DNA Polymerase Activity in Extracts of the Developing Chick Skin and Down Feather Organ.* (31960)

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The developing down feather of the chick embryo has recently become increasingly important in studies of organogenesis(1-4). Although the factors involved in feather induction and differentiation have not been precisely defined either morphologically or temporally, several investigators have described events which characterize different developmental stages.

Wessels(3) has found random incorporation of thymidine-H^3 into deoxyribonucleic acid (DNA) of the skin during the period prior to feather germ formation (stages 27 to 29). During this time, epidermal placodes are formed by a mobilization of the cells rather than by a differential mitotic process. Mesodermal condensations are formed only at stage 30 and are believed to arise from a brief phase of mitotic activity. A nonincorporating phase occurs from stage 30 to 32. After this, feather germ outgrowth occurs rapidly; at stage 36 [10 days(5)], it forms a shallow cap. At stage 37 (11 days) barb ridges begin to form on the inner epidermal surface of the distal third of the feather. As the entire organ elongates into a tapered cylinder, the ridges at the base of the feather lengthen. Growth ceases abruptly at the end of the 13th day. Subsequently, keratinization proceeds rapidly with concomitant resorption of the mesodermal pulp.

The incorporation of thymidine into DNA requires the sequential action of a number of enzymes. The initial thymidine utilization may represent merely one of the pathways for the salvage of degraded DNA components. A more direct index of DNA synthesis is the measurement of the activity of a synthetic pathway enzyme. These considerations led us to measure DNA polymerase activity (deoxynucleoside triphosphate: DNA nucleotidyl transferase, EC 2.7.7.7) in extracts from developing chick skin.

In extracts of mammalian tissues, almost all the measurable DNA polymerase activity has been found in the ultracentrifugal supernate (S-3) fraction of the tissue homogenate (6). If, however, the homogenization medium contains magnesium or calcium ions, at least part of the activity is associated with nuclei (7). The present paper reports that the DNA polymerase specific activity in the S-3 fraction of homogenates of chick skins removed at intervals throughout the period of down feather development undergoes a cyclic variation.

Methods. Preparation of extracts. Whole back skins were cleanly stripped off chick embryos at stages 26, 30, 31, 34, 35, 36, 37, 38, 39, and 40(8), encompassing a range in embryonic age of 5 to 14 days. The skins were collected on ice and kept as cold as possible during processing. Each stage was homogenized in 2 ml of media containing 0.22 M sucrose, 0.01 M Tris·Cl pH 8.0, and 6.0 mM KCl in small all-glass(potter-type homogenizers and centrifuged for 1 hour at 105,000 $\times$ g. The supernatants were transferred to clean test tubes and flash-frozen in a bath of acetone and dry ice. They were stored at —10°C until tested.

Methods. Assay for DNA polymerase activity. DNA polymerase activity was measured by the method of Furlong and Williams (9) using 15 μl samples of the soluble supernatants.

Assay for deoxyribonuclease. Since deoxyribonuclease activity can change the nature and rate of the DNA polymerase reaction

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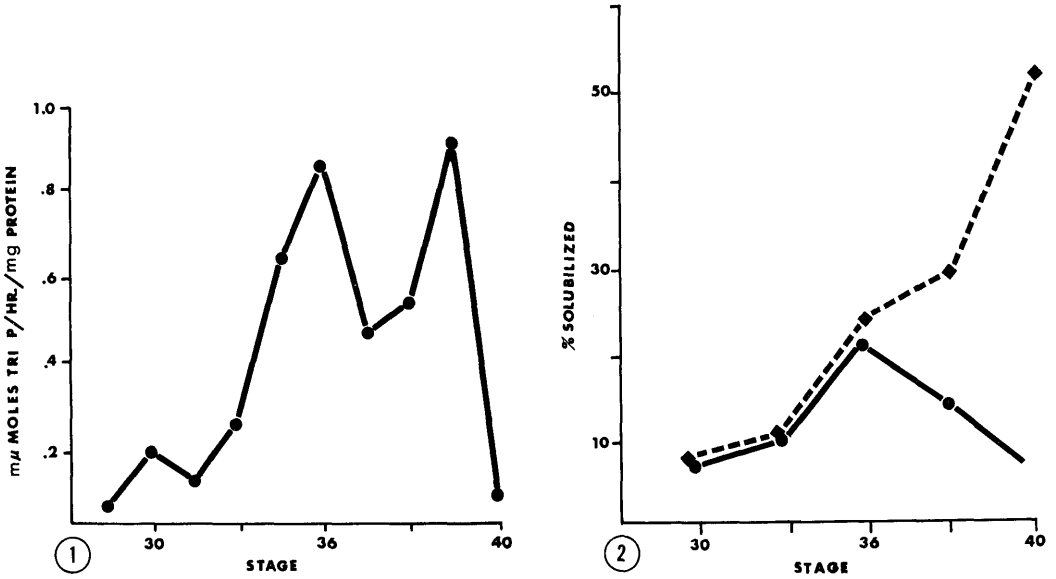

FIG. 1. Deoxyribonucleic acid polymerase activity in embryonic extracts. Values were determined by the method of Furlong and Williams(10).

