CHAPTER 3

MATERIALS AND METHODS
3.1 Experimental setup

The lizards, *Hemidactylus brooki* were collected from the villages near Kottayam town with the help of animal collector and were brought to the laboratory. The animals were kept in the laboratory for a fortnight, to get them acclimatized to the laboratory conditions. They were maintained on a diet of insects and water *ad libitum*.

For each experiment, thirty adult lizards of both sexes with normal tail, weighing 2 to 2.5 gms and measuring 4 to 5 cm snout-vent length were selected. They were then divided into 3 equal groups. Tail autotomy was induced in the lizard by pinching off the tail leaving two to three segments intact after the vent. For each set of experiment, five autotomised lizards were maintained in a well aerated cylindrical plastic jar (16 x 10 cm). The plastic jars with animals were placed in contact with north and south poles of a cylindrical solid ceramic permanent magnet (Plate 1. A). Control lizards were kept away from the magnetic field (Plate 1. B).

For the present study, ceramic cylindrical solid permanent magnets, with a diameter of 10 cm and thickness of 5 cm, procured from Dath Pethe, Magnets, XL/3440, T. D Road, North Ernakulam, Kochi were used. The measured strength of these magnets was non-uniform, but approximately 3000 Oersteds. The magnetic gradient
PLATE 1

A- Experimental Setup

B- Control
lessened further away from the magnet. The plastic jar containing lizards were placed on a wooden table in horizontal position with magnetic flux horizontal to the ground. Each experimental setup was kept away from each other in order to avoid magnetic interferences between them.

**Group I** - The lizards of these groups were served as control. These animals were kept away from the magnetic fields throughout the experimental period.

**Group II** - The lizards of these groups were exposed to north pole of the ceramic magnet throughout the experimental period.

**Group III** - The lizards of these groups were exposed to south pole of the ceramic magnet throughout the experimental period.

### 3.2 Time taken for attainment of various stages and rate of growth of tail regenerate under different magnetic fields

The time taken (in days) for the attainment of each of the stages of the tail regeneration of control and experimental animals were recorded. The growth rate of the regenerate was measured at ten days intervals, starting from the 10th day of the autotomy till the 80th day. Relative weight of regenerate per 100 gm body weight during different phases of regeneration, the rate of growth per day, percentage of inhibition and total percentage of tail replacement in control and experimental lizards were calculated.
3.3 Metabolites in regenerating lizards under different magnetic fields

3.3.1 Glycogen

Estimation of glycogen contents in the tail regenerate and liver of control and experimental lizards during different phases of their tail regeneration were carried out by Anthrone method of Seifter et al., (1950). The optical density of the solution was measured at 620 nm in a spectrophotometer. The amount of glycogen was expressed in mg / 100 mg wet tissue.

3.3.2 Blood Glucose

Estimation of glucose levels in blood in control and experimental lizards during different phases of regeneration were carried out by micro-method of Folin and Malmros (1929). The optical density of the colour was measured at 540 nm in a spectrophotometer. Glucose level was expressed in mg glucose / 100 ml of blood.

3.3.3 Total lipids

Total lipids were estimated based on the method of Bragdon (1951) in the tail regenerate and liver during different phases of tail regeneration in control and experimental lizards. The optical density of the coloured solution was measured at 580 nm in a
spectrophotometer. The amount of total lipids was expressed in mg / 100 mg dry tissue.

3.4 Haematology of regenerating lizards under different magnetic fields

3.4.1 Haemoglobin

Haemoglobin concentration was determined in the control and experimental lizards by the Cyanmethaemoglobin method (Baker and Silverton, 1976). 0.02ml of blood collected from the heart was mixed with Drabkins solution. It was then kept undisturbed for 10 minutes for full colour development. The optical density of the sample and the standard Cyanmethaemoglobin solution were measured in a spectrophotometer at 510 nm. The haemoglobin content was expressed in gm / 100 ml of blood.

3.4.2 RBC and WBC count

The number of red blood corpuscles (RBC) and white blood corpuscles (WBC) present per cubic millimeter (mm$^3$) were determined in the control and experimental lizards using a haemocytometer (Neubauer, Germany). Differential counts of leucocytes were carried out in thin blood smears, stained with Leishman stain (Gurr, 1956).
3.4.3. Packed Cell Volume (PCV)

The packed cell volume was estimated by the microhaematocrit method (Baker and Silverton, 1976). MCH, MCHC and MCV were calculated from PCV, RBC and haemoglobin values (Sood, 1990).

3.5 Ascorbic acid in regenerating lizards under different magnetic fields

For quantitative estimations of ascorbic acid, liver, kidney and tail of control and experimental animals were used. The tissues were quickly weighed and homogenized in 6% Trichloroacetic acid (TCA) in prechilled mortar. Aliquot samples of the extracts were utilized for estimation of total ascorbic acid, employing the Dinitrophenyl hydrazine method of Roe (1954). The optical density of the coloured solution was measured at 540 nm in a spectrophotometer. The quantity of ascorbic acid was expressed in mg / 100g wet tissue.

3.6 Acid and Alkaline phosphatases in regenerating lizards under different magnetic fields

3.6.1 Acid phosphatase

Acid phosphatase activity was measured by using the method of Gutman and Gutman (1940) in liver, kidney and tail at different phases of tail regeneration in experimental and control lizards. The
optical density was measured at 510 nm in a spectrophotometer. The enzyme activity was expressed as mg phenol / 1hr / gm tissue.

3.6.2 Alkaline phosphatase

Alkaline phosphatase activity was measured by using the method of Kind and King (1954), using Disodium phenyl phosphate as substrate in liver, kidney and tail during different phases of tail regeneration in experimental and control lizards. The optical density was measured at 510 nm in a spectrophotometer. The enzyme activity was expressed as mg phenol / 1hr / gm tissue.

3.7 Data analysis

The data thus obtained from the various parameters investigated were statistically analysed for possible significant differences, employing Students‘t’ – test, following the descriptions of Gomez and Gomez (1981).