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Effect of Diethylstilbestrol on the Development of *Drosophila melanogaster*: Studies on Nucleic Acids and Proteins

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Abstract: The present study is an attempt to evaluate the effect of Diethylstilbestrol (DES), a female non-steroidal hormone, on the development of *Drosophila melanogaster* at the temperature of 25±4°C. The various developmental stages were weighed and then used for the estimation of nucleic acids and total protein contents. In this study an attempt is being made to correlate the present results with the established role of DES on the vertebrates. The weights of various developmental stages are drastically affected by the DES treatment. The various macromolecular contents are also likewise affected. DNA and protein show negative trend between treatment groups (from 1st instar to adult stage) and differ non-significantly from one another but each developmental stage treated with different doses of hormone shows highly significant differences. It appears that DES has an inhibitory effect on the developmental stages of *D. melanogaster*.

Key words: Diethylstilbestrol, *Drosophila melanogaster,* nucleic acids, protein, larva, pupa.

INTRODUCTION

Estrogenic activity is a property shared by a great number of chemical compounds and in this respect the estrogens are unique among the hormones. Among the non-steroidal hormones, diethylstilbestrol (DES) is the most potent one. In contrast to the natural estrogens, it is highly active when given by mouth and the duration of action of a single dose is longer, due to its slower rate of degradation in the body (Goodman and Gilman, 1975). DES has been used most widely either in human and veterinary medicine or for growth promotion as an anabolic agent. This chemical has also undergone the most thorough investigations regarding its toxicity and potential carcinogenic effects (Lone, 1997).

Although different types of drugs and other chemicals have been frequently used to elucidate the mechanism of developmental processes in *Drosophila,* one of the sex hormones of the vertebrates, namely DES, has been used in the present investigation on *Drosophila melanogaster* for the first time. Moreover, as developmental processes are under direct genetic control, the effects of a carcinogen on such processes will be interesting from the viewpoint of gene activation during developmental processes. The present report describes the biochemical changes involved during the development of *Drosophila melanogaster* under the influence of estrogen (diethylstilbestrol) for the first time.

MATERIALS AND METHODS

A total of 200 male and 200 female *Drosophila melanogaster* were used for the experiment. Food preparation, maintenance of the *Drosophila* culture and the identification of the first, second, third and fourth instar larvae, pupae and adults (males and females) was according to the Strickberger's technique (1962). The drug was procured from Sigma (UK). A stock solution of the drug was prepared in ethyl alcohol and the required amount of this solution was added to the culture medium. The control medium was treated with the carrier only. The weights of different developmental stages were recorded and then used for the estimation of nucleic acids (DNA and RNA) and total protein contents. Nucleic acids from different developmental stages of the *D. melanogaster* were extracted according to Lone and Matty (1980). The proteins were determined by the method of Lowry *et al*. (1951) as modified by Schacterle and Pollack (1973).

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RESULTS

The effects of Diethylstilbestrol on the life cycle of *Drosophila melanogaster* have been studied in terms of changes in: i) weight, ii) total nucleic acids, and iii) total protein contents of various developmental stages at a temperature of 25±4°C. Figure 1A shows the effect of 4 doses of DES on the weight of various developmental stages. The first instar larvae of the control and 8.33, 10, 12.5 and 16.67 mg/kg food DES treated weighed 85, 65, 57, 50 and 45 µg / larvae, respectively. The maximum weight of these larvae is achieved at the 4th instar stage, just before they develop into pupae. These weights were 1.5, 1.45, 1.38, 1.21 and 1.19 mg in the control and treated larvae, respectively. The larval weight increases 18 times in the control, 22 times in 8.33 mg, 24 times in 10 mg, 24 times in 12.5 and 26.5 times in 16.67 mg DES treated groups. The weight of pupa decreases from the 4th instar in all doses. The DES treated groups showed negative trend in weight in different developmental stages as the dose of the hormone was increased. Highly significant differences in weight at each developmental stage treated with different doses of hormone were observed. Similarly analysis of variance carried out on adult (average weight for females and males were used in the analysis) weights showed significant difference.

During the development, the quantity of DNA/mg larva decreased gradually both in the control and hormone treated larvae (Fig.1B). In the control the first instar larva contains 462.6562 µg DNA/100 mg larval weight. During larval growth there is a distinct tendency towards a drastic decrease in the DNA content. These contents decrease to 218.82 µg DNA/100 mg larval weight at the 4th instar stage, just before it develops into pupa. This decrease is 52.7%. Similarly, there is 60%, 61%, 59.76% and 45% decrease of DNA content in the 4th instar of 8.33, 10, 12.5 and 16.67 mg DES treated larvae in relation to the DNA content/mg larvae (Fig.1B). In the pupal stage, the amount of DNA/mg pupa shows an increase in the treated as well as control experiments. The increase of DNA in pupa is 60, 63, 70, 70.57 and 4% with respect to 4th instar larva respectively in control and treated groups (Fig.1B). In each treatment group a negative trend in DNA amount was observed starting from 1st instar to adult stage. Each developmental stage treated with different doses of hormones showed highly significant differences in DNA amounts (ANOVA; $P<0.05$). Similar trend of DNA is observed for adults (average values of males and females were used in the analysis).

