ostracodes was sparse. The genus Tanella was fairly well represented in samples. Members of the genera Phlyctenophora; Paijenborchellina; Loxoconcha; Atjehella; Palmenella and Hemicytheridea occurred in limited numbers.

The various ecological parameters and their ranges of variation prevailing at the time of collection are: water temperature 28.6° to 40.0° C., salinity of the water nearly 0.00 to 34.0%, organic matter 0.1622 to 2.5062%, sand 41.02 to 87.92%, silt 3.50 to 34.30%, clay 5.82 to 41.94%.

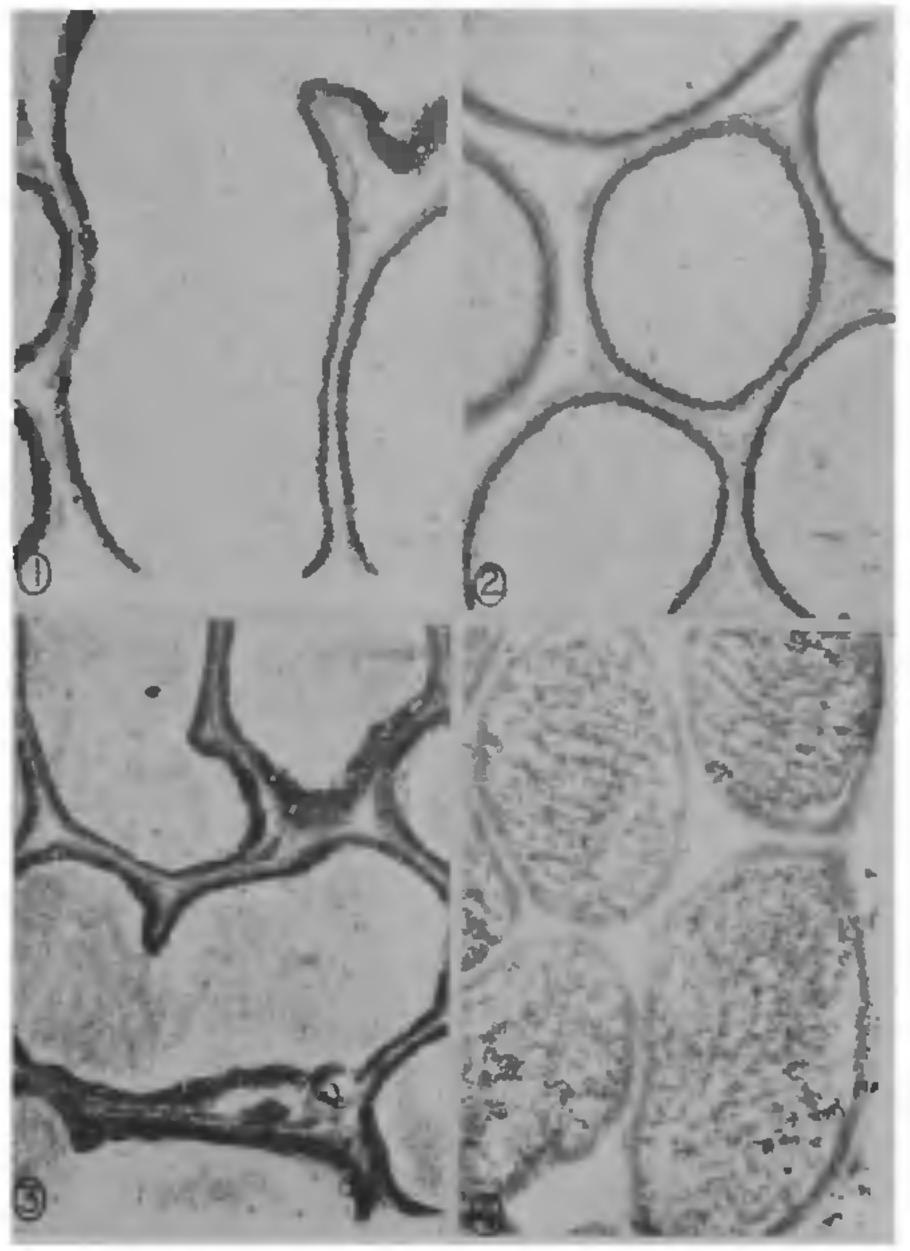
Thanks are due to Dr. D. V. Rama Sarma for his guidance, Prof. K. Hanumantha Rao, Head of the Department, for the facilities provided to carry out the present work, Dr. D. Srinivasa Rao for his assistance in sampling and finally to the U.G.C., New Delhi, for the award of fellowship.

