ENZYMORPHOLOGIC DEMONSTRATION OF Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE AND SUCCINIC DEHYDROGENASE IN THE CORTICAL AND MEDULLARY CELLS OF THE ADRENAL GLAND OF *TAPHOZOUS LONGIMANUS* HARDWICKE (MICROCHIROPTERA : MAMMALIA)

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ABSTRACT

Histochemical site and distribution of Δ^5-3β-hydroxysteroid dehydrogenase Δ^5-3β-HSDH and a key mitochondrial enzyme-succinic dehydrogenase (SDH) in the cortical and medullary cells of adrenal gland of a insectivorous microchiroptera–*Taphozous longimanus* revealed varying intensities of reaction. Patches of cortical cells in the substance of the adrenal medulla also displayed positive Δ^5-3β-HSDH activity.

These enzymes may be involved in steroid biosynthesis/interconversion; cellular metabolism leading to energy kinetics; and maintenance of a specific steroid hormone milieu. It is proposed that positive Δ^5-3β-HSDH activity in the "islets" of cortical cells positioned in the medullary region may be of metabolic significance and may have some modulating effects on the adrenal medullary cell dynamics, as they seem to be active steroidogenic sites with the potentiality of a role akin to cortical cell action. Differential SDH activity is a positive histochemical evidence for the energy turn-overs at the steroidogenic cellular sites. The gradual shift in the intensity of this enzyme from the cortical to the medullary tissues perhaps also signifies the metabolic adaptations at the sub-cellular levels.

INTRODUCTION

ADRENAL cortex and medulla of mammals differ not only in their embryological origin, but also in structure, secretory products and the regulating influences exerted by them on various organ systems under different physiological states including emergent conditions[1–2]. The adrenal cortex is the pivotal site for the biosynthesis and interconversion of vast array of steroid hormones through enzymatic interventions[3–6]

Limited information is available on the histology of the adrenal gland of some chiroptera belonging to Pteropidae, Magadermatidae, and Vespertilionidae[7–9], and the literature is virtually blank with regard to the adrenal gland enzymology[5].

In view of the nebulous information on the adrenal gland enzymology of Indian bats, we have initiated a comprehensive programme to study the paradigm of various classes of dehydrogenases, phosphatases and oxidases. The present extract deals with the histochemical site and distribution of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5 3β HSDH) and a key mitochondrial marker enzyme succinic dehydrogenase (SDH) in the cortical and medullary compartments of the adrenal gland of *Taphozous longimanus*.

MATERIAL AND METHODS

Adult males of *T. longimanus* were netted at dusk from their barnyard roost while emigrating for nocturnal activities. The animals weighing 23–25·5 gm were maintained in batches of 2–3 in steel cages with wire nettings for 24 h.

The animals were sacrificed by cervical dislocation. The adrenal glands were quickly dissected out surgically under semi-sterile conditions and washed off of blood in chilled mammalian ringer (at 4° C).

Fresh frozen sections were cut at 10 μM and processed as follows :

(i) Δ^5-3β-*HSDH*: activity was histochemically demonstrated according to the method of Baillie *et al.*[4], by incubating sections taken on coded slides at pH 7·5 in 0·15 M sodium-potassium buffer to which 0·5 mg of nitro blue tetrazolium/ml was added just before use. The substrate steroid used were epiandrosterone and pregnenolone acetate dissolved in dimethyl formamide (5 mg/ml) and added to give 0·5 mg/ml in the final reaction mixture. Control sections were incubated in similar medium but lacking the substrate and having dimethyl formamide. After incubation for 60 min., the sections were fixed for 15 min. in 10% neutral formalin and rinsed briefly in distilled water. The cover glass was mounted using an aqueous 25% glycerol solution.

(ii) *SDH* : was demonstrated by the method of Nachlas *et al.*[10], using nitroblue tetrazolium as an electron acceptor. The sections were incubated for 15 min. at 37° C. Blue diformazon granules were taken as indicator of SDH activity. Control sections were treated similarly but incubated in the medium without substrate.

