In vitro germination method

In this method mature and intact pollinia dissected out from flowers just opened of *C. gigantea* were used. The hanging drop technique was followed. All germination studies were carried out at room temperature (25 and 31 C) observing five replicates for 24 hr. To determine the percentage of germination on different media, the pollinia after 24 hr of incubation were teased out carefully with a needle to remove the pollinial wall and as many as 1000 pollen grains were studied. The percentage germination varied with the use of different media: 35 % in distilled water; 53, 81, 95, 96 and 99 % in 5, 10, 15, 20 and 25 % sucrose respectively; 85 % in Brewbaker and Kwack's medium[6]; 96 % in Modified Brewbaker and Kwack's medium (with 20 % sucrose and 200 mg/l boron); and 90 % in Malik and Chhabra's medium[7].

Alexander's staining method:—

Pollen viability was calculated using Alexander's stain[8], where the fertile pollen were stained red and the sterile ones green. The pollen grains could not be liberated by dissecting the fresh pollinia. Therefore, the pollinia were dipped in double-distilled water for 45 min (time required for initiation of first pollen tube) and then teased out carefully to liberate the pollen grains for staining. The pollen grains (78.9 %) stained red, indicating their viability.

Though earlier attempts[1] to culture the pollinia of *C. gigantea* through hanging drop technique did not succeed, it has been successfully carried out here. However, teased out pollen grains from the pollinia of *C. gigantea* cannot be germinated well on any media. A detailed study on the *in vitro* pollinial germination of *C. gigantea* will be published elsewhere.

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TERATOGENIC EFFECTS OF SODIUM DIETHYLDITHIOCARBAMATE IN THE GARDEN LIZARD, *CALOTES VERSICOLOR*.

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SODIUM diethyldithiocarbamate (DEDC) and related compounds are of considerable pharmaceutical value. They are used to treat heavy metal poisoning, aversion therapy for alcoholism and as sarcopticide in the treatment of scabies[1,2]. Since our earlier work showed that DEDC is a potent teratogen in frog embryos[3], the same was tested on lizard embryos for the degree of its potency.

The eggs were collected from the local garden, cleaned with distilled water and kept on cotton soaked in distilled water. Before experimentation the age of one embryo from a clutch was determined as described by Muthukkaruppan *et al*[4]. The eggs were treated with various concentrations of aqueous sodium diethyldithiocarbamate solution (DEDC) by keeping them on soaked cotton in petri dishes. Eggs kept on cotton soaked in distilled water were treated as controls. The experiments were conducted at room temperature (26 $\pm$3°C) and continued until the embryos in the controls hatched out of shell. During this period (40–44 days) cotton was soaked with fresh solution every 72–96 hr. The eggs which showed discharge of fluid from the shell were opened because eggs discharge fluid either when embryos are in hatching stage or are under stress[5]. The embryos were observed under binocular dissecting microscope and photographed.

Calotes eggs selected for the experiments were between stage 28 and 30 with 30 to 32 somite pairs. In stage 30 well-marked, swollen limb buds could easily be observed. The period required for complete development and hatching varied between 41 and 44 days. Throughout this period the eggs showed gradual increase in size. In any one clutch, all the eggs hatched within a period of 24 hr from the one that hatched first. All control lizards were normal in appearance and behaviour (table 1).

All experimental egg groups were opened with scissors since hatching failed. Eggs treated with 0.05 % and above concentrations showed prematrue discharge of watery fluid and embryos removed from the eggs were usually found dead or moribund and highly retarded in growth (figure 1). Some embryos died

Table 1 *Effects of various dithiocarbamate solutions on the embroys of the garden lizard*

Concentration	No. of Eggs treated	Mortality	Observations
0.0 % DEDC (control)	46	6.52 % till the end of experiment	No abnormalities in any hatching, total length of young lizard was 74 ±4 mm (snout to the tip of tail) and snout-vent length 25–30 mm.
0.01 % DEDC	35	14.28 % till the end of experiment	5 eggs showed premature discharge in 20–36 days, none of the remaining 30 hatched, when removed all embryos were living, 23 out of 30 eggs were considerably swollen and turgid than the controls, total length of the embryos 55 ±5 mm, snout-vent length 20–25 mm.
0.025 % DEDC	35	20 % till the end of experiment	Only 7 eggs showed premature discharge of fluid in 22–38 days, remaining 28 continued to develop but could not hatch, all 28 embryos were highly abnormal, 21 were living when removed from egg, total length of the embryos 30 ±5 mm.
0.05 % DEDC	25	100 % in 30 days.	Eggs showed premature discharge of fluid in 15–30 days, embryos removed were severely retarded and abnormal, 10 embryos showed haemorrhage, limb development was very much affected.

which may be due to severe haemorrhage in the dorsal region of head and trunk. The DEDC at 0.025 % was not highly embryotoxic and most of the embryos continued to develop without any discharge of fluid. All these embryos were highly abnormal (100 % abnormality) but most of them were living and showed movement when removed from egg. Some prominent abnormalities were: extreme retardation of growth, trunk and tail poorly developed as compared to head, limbs stumpy with subnormal number of digits, incomplete absorption of yolk sac, incomplete abdominal skin with gut region protruding out, lower jaw incomplete and abnormal bending of the body axis (figures 2–4). In a few cases total morphogenesis was affected and the embryos were just a mass of cells without any kind of differentiation.

Eggs treated with 0.01 % DEDC also developed without external sign of stress. In fact, the eggs often absorbed more water and were swollen and turgid than the control eggs. When pricked with a fine needle the watery fluid squirted out of the leathery egg cover. The embryos removed from these eggs were completely developed without any kind of striking abnormality. There was a slight retardation of growth and the yolk sac was incompletely absorbed. All such embryos were living and showed movement. None could however survive and assume normal posture from the curled-up condition in which they reside in the egg.

