·7
Tetracycline	11	10·4
Chlortetracycline	10	9·7
Chlormethylenecycline	11	11·1
None (control)	12	11·8

TABLE 2

ANALYSIS OF VARIANCE BETWEEN ACTIONS OF CHLORMETHYLENECYCLINE AND THE OTHER TETRACYCLINES

The values in Table 1 are analysed. $*P<0.05$ $\dagger P>0.05$

Drugs	Source of variation	Sum of squares	Degrees of freedom	Variance	Variance ratio (F)
Chlormethylenecycline and demethylchlortetracycline	Between samples	13	1	13	9·8*
	Within samples	28	21	1·33	
Chlormethylenecycline and tetracycline	Between samples	2·27	1	2·27	1·46†
	Within samples	31	20	1·55	
Chlormethylenecycline and chlortetracycline	Between samples	11·14	1	11·14	5·3*
	Within samples	40·10	19	2·11	
Chlormethylenecycline and controls	Between samples	2	1	2	2†
	Within samples	21	21	1	

TABLE 3

FREQUENCY AND NATURE OF MALFORMATIONS IN CHICK EMBRYOS

Drug	Malformations (%)	Deformities
Demethylchlortetracycline	91·6	Various limb deformities, syndactyly, celosomia
Tetracycline	63·6	Malformation of tarsometatarsus
Chlortetracycline	41·6	Malformation of tarsometatarsus, celosomia, brachygnatia inferior microphthalmia
Chlormethylenecycline	8·2	Celosomia, malformation of tibiotarsus and tarsometatarsus
None (control)	8·3	Celosomia

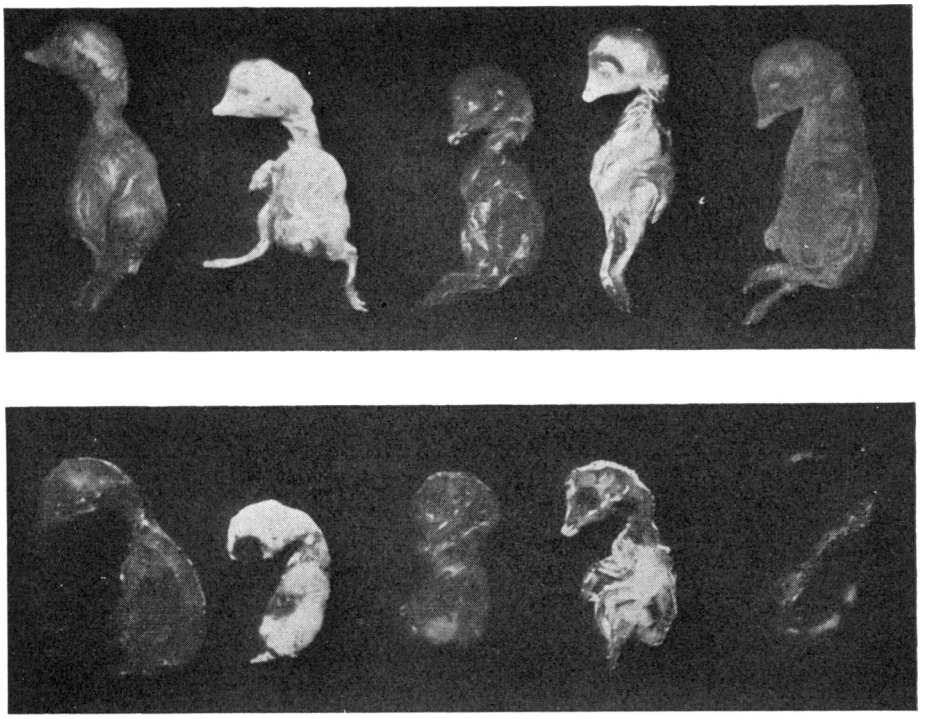

Fig. 1. 16-day chick embryos injected into the yolk sac on the eighth day after the onset of hatching with 1 mg of various tetracyclines, photographed under ultra-violet light. From left to right: embryos treated with chlormethylenecycline, demethylchlortetracycline, chlortetracycline, tetracycline and control. Top, whole embryos; bottom: sagittal sections of same.

Examination of embryos under ultra-violet light showed an extremely variable deposition in the skeleton according to the nature of the tetracycline. Fig. 1 shows sectioned and unsectioned embryos, treated with the four tetracyclines, under ultra-violet light. The examination of chick embryos in ultra-violet light showed a different amount of fluorescence, probably connected with the stability of each tetracycline ; moreover, embryos treated with chlortetracycline or chlormethylenecycline showed only the autofluorescence of tissues. It seemed worth while to investigate the possible relationship between stability and bone-persistence in the mammalian foetuses. To this end rabbit neonates, born from tetracycline-treated mothers, were carefully examined ; they did not show either significant variations in weight between the groups, or any malformation. The same results were obtained with Wistar albino rats.

When sagittal sections of the neonates were viewed under ultra-violet light the sections showed very different pictures depending on the nature of the tetracycline administered to the mother. Chlormethylenecycline did not cause any significant degree of fluorescence in any part of the body ; chlortetracycline caused a very low degree of fluorescence especially in the cranial bones, and tetracycline and particularly demethylchlortetracycline showed an intense brilliant yellow fluorescence of the skeleton and, occasionally, of some soft tissues.

Although the various tetracyclines have a different intensity of fluorescence, these differences do not seem to be sufficiently marked to impair the results significantly.

DISCUSSION

There appears to be direct relationship between the stability of a tetracycline and its concentration in the foetal skeleton as demonstrated by fluorescence ; this is probably related to a longer persistence in the foetal blood of more stable tetracyclines during the time of bone formation. Although it was not possible to find true malformations in our experiments with rabbits and rats, there is ample evidence in the literature on the influence of tetracyclines in delaying growth and causing malformations (Filippi & Mela, 1957 ; Bevelander, Goldberg & Nakahara, 1960 ; Bevelander, Nakahara & Rolle, 1960 ; Carter & Wilson, 1962 ; Cohlan, Bevelander & Bross, 1962 ; Cohlan, Bevelander & Tiamsic, 1963).

It is therefore suggested that the greater the stability of a tetracycline, the more it becomes localized in developing bone of foetus ; furthermore, accepting the results of Eidus, Maniar & Greenberg (1962) that the antibiotic potency *in vivo* of a tetracycline does not depend on its stability, it should be safer to use unstable tetracycline clinically, in order to avoid undesired effects on the foetus.

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