

The suppressant effect exerted by CPZ, DZP and PB on shock-induced fighting aggression in mice agrees with earlier reports^{3,5,6} but there is no report to substantiate that DDT or malathion augmented fighting behaviour. The mechanism involved, therefore, can only be speculated. Irritation aggression which resembles rage reaction is elicited by a wide range of external stimuli which in the present case is pain caused by electric shock. Hypothalamus and limbic forebrain structures such as amygdala through certain neurochemical changes have been implicated in the aggressive behaviour. Also, there is a greater activation or arousal of the autonomic and endocrine components⁷.

The authors thank Cyanamid India Limited, Bombay and Hindustan Insecticides Limited, Kerala for supplying technical grade of malathion and DDT, respectively. RPU is grateful to ICAR, New Delhi for the award of a fellowship.

26 May 1982

1. Hayes, W. J. Jr., Dale, W. E. and Lebeton, R., *Nature (London)*, 1963, 111, 1189.
2. Wooley, D. E., *Toxic. Appl. Pharmac.*, 1970, 16, 521.
3. Tedeschi, R. E., Tedeschi, D. H., Mucha, A., Cook, L., Mattis, P. A. and Fellow, E. J., *J. Pharmac. Exp. Ther.*, 1959, 125, 28.
4. Uppal, R. P., *Neurologic effects of insecticides and their interaction with drugs*, Ph.D. Thesis, Haryana Agricultural University, Hissar, 1979.
5. Chen, G., Bohner, B. and Bratton, A., *Archs. Int. Pharmacodyn. Therap.*, 1963, 142, 30.
6. Sofia, R., *Life Sci.*, 1969, 8, 705.
7. Mogenson, G. R., *The neurobiology of behaviour: An Introduction*, LEA Publishers, New Jersey, 1977, 239.

INSULIN TOLERANCE TEST IN THE NORMAL AND HYPOPHYSECTOMIZED TOAD, *BUFO MELANOSTICTUS* (SCHNEIDER)

M. S. MEHENDALE AND A. S. PADGAONKAR
Zoology Department, B.N.N. College, Bhiwandi
421 305, India.

THE hypoglycemic effect of insulin is very slow in poikilotherms as was reported in amphibians, *Bufo arenarum*¹, *Leptodactylus ocellatus*² and *Rana tigrina*³ and in snakes^{4,5} and crocodiles⁶. Hypophysectomized toads had a greater hypoglycemic action to insulin than normals⁷. This reaction could be reduced by the injection of extracts of the hypophysis, particularly the pars distalis⁸. The following experiment was performed to study the comparative tolerance to

insulin of normal and hypophysectomized toad, *Bufo melanostictus*.

The animals used for this study were kept in cages containing water for two days and during this period they were not fed. Hypophysectomy was performed under ether anaesthesia. The mouth of the toad was kept open as wide as possible with the help of rubber bands. A medial cut (2.5 cm) was then made in the mucous membrane of the palate. The margins of the cut were pulled apart to expose the parasphenoid bone. A small window was made in the parasphenoid bone just above the position of the pituitary with a sharp scalpel. The cartilage which thus became visible was removed using a pointed needle. The pituitary which now lay exposed was removed with pointed curved forceps. The square piece of the parasphenoid bone was then put back in place and the cut ends of the mucous membrane were sewn together with surgical thread. Neosporine antiseptic was applied over the wound to prevent infection.

Insulin tolerance test was performed on normal and hypophysectomized animals by injecting a dose of 10 IU/kg of insulin (insulin lente, insulin zinc suspension, Lot No. 618, The Boots Company (India) Ltd., Bombay). For these tests 27 normal and 27 hypophysectomized animals were used. The normal animals were sacrificed after 0, 0.5, 1, 2, 3, 4, 24, 48 and 96 hr. In the hypophysectomized animals insulin was injected 24 hr after the operation and the animals were sacrificed after 0, 0.5, 1, 2, 3, 4, 24, 48 and 96 hr. All injections were given in the dorsal lymph sac. The animals were sacrificed by pithing. Blood samples were withdrawn from the conus arteriosus and were received in oxalated tubes. Blood sugar was estimated by Folin-Wu method⁹, and the values obtained were plotted (figure 1).

