noticed by us but from the same anther, pollen with no B's are also seen. The present note is the second report from India about the presence of B's in sedges. We have not noticed the presence of SAT-chromosomes in *E. atropurpurea* and *E. palustris* although their presence was reported earlier[12]. No secondary constrictions on metacentric chromosomes were seen by us for *E. palustris* but earlier studies show their presence[13].

The above observations clearly indicate the high amount of genetic diversity of sedges within species concept, indicating that the evolution is operative at micro level. As far as sedges are concerned, karyotypic variations are not only conserved but are also multiplied because of vegetative reproduction—a common feature of this group of plants.

1. Bir, S. S., Sidhu, M. and Kamra, S., *Cytologia*, 1986, **51**, 95–106.
2. Bir, S. S., Kamra, S., Sidhu, M. and Cheema, P., *J. Cytol. Genet.*, 1988, **23**, 14–37.
3. Bir, S. S., Cheema, Paramjeet, and Sidhu, M., *Nucleus*, 1990, **33**(1, 2), 22–24.
4. Bir, S. S., Cheema, Paramjeet, and Sidhu, M., *Cytologia*, 1991, **56**, 645–651.
5. Bir, S. S., Cheema, Paramjeet, and Sidhu, M., *Indian Natl. Sci. Acad. Biol. Sci.*, 1992, **B58**, 63–70.
6. Kumar, V. and Subramaniam, B., *Chromosome Atlas of Flowering Plants of Indian subcontinent*, Botanical Survey of India, Calcutta, India, 1987, vol. II, pp. 499–510.
7. Wulff, H. D., *Jahrb. Wiss. Botanik*, 1939, 87.
8. Stebbins, G. L., Cold Spring Harbour Symp. Quant. Biol., 1958, **23**, 365–378.
9. Tanaka, N., *Cytologia*, 1939, **10**, 51–58.
10. Mosquin, T. and Hayley, D. E., *Can. J. Bot.*, 1966, **44**, 1209–1218.
11. Mehra, P. N. and Sachdeva, S. K., *Cytologia*, 1976, **41**, 585–590.
12. Sanyal, B. and Sharma, A., *Cytologia*, 1972, **37**, 13–32.
13. Sanyal, B., *Cytologia*, 1972, **37**, 33–42.
14. White, M. J. D., *Animal Cytology and Evolution*, Cambridge University Press, Cambridge, 1945.

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Gamma amylase activity—An alternate pathway of carbohydrate metabolism in animals

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An attempt has been made to establish the gamma amylase activity as an alternative pathway of glycogenolysis in starved condition of animal taking *Heteropneustes fossilis* as model. The enzyme activity was estimated in the liver, kidney, brain and muscle of diet supplemented (control), starved and refed fish groups. The maximum enzyme activity was noted at pH 4.8 and measured in the kidney followed by liver and brain in the control. But the highest activity of this enzyme was recorded in the liver of starvation-stressed fish. The reduction of the enzyme activity was noted in the tissues of refed group of fish suggests that the gamma amylase is responsible for the breakdown of glycogen to glucose other than phosphorylase pathway to meet the energy demand under certain stress.

THE gamma amylase activity other than phosphorylase[1] in mammalian and amphibian liver in diseased condition[2–5] have been reported to some extent. The gamma amylase can directly liberate glucose from glycogen by splitting both alpha 1, 4 and alpha 1, 6 linkages along with gamma dextrin[4] and appears as an important enzyme in the carbohydrate metabolism under certain stress. However, no reports are available on the gamma amylase activity in the starvation stress condition as it is in many ways similar to the diabetic condition[7]. We therefore studied the gamma amylase activity in different tissues under starvation stress as an alternate pathway of carbohydrate metabolism taking *Heteropneustes fossilis* as model.

The fish *H. fossils* was acclimatized in the laboratory and sorted into three groups. Group I, regularly fed on minced goat liver, earthworm and artificial diet, continued upto the 20th day was termed as control. Group II, kept without food upto the 20th day was termed as starved and group III, comprising of fishes starved upto the 10th day and thereafter supplemented with control diet, were named as refed group.