Department of Zoology, C. Annapurna. Andhra University, Waltair, June 5, 1979.

- 1. Glaessner, M. F., Principles of Micropalaeontology, Wiley (New York), 1945, p. 296.
- 2. Imbrie, John, Geol. Soc. America, 1955, 2, 449.
- 3. Teichert, Curt, Am. Assoc. Petroleum Geologists Bull., 1958, 24, 2718.
- 4. Kaesler, R. L., Univ. Kansas. Palaeont. Contr., 1966, 10, 50.
- 5. King, C. E. and Kornicker, L. S., Smith. Cont. Zool., 1970, 24, 92.

## HISTOCHEMICAL DEMONSTRATION OF STEROID METABOLISM IN THE EPIDIDYMIS OF CHAMELEON CALCARATUS (BOULENGER)

Repriles are the first to become completely terrestrial and to acquire internal fertilisation and accessory sex organs. The epididymis is one male sex accessory to appear for the first time in reptiles. It is made up of contorted tubules, their lumen being lined by single layered columnar epithelium and as is the case with mammals, it serves in the storage and physiological maturation of spermatozoa. Histochemical and biochemical studies have shown the occurrence of steroid metabolism enzymes in the mammalian epididymis<sup>1,2</sup>. Several hydroxysteroid dehydrogenases have been method of Altman and Chayen Lipids were localocalized in the epididymis of mammals<sup>3-5</sup>. The lizard epididymis also seems to possess the capacity for steroid metabolism. Steroid dehydrogenases involved in steroid metabolism have been demonstrated histochimically both in the epithelium and spermatozoa of the epididymis of a few lizards. The present report describes the localization of / 5-3 p-tydroxysteroid dehydrogenase ( $\Lambda^{6}$ -3 $\beta$ -HSD11), 17 $\beta$ -bydroxysteroid dehydrogenise (17\beta-HSDH), glucose-6-phosphate dehydrogenase (G-6-PDH), reduced nicoti-



Figs. 1-4. Cryostat section of the epididymis NADH<sub>2</sub>  $\triangle$  5-3 $\beta$ -HSDH (Fig. 1), 17 $\beta$ -HSDH (Fig. 2), showing diaphorase (Fig. 3) activities and lipids (Fig. 4) in the epithelium and lumen, × 80.

namide adeninedinucleotide (NADH<sub>2</sub>) diaphorase and lipids in the epididymis of Chameleon calcaratus.

Sexually mature male Chamelean were collected during the breeding season. These animals were decapitated, the epididymis were removed and immediately frozen at  $-20^{\circ}$  C. Air-dried cryostat sections were incubated in serological water bath at 37° C for one hour in appropriate incubation media containing different substrates, co-factors and tetrazolium salt,  $\triangle^{5}$ -3 $\beta$ -HSDH (substrates; pregnenolone and dehydroepiandrosterone) and  $17\beta$ -HSDH (substrates; estradiol-17\beta and testosterone) were localized according to the method of Baillie et al,8. Similarly G-6-PDH and NADH<sub>a</sub> diaphorase were demonstrated as per the lized using sudan black B method of Pearse<sup>11</sup>. Suitable control sections were also incubated in appropriate incubation media without the substrate or ade linedinucleotide/adeninedinucleotide phosphate (NAD/ NADP). After incubation sections were washed, fixed in 10% neutral formalin and mounted in glycerol jelly or PVP mounting medium.

Intense positive reaction for  $\beta-3\beta$ - and  $17\beta$  HSDHs was seen in epididynial epithelium (figs. 1 and 2) and weak reaction was noticed in the luminal contents. There is substrate specificity for both  $\triangle^{5}-3\beta$ - and  $17\beta$ -HSDHs. DHA and testosterone were more preferentially utilized than pregnenolone and estradiol-17 $\beta$ . The localization of G-6-PDH, NADH<sub>2</sub> diaphorase (Fig 3) and lipids (Fig 4) were similar to that of HSDHs.

