ing factor for O_2 diffusion at the secondary gill lamellae of fish, the increase in thickness lowers the O_2 uptake capacity of the gills resulting 40 % drop in VO_2 through the gills[11]. Anatomical studies[13] showed the development of air-breathing organs at the same age and size of the fish which is responsible for the fulfilment of decreased amount of O_2. Hughes *et al.*,[14] while determing the diffusion capacity of *A. testudineus* concludes that the thickness of water-blood or air-blood barrier is of much importance since it particularly influences the O_2 tension difference (PO_2) which provides the driving force for the diffusion of the O_2 into the blood.

Duffusion distance (6 μm) obtained in this study for a fish of 2 g is far greater than the value 2.07 μm reported[4] for a fish of 10 g. This difference in thickness of the diffusion barrier for the same species of fish might be counted for the occurrence of two varieties of fish even in the same species (narrow-trunked and broad trunked[4].) In the present study, the fish taken was of broad-trunked variety in which the diffusion distance has been reported to be 15–20 μm in adult specimens[7,10]. Very thin diffusion distance (0.5 μm) in the beginning is quite logical since at this stage the fish is very active and inactive fish this distance is reported[2] to be as low as 0.553 to 0.598 μm.

The diffusion distance increased by a fractional power of 0.3611 and 0.9862 in relation to body weight and length. After achieving a body weight of 30 mg and 12 mm length, the gills become incapable of meeting the total O_2 demand of the body as growing body size reduces the functional efficiency of gaseous exchange by increasing the blood-water pathways. Similar suggestions for weight specific metabolism have been made in *Tench*,[3] *Anabas*[4] and *Saccobranchus*[15].

In spite of increasing body size the decrease in slope values for weight and length after functioning of the air breathing organs, is suggestive of a rapid growth in weight and length of the fish as compared to increase in the diffusion distance.

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SOME OBSERVATIONS ON THE ACTION OF URETHANE IN CHICK EMBRYOS CULTIVATED *IN VITRO*

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URETHANE is well known for its diverse biological effects. Studies on its action on nucleic acid metabolism assume a special significance as these play an important role in animal growth and differentiation[1]. Lombardi[2] stated that urethane inhibits DNA and RNA synthesis. Prodi *et al*[3] showed that urethane exerts a strong inhibitory effect on DNA synthesis in lymphoid organs and the bone marrow of the rat. Diwan and Mulherkar[4] along with others[1,3] while studying the effects of urethane on chick embryos also suggested the possible urethane interference with DNA synthesis. The present studies underline the mechanism of action of urethane and certain steps to understand the effects on nucleic acids.

Fresh fertilized eggs of white leghorn hens were incubated to obtain the mid-primitive streak stage or definitive primitive streak stage[5] (stages 3$^+$ and 4). The

glassware and metallic instruments were sterilised and the solutions [Pannet Compton's saline (PC)] were autoclaved. The embryos were explanted *in vitro* as described by New[6]. Of the explanted embryos, half as controls were grown in PC saline. The other half—experimental set—was treated with 0.168 M urethane solution and was kept at room temperature for 45 min to facilitate proper diffusion of the chemical into the embryo. The embryos were then kept at 37.5 ± 1°C for 4 hr after which they were washed thoroughly by PC saline. Both the sets were grown further under the same conditions for 18–19 hr. The embryos were washed in 0.9% physiological saline to remove any yolk particles still adhering to them. The sets were then separately weighed. Eight replicates, each comprising of five blastoderms in both the sets, were then homogenised and the extracts were stored frozen until further use. DNA and RNA were separated by Schmidt-Thannhauser procedure[7] modified by Schneider[8]. Protein was extracted from the residue remaining after nucleic acid removal by overnight incubation at 30°C with 0.5 N NaOH. The extracted fractions were made upto 50 ml and used for further analysis. DNA was estimated by diphenylamine method[10], RNA by Bial's orcinol reaction[11] and the protein by the method of Lowry *et al.*[12].

From the results it is obvious that there is an appreciable decrease in the wet weight of the embryos after the urethane treatment (table 1). This could partly be ascribed to the decrease in cellular constituents *viz.* DNA, RNA and protein content. Moreover, it is established that the weight changes in whole embryos represent the sum of chemical changes taking place in the developing organism[13].

