CHAPTER V

ABNORMAL DEVELOPMENT IN CHICK EMBRYO AFTER ADMINISTRATION OF 4-DAB
Normal embryogenesis rests on a co-ordinated sequence of large number of processes and this harmonious integrity is maintained by elaborate systems of physiological control throughout life. The normal existence of a multicellular organism is thus governed by the co-ordination of growth and maintenance of the tissues. Differentiation and growth of tissues and organs in the embryo are inseparable. However, under certain circumstances the delicate interconnection of differentiation and growth may be dislocated and dis-co-ordinated development proceeds resulting in some atypical growth in sharp contrast with the normal. In these circumstances differentiated tissues are present in chaotic arrangement bearing very little resemblance with the same type of tissues in normal individual. These type of dis-co-ordinated tissues are termed as teratomas. Differentiation and growth, both the process in teratomas are severely dis-co-ordinated simultaneously. But more often conditions of well co-ordinated differentiation with a severe dis-co-ordination in growth process are encountered with resulting malformation of the involved organ during the period of embryogenesis.

These developmental anomalies may occur spontaneously. In recent years much work has been done on this line and some genetic strains have been thought to be responsible for producing these abnormalities. Spontaneous malformations are considered to be only those which occur independent of any
stimulus. The deviations from normality occur due to the general influence of undetermined genetic constitution or as a response of the embryo to deviations of intrinsic or extrinsic environment during development.

Congenital malformations in birds in general are caused by genetic factors. A variety of these hereditary defects are determined by factors transmitted through the germ cells from generation to generation (Romanoff, 1972). However, both physical and chemical environment may influence to modify the susceptibility of these hereditary abnormalities.

Teratogenesis:

Malformations can be induced in experimental animals by using variety of physical and chemical agents. Induction of malformation during development by chemical agents is one of the methods frequently used by workers. Sometimes nutritional deficiencies may disturb the embryonic development leading to various malformation and prenatal death (Romanoff, 1972). Each chemical compound in certain doses, may cause diseases, malformation and prenatal death. There is a wide variation of sensitivity to a particular compound in teratological and lethal responses. Romanoff (1972) observed that the anomalies induced in chicken embryos at a certain stage of development are often always same for different chemical teratogens. At the same time the anomalies induced by a certain
chemical teratogen are different in nature when the developing embryo is exposed to the agent at different stages of development. But the sensitivity period for each compound varies widely, giving a characteristic anomaly for the compound used which depend on the sensitive period of that compound. A teratogen may induce different types of anomalies depending on the stage of development at the time of treatment. From this observation it can be remarked that the chemical nature of teratogenic agent primarily determines its sensitive period on the stage of development and the stage at which the embryo is exposed to the agent, determines the type of abnormality produced.

The development of avian embryo is also disturbed by the physical components of the atmospheric environment. Dareste (1891) noted that abnormal temperature causes prenatal malformations in avian embryo. Further, Romanoff (1938) have reported that when chick embryos were exposed to temperature ranging from 33.5 to 40.5°C cause off colour liver, deformed feet, abdominal oedema and other malformations. At present, it is known that a small deviation in temperature from optimum (37.5°C) produces abnormal chick embryos, the percentage of which is quite noticeable. Ridgway and Karnofsky (1952) reported that induction of malformation by chemical compounds like cobalt chloride in chick embryos causes softening of brain and slightly oedematous embryos with enlarged liver. Other chemical
12TH DAY OLD MALFORMED EMBRYO
(AFTER ADMINISTRATION OF 4-DAB)

K 12TH DAY OLD EMBRYO (CONTROL)

L 16TH DAY OLD EMBRYO (CONTROL)
Compounds like ethionine induce malformation in chick embryos manifested by growth inhibition, enlarged fatty liver and severe oedema (Dessi et al. 1959). Severe retardation of growth (28%), enlargement of liver (9%) and other abnormalities by floxuridine was noticed by Kury and Crosby (1967). Antibiotic, such as, mitomycin C causes defects of abdominal wall (23%), cerebral haemorrhage (17%), abnormalities of beak (44%) and other malformations as reported by Kury and Craig (1967). Among the carcinogens and azo dyes, 3-methylcholanthrene causes retardation of growth and effects the brain (Beskrovnii, 1941). Methylene blue causes partial absence of roof of skull, malformation of brain, abdominal defects and other abnormalities (Kirrmann, 1961). Trypan blue causes hematomas in brain, eyes and limb buds, with other malformations (Kaplan and Johnson, 1970). Aflatoxin B causes reduction of weight in chick embryo, growth retardation and decrease in weight of liver parallel to the decrease in weight of the embryo (Shibko et al. 1968). Armstrong and Ham (1947) have reported that the liver and heart of tumour bearing chick embryos are found to be larger than those of the controls. Among the chief pathological symptoms in the affected embryos are enlarged flabby hearts and enlarged liver showing peripheral necrosis (Armstrong and Ham, 1947).

