ENZYMOPHLOGIC DEMONSTRATION OF $\Delta^{5}$-3$\beta$-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 $\Delta^{5}$-3$\beta$-hydroxysteroid dehydrogenase $\Delta^{5}$-3$\beta$-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 intensi-
ties of reaction. Patches of cortical cells in the substance of the adrenal medulla also displayed
positive $\Delta^{5}$-3$\beta$-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 $\Delta^{5}$-3$\beta$-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-over
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$^{1-3}$. The adrenal cortex is the pivotal site
for the biosynthesis and interconversion of vast array
of steroid hormones through enzymatic interven-
tions$^{5-6}$.

Limited information is available on the histology of the adrenal gland of some chiroptera belonging to
Pteropidae, Magadermatidae, and Vesperilionidae$^{7-9}$,
and the literature is virtually blank with regard to the
adrenal gland enzymology$^{10}$.

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 histochem-
ical site and distribution of $\Delta^{5}$-3$\beta$-hydroxy-
sterol dehydrogenase ($\Delta^{5}$ 3$\beta$ HSDH) and a key
mitochondrial marker enzyme succinic dehydrogenase
(SDH) in the cortical and medullary comaprtments
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 surgic-
ally under semi-sterile conditions and washed off of
blood in chilled mammalian ringer (at 4°C).

Fresh frozen sections were cut at 10 $\mu$M and processed
as follows:

(i) $\Delta^{5}$-3$\beta$-HSDH: activity was histochemically demonstr-
ated 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 epiprosterone 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 diphosphonate granules were
taken as indicator of SDH activity. Control sections
were treated similarly but incubated in the medium
without substrate.
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, infiltrated 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^8-3β-HSDH and SDH in the cortex and medulla of the adrenal gland of a insectivorous microchiroptera Taphozous longimans. For the sake of brevity, meaningful interpretation and comparison the two enzymes are discussed separately:

(i) Δ^8-3β-HSDH: has been histochemically demonstrated using epandrosterone and pregnenolone as substrates. The steroidogenic cellular sites showed

Figs. 1–6. Δ^8-3β-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^8-3β-hydroxysteroid dehydrogenase (Δ^8-3β-HSDII) and succinic dehydrogenase (SDH) in the cortical and medullary cells of the adrenal gland of Taphozous longimans.

Cur. Sci.—3
similar intensities of enzyme reaction with both classes of substrates. The intensity of difformazon 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 Δ⁵-3β-HSDH activity (Figs. 1–6).

Δ⁵-3β-HSDH has been defined as an enzyme that catalyses the reaction of 3β-hydroxysteroid with NADH to yield ketosteroid and NADH. The positive enzyme reaction as observed in the present study clearly shows the steroidogenic cellular sites and their ability to utilize androstosterone 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 Δ⁵-3β-HSDH activity in the "islets" of cortical cells positioned in the medullary region may be interpreted to mean that steroids emanating 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-Yesperugo pipistrellus, the adrenal cortex consists of all the three zones instead of two as are found in Taphozous longimanus. Uniform Δ⁵-3β-HSDH activity was observed in the adrenal cortex and also in the cortical cells positioned in the medullary region. The two species of bat thus have some similarity but also differences. Our results are also at variance with the previous observations on human and primate adrenal cortex, Δ⁵-3β-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. Other results of histochemical staining studies have shown considerable variability. Thus, both strong and weak activities in the rat 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 Δ⁵-3β-HSDH activity).
glomerulosa have been reported and such inconsistencies have been discussed. Similar conflict of observations in the literature pertaining to the utilization abilities of cortex in relation to pregnenolone and epiandrosterone have been observed. Thus, while our results are in agreement with Baillie et al. other investigators observed low Δ⁵-3β-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. 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. The importance of the mitochondrial enzyme system at steroiogenic sites is well established and in the adrenal cortex of male and female rats these organellas have been shown to exhibit different fine structural characteristics. 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 steroiogenic 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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