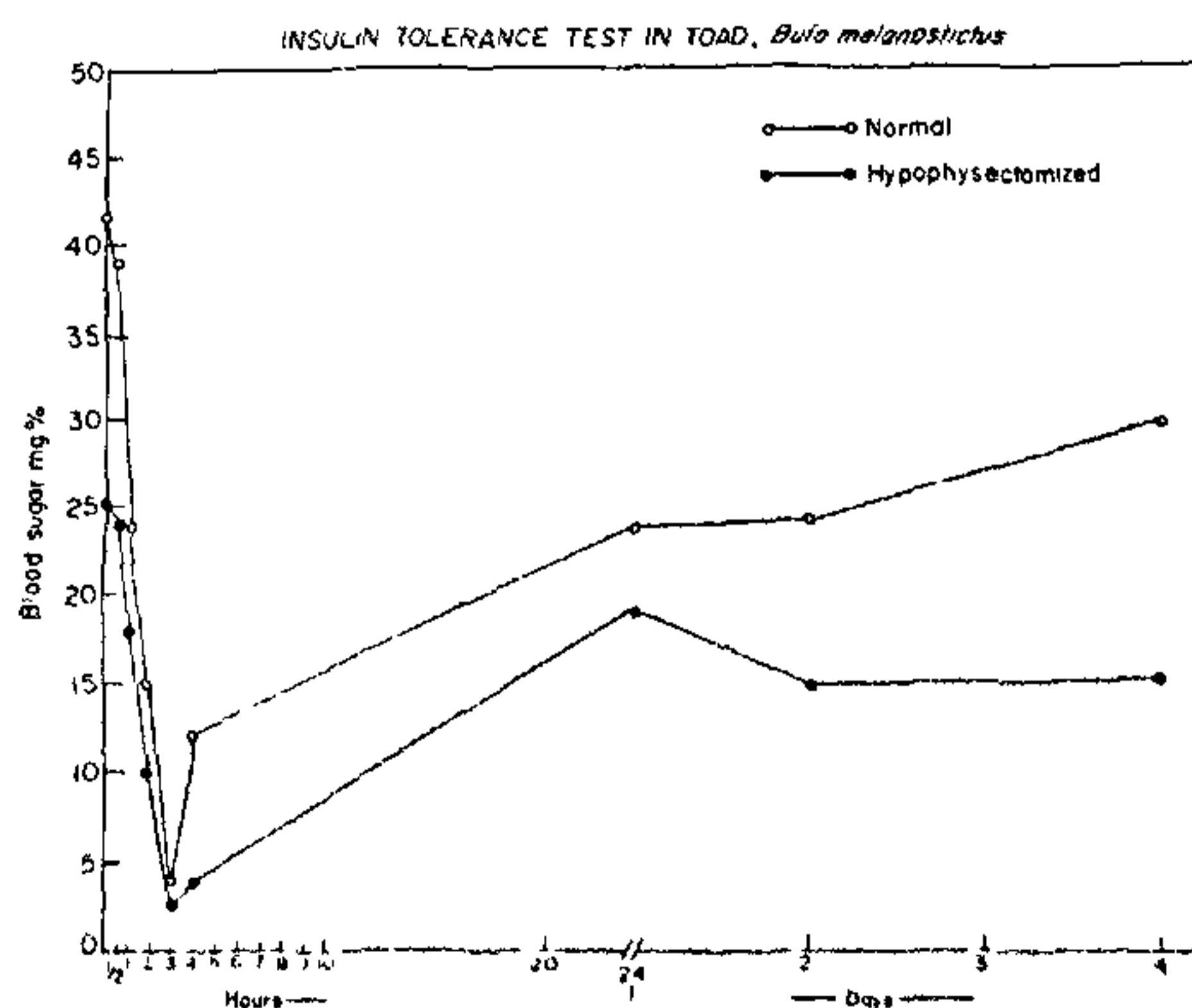


Figure 1. Hypoglycemic action of insulin (10 IU/kg) in normal and hypophysectomized toads (each point on the graph represents the average of three blood sugar values).

On comparing the graphs of the hypophysectomized and normal toads after a dose of 10 IU/kg of insulin, it is evident that the glycemia in the latter group is at a higher level. The maximum hypoglycemic response was observed in both groups at 3 hr stage; the blood sugar in the hypophysectomized toads dropped as low as 2.7 mg%. This indicates that in the toad, *B. melanostictus* sensitivity to insulin is increased after removal of hypophysis. This observation conforms with the results obtained earlier in the frog, *Rana tigrina*⁷, turtles¹⁰ and the toads and mammals¹¹. In agreement with the observations made in toads and mammals¹¹, the hypersensitivity to insulin exhibited by the toad, *Bufo melanostictus* following hypophysectomy, is related to the removal of diabetogenic factor present in the hypophysis. However, the lizard, *Varanus monitor*³ when similarly operated became insulin-resistant and the blood sugar level was found to be at a higher level than that in the insulin-treated and the blood sugar level was found to be at a higher level than that in the insulin-treated normal animals for about 21 hr. This resistance to insulin was suggested as due to the discharge of endogenous glucagon. This is another example of important metabolic difference existing between the toad, *B. melanostictus* and lizards.