Observation and Discussion:

In the present study, during the entire period of investigation, a very peculiar observation was noted. In the
16TH DAY OLD MALFORMED EMBRYO
(AFTER ADMINISTRATION OF 4-DAB)
control groups, 170 numbers of fertilized eggs were incubated and no malformations or abnormalities in any instance were observed. Similarly, in the test groups, 195 numbers of fertilized eggs were incubated and on the 4th day of incubation a single dose of 0.07 ml. of 4-DAB (0.1 mg/ml.) was inoculated. On the 12th and 16th day of incubation, in the test group, 4 embryos were observed with liver defects and malformations of abdominal wall, to only one embryo on the 12th day of development was observed with severe malformation having incomplete development of abdominal wall and hematomas on the head. On the 16th day of development in the same group 3 cases of malformed chick embryos were noted with incomplete abdominal wall, exposed viscera, enlarged liver and grossly enlarged gall bladder. (Malformed embryos - in the Photographs).

Each embryo is the product of hereditary and environmental influence. Generally, great majority of congenital malformations are caused by genetic factors. These hereditary abnormalities, however, are susceptible to modification by the influence of both physical and chemical environments (Romanoff, 1972). Landauer et al. (1954) earlier believed that non-hereditary abnormalities and hereditary mutations are modification with end effects morphologically similar to genetic syndromes which can be produced by various physical and chemical agents. Reports are available for various
malformation of embryos induced by chemical agents. Dessi et al. (1959), Martin (1975), Jacob (1978), Kury and Crosby (1967), Kury and Craig (1967), Beskrovni (1941), Kirrmann (1961), Armstrong and Ham (1947) reported that various malformations such as inhibition of growth, abdominal defects, defects in brain, liver, hearts, limb-buds etc., the effect can be induced by number of teratogen. In the present investigation, embryos were exposed to 4-DAB and the observed abnormalities with defective growth and malformation are likely to be due to the effects of genetic anomalies caused by 4-DAB. 4-DAB binding with the proteins ultimately attacks the hereditary materials (Miller, 1945, 1970) and may lead to the development of the malformation and enlargement of liver and gall bladder (Photographs: malformed embryos on 16th day).

Instances of malformations in chick embryos are well known phenomenon as reported by many workers (Shibko et al. 1968; Kaplan and Johnson, 1970, Ridgway and Karnofsky, 1952). It is also known that abnormalities can be induced by different physical factors like temperature (Dereste, 1891), nutritional factors (Romanoff, 1972), genetic strains as well as mutants (Landauer, 1954) etc. Certain workers have also stressed the importance of various chemicals for embryonic malformations. In the present investigation both the control and the test groups were incubated under identical conditions and hence the physical factors like temperature, nutrition were unlikely to
influence the abnormal growth and development and no anomalies in the process of development and growth in the control group were noted. The abnormal development observed in a few numbers of embryos in the test group on the 12th and 16th day of treatment with 4-DAB certainly thus seemed to emphasize the importance of chemicals. However, it would be far-reaching conclusion to infer the influence of chemicals definitely at this stage of development and the mechanisms of the abnormalities encountered during the present investigation. Moreover, the present study was not designed to make an in detail study of such malformation. It is thus apparent that more than one factor may be involved to manifest for such abnormalities and 4-DAB could be one of such factors. With the present evidence it can only be said that a more detailed and systemic study should be undertaken before any firm conclusion can be drawn.
1) During the period of investigation, 170 numbers of fertilized eggs for the control groups and 195 numbers of fertilized eggs for the test groups (i.e. treated with 4-DAB) were incubated and the development was observed in both the control and test groups (after administration of 4-DAB on the 4th day of development) for the study of the project.

2) No abnormal formation was observed either in the control or in the test groups on the 6th and 8th day of incubation during the process of development.

3) On the 12th day of incubation in the test group malformations of hematoma and abdominal wall defect were observed only in one case. However, no such malformation was noted in the control group during the whole period of incubation and growth.

4) On the 16th day of incubation in the test group malformations of abdominal wall with the exposed viscera and enlarged liver and gall bladder were observed. No such malformation was recorded in the control group during the whole period of incubation and growth.