The general pattern of changes in the RNA content of various developmental stages is the same in control and treated groups. The amount of RNA/mg larval weight gradually decreased during larval period (Fig.1C). In controls the 1st instar larva contains 2180.72 µg RNA/100 mg weight of larva. Just before initiation of pupation, the quantity of RNA decreased by 64%. DES treatment resulted in an increase in RNA contents. In 8.33, 10, 12.5 and 16.67 mg Diethylstilbestrol treated groups, the quantity of RNA/100 mg larva of the first instar was 2656, 2416, 1728 and 2930 µg, respectively. Just before the start of pupation the RNA, when compared with those of 1st instar larvae showed a decrease of 65.6% in 8.33 mg, 53.2% in 10 mg, 72.7% in 12.5 mg and 76% in 16.67 mg DES treated groups. A positive trend in RNA amount was observed in each developmental stage when treated with different doses of hormone. This increase in RNA amount with increase in hormone dose is highly significant (ANOVA; $P< 0.05$).

The quantitative change in the protein content of various developmental stages is the same in control and treated groups. The amount of protein/mg larval weight gradually decreased during larval period (Fig.1D). The 1st instar larva in control contained 5.58 mg protein/100 mg larval weight. These contents decreased by 51.6% in the 4th instar, which had only 2.70 mg protein/100 mg weight of larva. Diethylstilbestrol treatment resulted in decrease in protein content. This decrease in protein contents of the larvae is 53%, 49.25%, 52.45% and 53% respectively (Fig.1D). A highly significant decrease in protein amount with increase in hormone dose at each developmental stage was observed ($P< 0.05$).

DISCUSSION

The eggs after hatching emerge into larvae, which then pass through the growth periods of 9 days