In the toad, *Bufo melanostictus*, hypophysectomy produced within 24 hr, a significant decline in the glycemic level (33.3 ± 3.66 in control and 20.9 ± 2.19 in experimental toads, unpublished data). It is therefore evident that this immediate post-operative fall in blood sugar level is mainly due to the deprivation of the hypophyseal diabetogenic action, suggesting the pivotal role played by the pituitary in the blood sugar homeostasis of this animal. The hypersensitivity to insulin shown by the hypophysectomized toad in the present experiment gives further support to our above observations made earlier after hypophysectomy.

6 July 1982

1. Houssay, B. A., Sordelli, A. and Mazzocco, P. 1923 (As cited by Lopes *et al.* 1954).
2. Houssay, B. A. and Rietti, C. T., *C. R. Soc. Biol. Paris*, 1924, 91, 27.
3. Rangneker, P. V. and Sabnis, P. B., *J. Anim. Morphol. Physiol.*, 1964, 11, 173.
4. Prado, J. L., *Rev. Can. Biol.*, 1947, 6, 255.
5. Padgaonkar, A. S. and Rangneker, P. V., *J. Anim. Morphol. Physiol.*, 1975, 22, 38.
6. Coulson, R. A. and Hernandez, T., *Endocrinology*, 1953, 53, 311.
7. Houssay, B. A., Mazzocco, P. and Rietti, C. T. *C. R. Soc. Biol. Paris*, 1925, 93, 967.
8. Houssay, B. A. and Potick, D., 1929. (As cited by Lopes *et al.* 1954).

9. Hawk, P. B., Oser L. and Summerson, W. H., *Practical physiological chemistry*, Maples Press Company, New York, 1964.
10. Lopes, N., Wagner, E., Barroes M. and Marques, M. *Acta Phys. Latinoamericana*, 1954, 4, 190.
11. Houssay, B. A., *Endocrinology*, 1942, 30, 884.

STEROID HORMONE PRODUCING SITES IN THE OVARY OF THE TWO SPECIES OF OTTERS, *LUTRA LUTRA* AND *AONYX CINEREA*: A HISTOCHEMICAL STUDY

S. MURALI, D. THEERTHA PRASAD AND H. B. DEVARAJ SARKAR

Department of Post-Graduate Studies and Research in Zoology, University of Mysore, Manasagangothri, Mysore 570 006, India.

Δ^5 3 β -HSDH and 17 β -HSDH are key enzymes involved in the steroidogenesis and have been demonstrated histochemically in the ovary of a number of species ranging from fish to mammals¹⁻⁴. It is well understood that the biosynthesis of all the hormonally active steroids involve the conversion of Δ^5 3 β -hydroxy steroids to Δ^4 3 β -keto steroids. The reaction is carried out by Δ^5 3 β -hydroxy steroid dehydrogenase (HSDH) with the help of an isomerase which in turn uses NAD⁺ as the cofactor which effectively participates in the transfer of hydrogen to tetrazolium salt⁵. 17 β -HSDH is another important steroidogenic enzyme which catalyzes the oxidative interconversion of sex steroids like androgens and estrogens. Glucose-6-phosphate dehydrogenase (G-6-PDH), one of the potential generator of NADPH, which is utilized for the hydroxylation reaction in steroidogenic sites and essential for the transfer of hydrogen from reduced NAD to tetrazolium salt, provide additional evidence to identify steroidogenic sites. Since there are no reports on the localization of these enzymes in the ovaries of the otters, the present study was undertaken.

Adult female otters, *Lutra lutra* (clawed otter) and *Aonyx cinerea* (Clawless otter) were collected from Cauvery river basin (150 km from Mysore city) and used for the present study. The ovaries were dissected out free from connective tissues and one ovary was frozen at -20°C in a cryostat. The other ovary is processed for histological observations. Frozen sections of 16 μ thick were cut and transferred to coverslips, air-dried and incubated aerobically in appropriate media in a serological water bath at 37°C for various enzymes. After incubation the sections were thoroughly washed in distilled water and post fixed in 10% neutral formalin and mounted in glycerine jelly. Histochemical procedures employed localize Δ^5 3 β -HSDH, 17 β -HSDH, G-6-PDH