MATERIAL AND METHODS

PROCUREMENT OF RATS

Mated female rats of Charles foster strain were purchased from Sarabhai Research Center, Baroda, and were maintained in a well ventilated animal house. The animals were given standard food and water *ad libitum*. When the mated females delivered pups, males and females were separated and the respective treatments/experimental schedules were started. Efforts were made to maintain more or less a constant litter size in order to minimize variations related to the number of pups per mother rat.

EXPERIMENTAL SETUP

The neonatal male pups were divided into the following six groups:

Group I: New born rat neonates, maintained as control (Con). This consisted of four subgroups (a) given normal drinking water, (b) injected intraperitoneally (i.p.) with saline, (c) subjected to sham pinealectomy (Px) and (d) sham Px and injected with saline.

Group II: New born rat neonates, subjected to transient hypothyroidism (HPOT) by feeding mothers with 0.1% 6-propyl-2-thiouracil (PTU) in drinking water for 21 days of preweanling period.

Group III: New born rat neonates, subjected to transient hyperthyroidism (HPRT) by daily i.p. injections of 0.09 μg thyroxine (T_4) in saline for 21 days of preweanling period.

Group IV: Pinealectomised (Px) rat neonates in which pineal was surgically removed on the fifth neonatal day.

Group V: Rat neonates subjected to a combination of hypothyroidism and pinealectomy (HPOT+Px).

Group VI: Rat neonates subjected to a combination of hyperthyroidism and pinealectomy (HPRT+Px).

In group I since there were no significant differences in any of the parameters studied between subgroups (a) to (d), data of only (a) subgroup of rats is given as control data in the studies throughout.

PARAMETERS AND METHODOLOGY OF EVALUATION

The respective treatments were discontinued from day 22 and, at the end of 35, 45, 60, and 90 days of age, the rats from all the six groups were weighed and sacrificed under mild anesthesia taking maximum care to avoid any stress during handling.

The viscera was cut open and the organs were quickly excised, blotted free of blood and tissue fluids and morphometric evaluation of the organs was carried out using digital Mettler balance. Organs were weighed accurately up to 0.01 mg. The absolute weights thus obtained were converted to relative weights and expressed in terms of percentage body weight.

I. Biochemical Analysis

I.1. Blood glucose: Prior to sacrificing, 0.1 mL of blood was drawn from the jugular vein by a minute puncture. Blood glucose level was estimated by the glucose oxidase method of Winckers and Jacob (1971). The glucose concentration was expressed as mg/dL blood.

I.2. Glycogen: The tissues were digested in 30% KOH and glycogen was precipitated with 95% ethanol. The diluted precipitates were treated with anthrone reagent and the estimation was done by employing the method of Seifter *et al.* (1950). The colour intensity developed was read colorimetrically at 620 nm. Glycogen content of liver and testes was expressed as mg/100 mg and μg/100 mg wet tissue respectively.

I.3. Phosphorylase (EC 2.4.1.11, 4-α-D-Glucan:Ortho-phosphate-α-D-glucosyltransferase): Assay of phosphorylase was made by the modified method of Cori *et al.* (1943) as adapted by Cahill *et al.* (1957) using di-potassium salt of glucose-1-phosphate (G-1-P) as the substrate. The inorganic phosphate liberated was estimated by the method of Fiske and SubbaRow (1925). The intensity was read at 660 nm and the enzyme activity in liver and testes was expressed as μg phosphorous released/mg protein/10 minutes.

I.4. Glucose-6-Phosphatase (G-6-Pase) [EC 3.1.3.9]: Homogenate for estimation of this enzyme activity was prepared in cold citrate buffer at pH 6.5. Enzyme activity was assayed by the method of Harper (1960), using di-sodium salt of glucose-6-phosphate (G-6-P) as the substrate. Inorganic phosphate released was measured as per the method of Fiske and SubbaRow (1925) and the color intensity was read at 660 nm. The enzyme activity in liver was expressed as μg phosphate released/mg protein/10 minutes.

II. Histochemical Localization

Steroid Dehydrogenases in Testes: The testis from each rat was quickly excised after decapitation under mild anesthesia and transferred to a cryostat microtome maintained at -20°C.

Fresh frozen sections of 15-20 μ thickness were taken on a clean slide and thawed. 17-ß-hydroxy steroid dehydrogenase was localized employing the method of Kellog and Glenner (1966) using testosterone as the substrate. 3-ß-hydroxy steroid dehydrogenase was localized according to the method of Wattenberg (1958) using dehydro-epiandro-sterone (DHEA) and pregnenolone(P) as the substrates while, 3-α-hydroxy steroid dehydrogenase was localized histochemically as per the method of Balough (1966) using androsterone (A) as the substrate. Incubation of the sections was carried out at 42°C. Nicotine-amide-dinucleotide (NAD) was used as a coenzyme and Nitro blue tetrazolium salt (NBT) as the hydrogen acceptor. The stained sections were washed thoroughly in distilled water and then fixed in 10% neutral formalin for 15 minutes, washed again in distilled water and mounted in glycerine jelly. In all the histochemical observations, the tissue sections incubated in a substrate blank medium served as controls.

III. Histological Techniques

Testes, epididymis, prostate , seminal vesicle, and thyroid were fixed (immediately after decapitation) in Bouin's fluid and processed for routine histological studies. Paraffin wax sections of 5 μ thickness were cut on a microtome and stained with hematoxylin-eosin. The stained sections were mounted in DPX. The measurements of the diameter of seminiferous tubules of testis and cell heights of both caput and cauda of epididymis were made using micrometer and expressed as μm.

IV. Radioimmuno Assay

IV.1. Triiodothyronine (T_3): Circulating levels of T_3 was analyzed using RIA kit (RIAK 4/4A) procured from the Board of Radiation and Isotope Technology, BARC, Bombay. The level of T_3 was expressed as ng/mL.

IV.2. Thyroxine (T_4): Circulating levels of T_4 in serum was assayed by RIA kit (RIAK 5/5A) purchased from the Board of Radiation and Isotope Technology, BARC, Bombay. The level of T_4 was expressed as ng/mL.

IV.3. Testosterone (T): The levels of circulating testosterone was assayed in serum using RIA kit by the method of Sufi *et al.*(1986) and the values were expressed in ng/mL of serum.

V. Statistical Analysis

All the results were statistically analyzed and are reported as the mean $\pm$ standard deviation. The data were also subjected to Students' 't' test with a 95% confidence limit.