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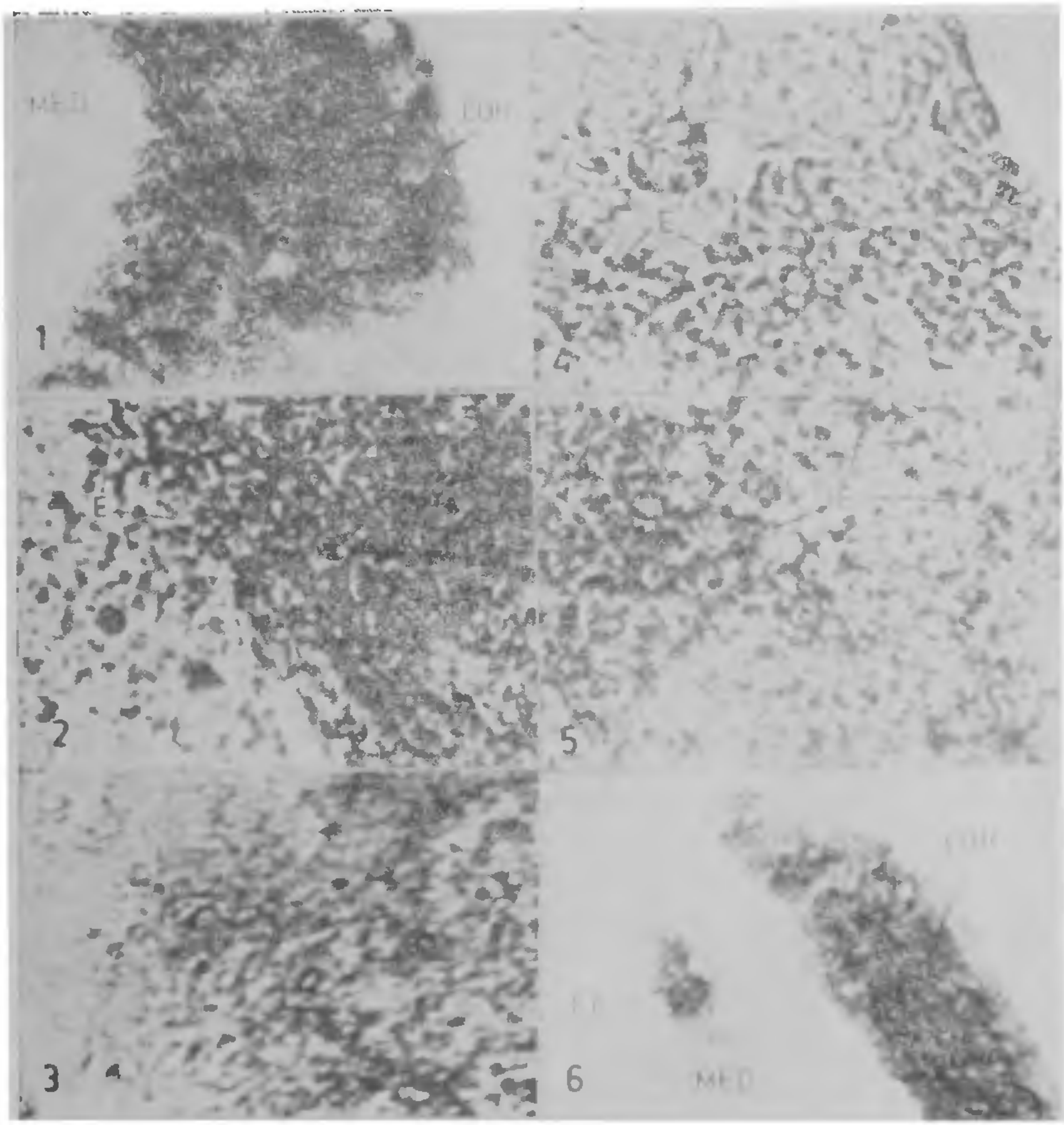
Relative intensities of the two enzymes in the cortical and medullary cells were visually scored as described earlier.

For histological orientation of cortical zonation and medullary region characteristics, adrenal gland from some animals were fixed in aqueous Bouin's fixative for 8–10 h, washed off of fixative, dehydrated in graded ETOH series, cleared in xylene, infilterated with paraffin wax and blocked in histo-wax. Paraffin sections cut at 7 μM were stained with haematoxylin–eosin.