Mechanism of action of any chemical carcinogen is always closely linked with its effects on critical macromolecular components *viz.* proteins and nucleic acids in the animal. Our results (table 1) indicate a significant decrease in DNA, RNA and protein after the urethane treatment. The chemical treatment seems to affect the DNA synthesis most. The decrease in the macromolecular components may presumably be due to an interference of urethane with the biogenesis of folic acid. Recently we reported that urethane treatment brings about a significant decrease in the total folate content of chick embryos[14]. In addition to the significant role played by one-carbon units in the metabolic processes, mediated by folic acid, the one-carbon units are also known to be involved in the methylation of proteins like histones[15-17] which regulate DNA replication. Similarly, one-carbon unit is known to play a significant role in the methylation of *t*-RNA and certain DNAs[18, 19]. These processes constitute key reactions in the formation of *N*-formyl methionine which is involved in the initiation and the formation of new proteins and nucleic acids. The antifolate action of urethane as understood from the above discussion is of great interest in view of the fact that urethane acts as an antileukemic agent under certain conditions.

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Table 1 *Total DNA, RNA and protein content of urethane untreated (control) and treated (experimental) chick embryos.*

Wet weight of embryos (mg)		µg DNA/g of protein		µg RNA/g of protein	
Control	Experimental	Control	Experimental	Control	Experimental
125	85	34	17	68	44
130	79	33	17	68	46
137	82	38	14	67	37
128	78	35	13	73	44
122	92	38	18	69	49
125	81	35	15	69	47
124	86	34	14	66	44
118	96	36	16	70	38

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OBSERVATIONS ON 17β-HYDROXYSTEROID DEHYDROGENASE IN THE BROOD PATCH OF HOUSE SPARROW, *PASSER DOMESTICUS* L.

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BROOD patches, also otherwise termed as incubation patches, of birds have been extensively investigated by avian endocrinologists interested in the mode of hormonal action, notably sex steroids and prolactin in the patch formation[1-7]. In the female house sparrows the brood patch is formed by a sequence of defeathering, hypervascularization and development of edema in the ventral skin during a nesting cycle. Such naked, well-vascularized patch of skin aids in incubation of the eggs by facilitating efficient heat transfer from the body. It is believed that the incubation patch formation is completed through the influence of prolactin on the 'estrogen-primed' skin[7,8]. Prolactin administration to laying hens produces patch formation[8]. Further, Jones *et al*[9] confirmed the role of estrogen and prolactin by exogenous administration, in the formation of incubation patch in immature chickens. The present investigation was undertaken to study the localization and distribution of 17β-hydroxysteroid dehydrogenase and the possible role of the steroids in the formation and maintenance of the incubation patch in female house sparrows.

Female house sparrows were collected from the nest boxes kept under regular observation in 1981. The birds were collected at various stages of incubation patch formation during their breeding season, which spans from February to May as well as from June to October each year. Various stages in incubation patch were recognised according to previous descriptions[10]. The birds were weighed, decapitated under mild anaesthesia and the skin from ventrum removed after trimming the feathers when present. The skin was immediately fixed on the cryostat chuck maintained at −20°. Sections of 10–20 mM thickness were cut and transferred to incubation media. For demonstrating 17β-hydroxysteroid dehydrogenase activity, both testosterone (T) and estradiol-17β (E) were utilised as substrates and NAD as cofactor[11]. Control sections were incubated in the medium devoid of the substrate. Incubation was carried at 37° for 30 min after which the sections were thoroughly washed, post-fixed in 10% formalin, rewashed and mounted in glycerine jelly.

In general, the epidermis and feather follicles registered high activity as compared to the dermis (figures 1–6). The enzyme intensities have been graded as minimum (+), moderate (+ +) and high (+ + +) (table 1). During the pre-breeding season the enzyme activity in the patch forming skin was low, however with testosterone the activity was relatively more(+ +, figures 1, 2). The enzyme activity with estradiol showed an increase reaching to peak(+ + +) during defeathering and fully formed patch phases, decreasing more or less to pre-breeding phase thereafter (figures 3, 5). With testosterone, the activity remained the same from nest building phase up to fully formed patch phase (peak intensity + + +, figure 4). Thereafter, the skin revealed a decreased activity during the regression and refeathering phases which were comparable to those seen during the pre-breeding season. In general, therefore, the enzyme activities were highest during defeathering and fully formed patch phases with both the hormones.

The results indicate that the hormone metabolising enzyme of the target skin shows an adaptive nature. According to van Tienhoven[12], estrogen levels rise along with the increases in ovary and oviduct weights

Table 1 *Intensity of 17 β-HSDH response at various phases of patch formation*

Enzymes	17β-HSDH- (E)	17β-HSDH- (T)
Pre-breeding phase	+	2+
Nest building phase	2+	3+
Defeathering phase	3+	3+
Fully formed patch phase	3+	3+
Regressing phase	2+	2+
Refeathering phase	2+	2+