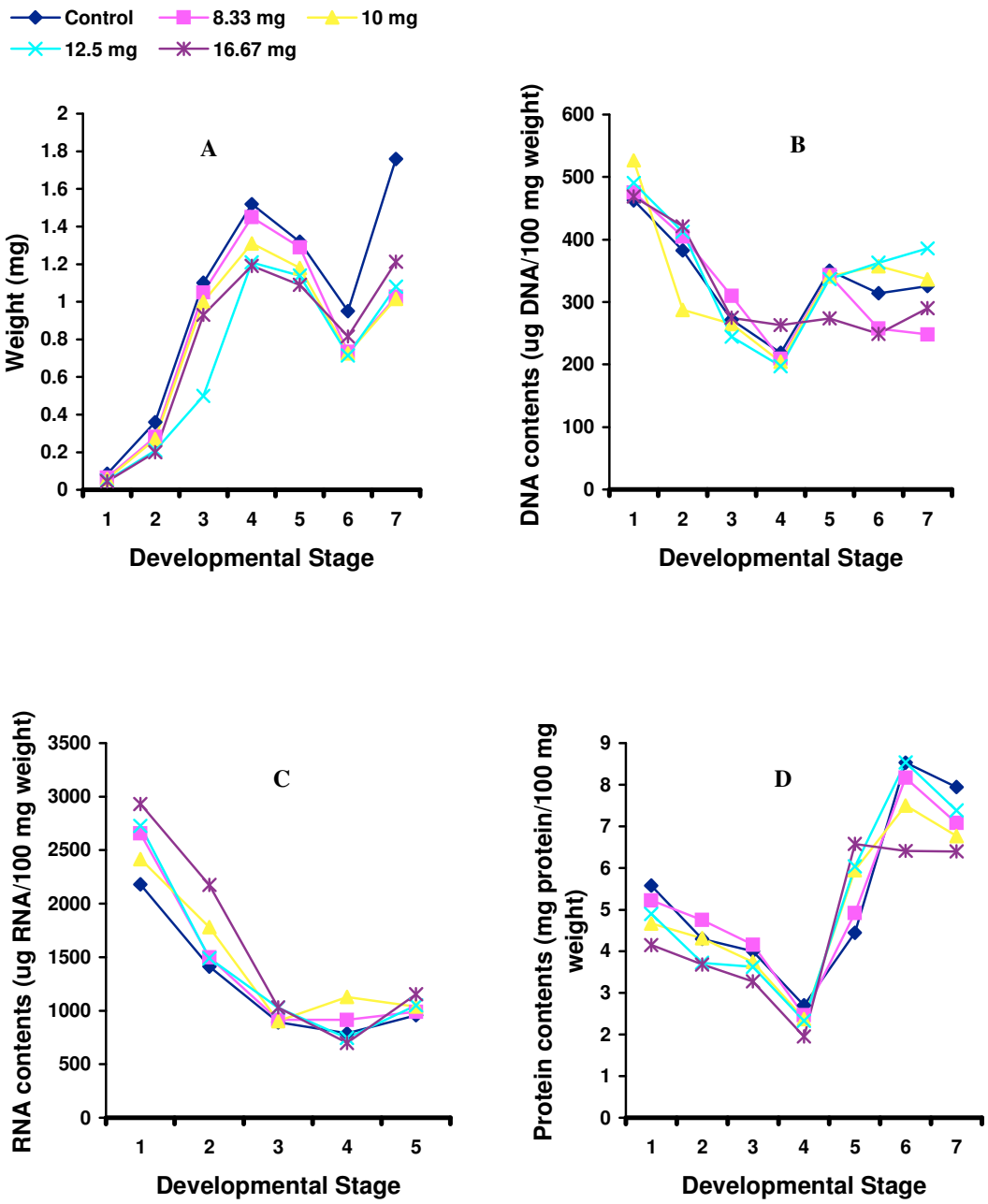

Fig. 1. Effect of diethylstilbestrol added in the culture medium on the various developmental stages of *Drosophila melanogaster* during development at 25±4°C. A, Weights; B, DNA; C: RNA and D, protein contents. 1, 1st instar larvae; 2, 2nd instar larvae; 3, 3rd instar larvae; 4, 4th instar larvae; 5, pupae; 6, males; 7, females.

to become pupae. During this period, not only the weight of larvae is gradually increasing, the various molecular contents, like RNA, DNA and total proteins also increase. The degree of increase and the speed with which the synthesis of these molecules takes place depends very much on

temperature, at which the development is being carried out. In spite of the differences in the absolute values of the biochemical parameters under different temperature conditions, the general pattern of changes in the biochemical contents during successive stages of development, remain the same. Since the weights of the larvae are increasing at a greater speed, the various macromolecular contents, when considered in terms of contents per mg larval weight show an overall decrease during the larval growth period. Pupa, though apparently a very stable and non-growing stage of development, is characterized by very intensive cellular and metabolic re-organization. During pupation the body weight starts decreasing. Closely linked with this is the increase of total protein, DNA and slight decrease of RNA contents. All these biochemical changes, when considered as per mg pupal weight, show an increase to various degrees during pupation period. In adult flies these components show a definite trend towards higher values. The various changes mentioned in the present report are consistent with those of Church and Robertson (1966) and Shakoori and Bakhtiar (1979).

The administration of DES did not induce any major qualitative deviation from the control pattern of biochemical changes during development. No morphological abnormality was observed after treatment during the development of *D. melanogaster.* In this regard the influence of DES is opposite to the effect of certain chemicals like thioacetamide (Shakoori and Butt, 1979), which is also a carcinogen.

Out of the 4 different doses of DES administered, the highest dose (16.67 mg) seems to have caused the maximum effect on DNA, RNA and protein contents of *D. melanogaster* during development, while the lowest (8.33 mg) dose had the minimum influence. The various developmental stages, *i.e*., larvae, pupae, and adults were found to be lighter in weight and shorter in size when treated with DES.

After reviewing the literature, Lone (1997) reported that DES increased weight gain up to 30% in lambs, steers, bulls and poultry. The effect of DES on *D. melanogaster* is opposite to that of vertebrates as regards the weight. However, oral administration of DES had no effect on reproduction in vertebrates (Wickersham and Shultz, 1964). In vertebrates factors affecting actions of anabolic agents are many. The effect of DES is clearly dependent upon the species used and sometimes differs even between the sexes (Lone, 1997).

Generally speaking DNA, RNA and total protein contents per mg larva weight decreased during the larval growth period in the control as well as with experimental animals. The degree of decrease is variable for different biochemical parameters. The rate of decrease of DNA and RNA contents per mg larva is greater with the increasing concentrations of DES. This obviously is an indication of the inhibition of RNA synthesis. Likewise the rate of decrease in total protein contents per mg larva weight increased during larval growth period with the increasing doses of DES. When the absolute values of all these biochemical components in the controls are compared with the treated groups for the various stages of the same age groups, the treated developmental stages have relatively lower values than the control. This deviation probably shows that the synthesis of all the biochemical components is inhibited with the increasing concentrations of DES.

During pupation the synthesis of various biochemical components seems to have enhanced. The rate of increase of biochemical contents here is highly variable depending upon the concentration of DES. DNA and RNA contents per mg pupa in DES treated groups are lower than that of the control group. The total protein content shows an increase during the pupation period. All these biochemical events can be explained in the light of two very important processes going on during pupation period *i.e*., histolysis and synthesis of new adult proteins including diverse types of enzyme systems (Pasteur and Kastritsis, 1973). Immediately after metamorphosis, once the foundations of definitive organs have been laid, the process of growth of body sets in as a result of which the various biochemical components increase in quantity. The DNA contents of adult fly increase after DES treatment, while the RNA contents and total protein contents rather show decrease under these circumstances. These changes point to the fact that growth in the adult stage is more due to hyperplasia rather than hypertrophy.

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