Results and Discussion

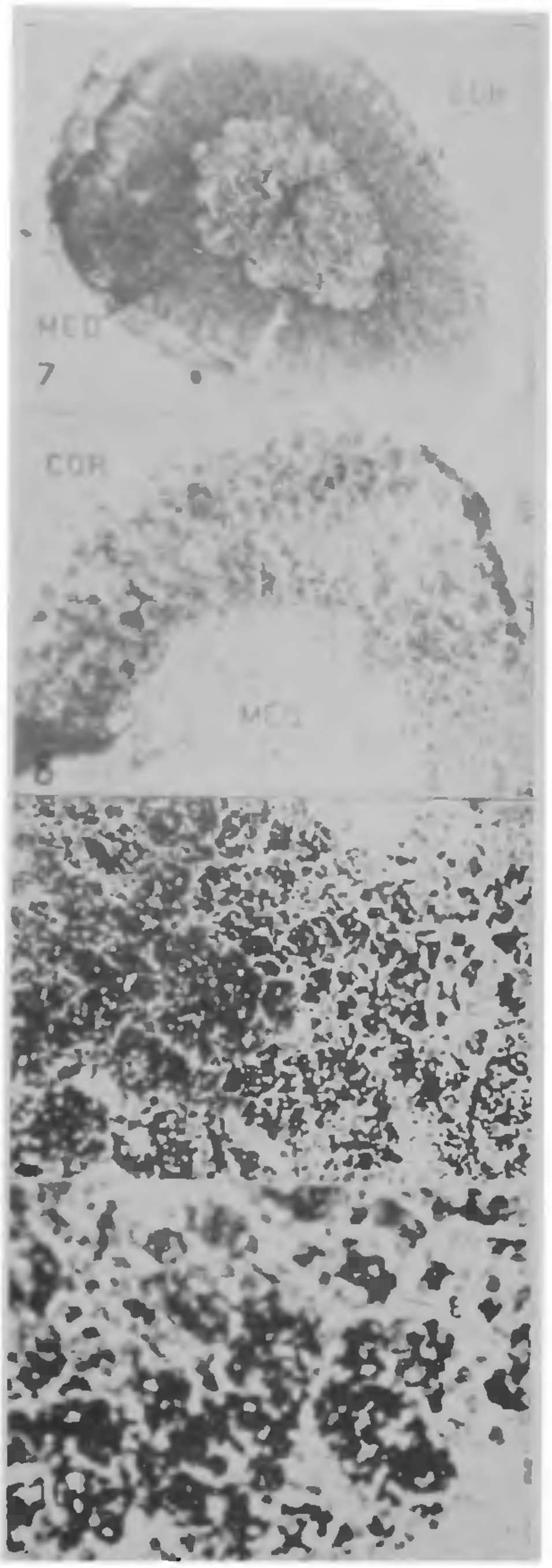
The present studies highlight the site and distribution of Δ^5-3β-HSDH and SDH in the cortex and medulla of the adrenal gland of a insectivorous microchiroptera *Taphozous longimanus*. For the sake of brevity, meaningful interpretation and comparison the two enzymes are discussed separately :

(i) *Δ^5-3β-HSDH* : has been histochemically demonstrated using epiandrosterone and pregnenolone as substrates. The steroidogenic cellular sites showed



Figs. 1–6. Δ^5-3β-HSDH activity in the zona glomerulosa, zona fasciculata and patches of cortical cells position in the medulla. Note the absence of enzyme activity in the medulla and the nearly uniform enzyme reaction in the cortical cells.

Enzymorphologic demonstration of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH) and succinic dehydrogenase (SDH) in the cortical and medullary cells of the adrenal gland of *Taphozous longimanus*.



FIGS. 7–10. Fig. 7. T.S. of adrenal gland showing disposition of cortical and medullary zones. Zona reticularis is absent (H E. staining). Figs. 8–10. SDH activity in the adrenal cortex and medulla. Note the differential enzyme reaction in the cortical zones as well as in the medulla.