The enzymes  $\frac{5-3}{3}$ -and  $17\beta$ -HSDHs play an important tole in steroid metabolism. The former is involved in the exidative conversion of  $^{5}-3\beta$ -bydroxysteroids to -14-3 ketosteroids and the latter in oxidative interconversion of androgens and estrogens. Presence of HSDHs was demonstrated for the first time in the epididymis of hamster3. Later several HSDHs have been found in the epididymis of other mammals2,4,6. These steroid dehydrogenases are said to be involved in metabolism of steroids and in the maturation of sperms<sup>8</sup>. The strong activity of  $\angle 5-3\beta$ - and  $17\beta$ -HSDHs in the epididymal epithelium of Chameleon indicates that it is the site of steroid metabolism as in mammals. The G-6-PDH activity in the epididymal epithelium clso indicates its involvement in anabolic activities like lipogenesis or steroidogenesis. The localization of NADH2 diapherase which is ubiquitous to all steroidogenic sites and lipids which are the precursors of steroid hormones further confirms the capacity of Chameleon epididymis for steroid metabolism.

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Department of Zoology, G. R. Shivakumar.
University of Mysore, H. B. Devaraj Sarkar.
Manasa Gangotri, B. M Sekharappa.
Mysore 570 006, August 30, 1979.

- Hamilton, D. W., J. Reprod. Fert. Suppl., 1971, 30, 89.
- Dinkar, N., Arora, R-Dinkar, Kumar, P., Talesara, C. L. and Prasad, M. R. N., Ind. J. Exp. Biol. 1977, 15, 865.
- 3. McGadey, I., B illie, A. H. and Ferguson, M. M., Histochemic., 1966, 7, 211.
- 4. Moniem, K. A., J. Reprod. Fert., 1972, 28, 461.
- Rastogi, R. K., Saxena, P. K. and Iela, L., Hormone Res., 1976, 7, 43.
- 6. Shivanandappa, T. and Sukar, H. B. D., Gen. Comp. Endocrinol., 1978, 34. Abstract No. 97.
- 7. Shivakumar, G. R., Serker, H. B. D. and Sekharappa, B. M., Ind. J. Exp. Biol., 1979, 17, 826.
- 8. Baillie, A. H., Ferguson, M. M. and Mck Hart, D., Developments in Steroid Histochemistry, Academic Press, London and New York, 1966.
- 9. Altman, F. P., Progr. in Histochem. Cytochem. Fischer, Stuttgart, 1972.

- 10. Chayen, J., Bitensky, L. and Butcher, R. G., Practical Histochemistry, John Wiley and Sons, London, 1973.
- 11. Pearse, A. G. E., Histochemistry, Theoretical and Applied, Vol. I. J. and A. Churchil Ltd., London, 1972.

# EFFECT OF EXPERIMENTAL RUMINAL ACIDOSIS ON BLOOD GLUCOSE AND PLASMA INSULIN CONCENTRATION IN BUFFALO CALVES

#### Introduction

The concentration of glucose in blood is maintained within a fairly narrow optimal range during health and is influenced by several factors. Increase in glucose and insulin concentration in blood has been reported by different workers in cattle and sheep fed high grain diets<sup>2</sup>,<sup>3</sup>. However, there is scanty information in the literature regarding the effect of acute ruminal acidosis on blood glucose and insulin concentrations. The present experiment is designed to study the changes in the above parameters in buffalo calves following experimental induction of rumen acidosis.

### Materials and Methods

Experiments were conducted on five fistulated two years old buffalo calves. To establish the normal values, blood samples were collected in the morning before feeding on alternate days for a week. Blood glucose was estimated using Haden modification of Folin-Wu-mehod as described by Frantel et al.\*. Circulating level of immuno-reactive insulin (IRI) was determined with the help of RIA Kit (Bhabha Atomic Research Centre, Bombay).

To induce acid indigestion, crushed wheat grains were given intra-ruminally at the rate of 50 gm/kg body weight to each animal. Following the induction of rumen acidosis, blood samples were collected at 24 hour interval upto 120 hours.

#### Results and Discussion

The average biochemical values on blood samples collected from buffalo calves before and after the experimental induction of rumen acidosis are presented in Table I.

A significant rise in blood glucose concentration, observed in the present study, might have been either due to increase in glycogenolysis or gluconeogenesis or due to decreased utilization of glucose by peripheral tissues<sup>5,6</sup>. In advanced stages of rumen acidosis, the hyperglycremia may be due to the reduction in glucose utilization as a consequence of decreased level of circulating IRI. Sections of liver tissues stained with PAS (Periodic Acid Schiffs) method for demonstration of glycogen revealed depletion of glycogen in the vacuolated hepatocytes. Sections of