FIG. 2. Deoxyribonuclease activity in embryonic extracts. - - -■- - - Acid DNase Activity ——●—— Neutral DNase Activity.

(10), assays of deoxyribonuclease activity in the extracts were carried out under neutral conditions much like those in the DNA polymerase incubation. The degradation of DNA-P^{32} by nuclease was measured by adding 10,000 counts/minute of DNA-P^{32} to a medium identical to the one used for the polymerase assay except that the triphosphates were omitted. The DNA-P^{32} (specific activity approximately 20,000 counts/minute/mμmole) had been isolated from *Escherichia coli* grown in a medium containing $K_2HP^{32}O_4$. Nonlabeled DNA primer was also present as in the normal polymerase assay. Aliquots of 10 μl were removed from a total volume of 125 μl at time intervals of 0, 15, 60, and 120 minutes and placed on paper discs pretreated with 25 μl of 0.1 M sodium pyrophosphate. The discs were washed twice in ice-cold 5% trichloroacetic acid (TCA), twice in 95% ethanol, and then dried as in the polymerase assay. At 120 minutes, 5 μl of 6 M TCA was added, and the tubes were capped and incubated at 85°C for 15 minutes. An aliquot of 10 μl of this hydrolysate was placed on a paper disc and washed as before. An additional 10 μl were placed on another disc and dried without washing. The hydrolyzed and washed sample provided a check for nonspecific adsorption to the disc; this value was approximately 10% of the total counts. The hydrolyzed unwashed samples served as a check on the acid insolubility of the 0-minute sample; the two values were found to agree within 5% on all samples. The deoxyribonuclease activity was calculated as the per cent solubilized per hour.

The extracts were also tested for DNase activity under acidic conditions by incubation of 15 μl of the fraction to be tested in a solution (total volume 135 μl) containing 10 μmoles pH 4.9 acetate buffer, 0.1 μmole $MgCl_2$, 50 μg DNA, and 10,000 counts/minute of DNA-P^{32}.

Results. The average values for DNA polymerase activity for duplicate sets of experiments are given in Fig. 1. The specific activity remains relatively low until after stage 31 when a progressive increase is noted; maxima are reached at stages 36 and 39 with a dip in the 2 intermediate stages. Finally at stage 40, enzymic activity drops again to values similar to those in the earliest stages. The shape of the DNA polymerase specific activity curve bears an interesting relationship to the morphological events de-

scribed for this organ by Wessels(3) and Hamilton(5). The small rise of activity at stage 30 coincides with the brief mitotic pulse accompanying mesodermal condensations. The first large rise is simultaneous with feather germ outgrowth, reaching a maximum at stage 36. The dip at stage 37 occurs at the time of barb-ridge formation which may represent a rearrangement rather than a new growth. At stage 39, there is an increase in specific activity of polymerase occurring simultaneously with feather elongation. Elongation ceases with the onset of keratinization at stage 40, and polymerase activity drops abruptly at the same time.

The possibility that variation in DNase activity plays a role in the observed DNA polymerase assays was tested by the data given in Fig. 2. Neutral DNase activity increases approximately 2-fold by stage 36, and then drops again to original values by stage 40. A doubling of the DNase activity would not account for the 9-fold increase in polymerase activity found in the stage 36 extracts(10). Furthermore, the second rise of polymerase specific activity found at stage 39 is not related at all to neutral DNase activity.

Acid DNase activity mirrors the activity of neutral DNase until stage 36, after which acid DNase rises to a maximum by stage 40 while neutral DNase activity decreases uniformly.

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Mitigated Teratogenicity of Thio-TEPA in Goldthioglucose Obese Mice.* (31961)

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Recently much attention has been paid to the effect of maternal metabolic states upon the susceptibility of the offspring to a teratogen(1-6). Kalter(6) demonstrated that heavier mice are more resistant to the cleft-palate inducing properties of cortisone than lighter animals and related this finding to maternal fat metabolism. Nevertheless, obesity induced by goldthioglucose (GTG) in mice did not alter the frequency of fetal abnormalities(7). However, the susceptibility of the fetuses of GTG obese mice to a known teratogen, triethylene thiophosphoramide (thio-TEPA) has not been examined and these results are here presented.

Materials and methods. Colony bred ICR-JCL mice from Japan CLEA Co. (Tokyo) were used. They were fed OA-2 pellets made by Japan CLEA Co. and given fresh water *ad libitum*. Virgin females of 12 weeks of age were caged separately and after one week were randomly divided into 4 groups. Two groups were given GTG in a single

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