(Legend : COI = Cortex, Med = Medulla, E = sites of enzyme activity, CC = islets of cortical cells positioned in the adrenal medulla showing positive Δ^6-3β-HSDH activity).

similar intensities of enzyme reaction with both classes of substrates. The intensity of diformazon granule depositions in the zona glomerulosa and zona fasciculata were uniform in both these cortical zones, which were characterised by their large number and size. The medulla was totally devoid of any enzyme reaction. Islets of cortical cells dispersed in the medulla showed positive Δ^5-3β-HSDH activity (Figs. 1–6).

Δ^5-3β-HSDH has been defined as an enzyme that catalyses the reaction of 3β-hydroxysteroid with NAD to yield ketosteroid and NADH[11]. The positive enzyme reaction as observed in the present study clearly shows the steroidogenic cellular sites and their ability to utilize epiandrosterone and pregnenolone with equal efficiency. It is interesting to record that the adrenal cortex of *T. longimanus* lacks zona reticularis (Fig. 7), but 'islets' of cortical cells exhibiting, positive enzyme reaction in the medullary region are present. Further, our results also suggest that considerable biosynthetic activity and steroid interconversion occur in these zones.

Positive Δ^5-3β-HSDH activity in the "islets" of cortical cells positioned in the medullary region may be interpreted to mean that steroids emnating from these cortical islets may have some metabolic influence on cellular dynamics of medullary cells. This interrelationship is reasonable owing to the close approximation of these two types of tissues.

A comparison of our results with other mammalian species shows interesting parallels as well as sharp differences with many. Thus, in the bat–*Vesperugo pipistrellus*, the adrenal cortex consists of all the three zones instead of two as are found in *Taphozous longimanus*. Uniform Δ^5-3β-HSDH activity was observed in the adrenal cortex and also in the cortical cells positioned in the medullary region[6]. The two species of bat thus have some similarity but also differences. Our results are also at variance with the observations on human and primate adrenal cortex, Δ^5-3β-HSDH paradigm. In these, the enzyme activity is restricted only in the outer part of the zona fasciculata and to a lesser extent in the zona reticularis. However, in hamster and rat an intense enzyme activity has been reported which was conceded to be in conflict with the histologic zonation concept for the formation of glucocorticoids[9,12]. Other results of histochemical staining studies have shown considerable variability. Thus, both strong[13] and weak[14] activities in the rat

glomerulosa have been reported and such inconsistencies have been discussed. Similar conflict of observations in the literature pertaining to the utilizational abilities of cortex in relation to pregnenolone and epiandrosterone have been observed. Thus, while our results are in agreement with Baillie *et al.*,[4] other investigators observed low Δ^5-3β-HSDH activity when pregnenolone was used as a substrate; and with epiandrosterone as the substrate, the human adrenal cortex displayed moderate staining in zona fasciculata, while little or no reaction was discernible in zona reticularis. Replacement of epiandrosterone by the same concentration of pregnenolone gave a weaker reaction[11,16]. Perhaps these variations indicate species specific abilities.

(ii) *SDH* : is the only enzyme of the citric acid cycle that is bound to the inner membrane of the mitochondria. It is also one of the three flavo-protein known in which the flavin is covalently linked to the protein[17]. The importance of the mitochondrial enzyme system at steroidogenic sites is well estiablished and in the adrenal cortex of male and female rats these organelles have been shown to exhibit different fine structural characteristics[18]. The differential SDH activity as observed in the zona glomerulosa, zona fasciculata, islets of cortical cells positioned in the medulla and the medullary cells (Figs. 8–10) of *Taphozous longimanus* can be construed as an indication of variable status of oxidative metabolism in these regions. This probably also represents the preferential utilization of glycolytic and Kreb's cycle intermediates like succinates. It is proposed that the differential SDH activity in the adrenal cortex and medulla represents the threshold levels of this enzyme which ensure a sustenance of basal metabolism conducive to the integrity of the various histological constituents of the adrenal gland and the energy requiring function of its cell.

The relatively intense SDH activity in the cortical zones *vis-a-vis* medullary cells indicates that energy turnovers in the steroidogenic cellular sites are more profound as compared to medullary cells. These findings also denote that there is a gradual shift in the loci of this enzyme from the cortical zones to the medullary tissues of *T. longimanus*. Such a gradient perhaps also signifies the metabolic adaptations at the sub-cellular levels in the adrenal gland of this species.

No tangible information on the *SDH profile* of chiropteran adrenal has been discerned in the literature.

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