From the result of these experiments it is clear that the developing eggs take up DEDC along with water. Water is absorbed during normal development of *Calotes* and the egg membranes have been shown to be permeable even to the dye Trypan blue[6]. The experiments further prove that DEDC is a potent teratogen.

Many related compounds have also been reported to be teratogenic in vertebrates. DEDC, tetraethylthiuram mono and disulphide were teratogenic in frog inducing specific notochordal anomaly[3,7], while tetramethylthiuram mono and disulphide as well as tetraethylthiuram disulphide (TETD) induced eye defects and open coelom in chick embryos[8]. In mammals TETD (with dimethyl sulphoxide as a solvent) was shown to cause multiple skeletal abnormalities as well as limb and tail defects[9].

Dithiocarbamates are very reactive compounds possessing strong metal-binding characteristics and they have a wide variety of biological effects as reviewed by Fishbein[10,11]. Since these chemicals are widely used, the studies to understand the mechanism of their teratogenicity warrant attention.

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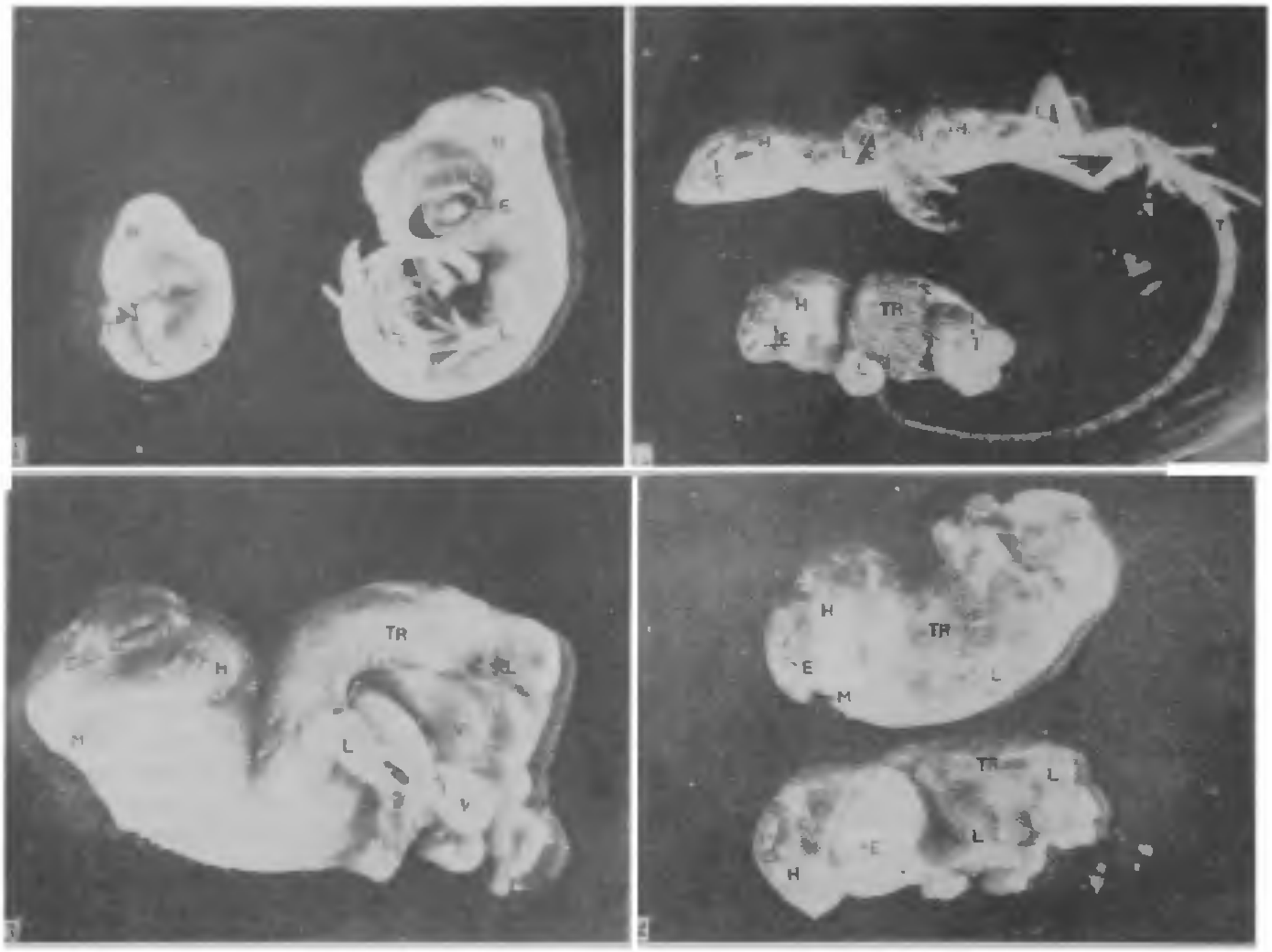

Figures 1–4: 1. *Calotes* embryos removed prematurely from the egg. Control embryos (on the right side) shows development of head, eyes, limbs and tail. The experimental embryos (on the left) is of the same age but shows retardation of growth due to treatment of 0.05% DEDC. **2.** Control *Calotes* hatching (above) and experimental embryo (below). Note abnormal development of head, trunk as well as tail due to 0.025% DEDC. **3.** Experimental embryo after treatment of 0.025% DEDC. See viscera hanging out of the open body cavity, stumpy limbs with claws. **4.** Two highly abnormal embryos treated as above. Note poor development of trunk region and tail. (*Abbreviations*: E—Eye, H—Head, L—Limb, M—Mouth, T—Tail, TR—Trunk, V—Viscera.)

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