A batch of 6 fishes from each set were sacrificed and the liver, kidney, brain and muscle on the 5th, 10th, 15th and 20th days were taken out from the control and starved groups. Similarly, the aforesaid organs of the refed group were taken out on the 10th (just one hour of diet supplementation), 15th and 20th days. The enzyme gamma amylase was isolated[5] and the activity was determined following the procedure of Rosenfield[6]. The glucose[8] and the protein[9] were estimated for the calculation of the enzyme activity (EU) which is expressed as

$$EU = \frac{\text{mgm of glucose/100 ml}}{\text{mgm of protein/ml}}.$$

To evaluate the optimum pH of the medium (acetate buffer) for the maximum activity of the enzyme, a series of buffer solutions of pH 4.5, 4.6, 4.7, 4.8, 4.9, 5.0 and

5.1 were prepared. The maximum gamma amylase activity was recorded at pH 4.8 in the liver, kidney and brain, while in the muscle, highest activity was obtained at pH 4.9 (Figure 1).

The highest activity of gamma amylase was noted in the kidney followed by liver (Figure 2) and then in the brain and muscle (Figure 3) of the control sets of fish. The maximum gamma amylase activity was recorded in

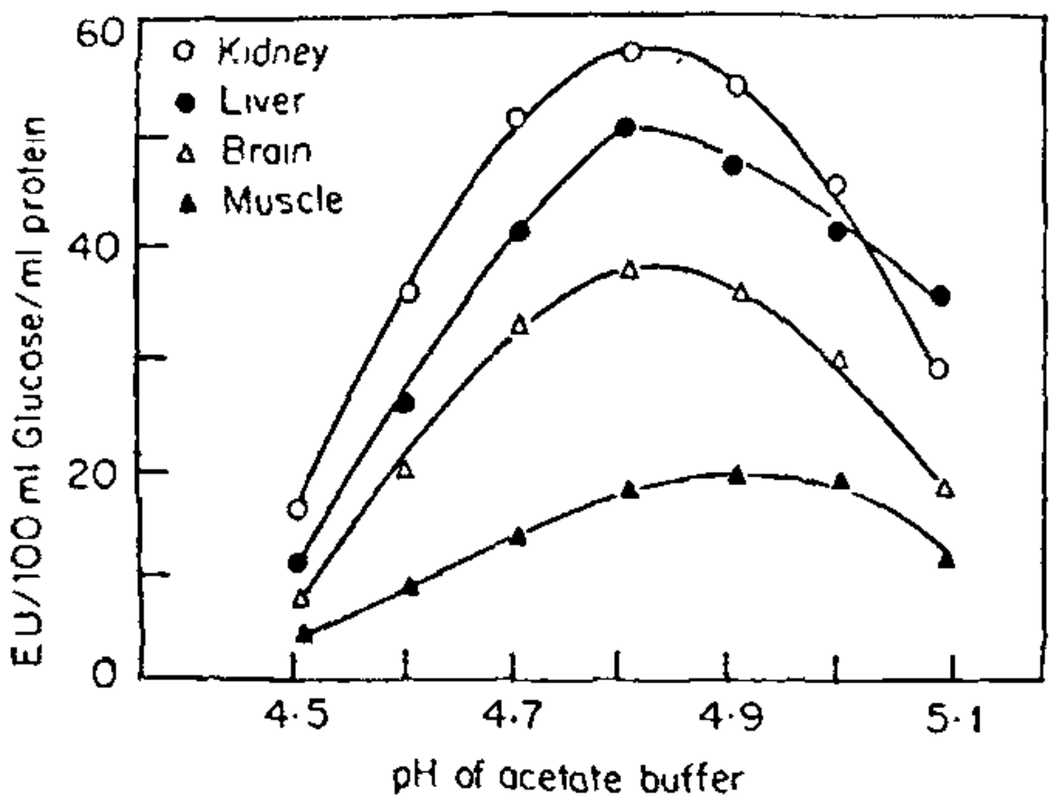

Figure 1. Gamma amylase activity (EU) at different pH of different organs of *H. fossilis*.

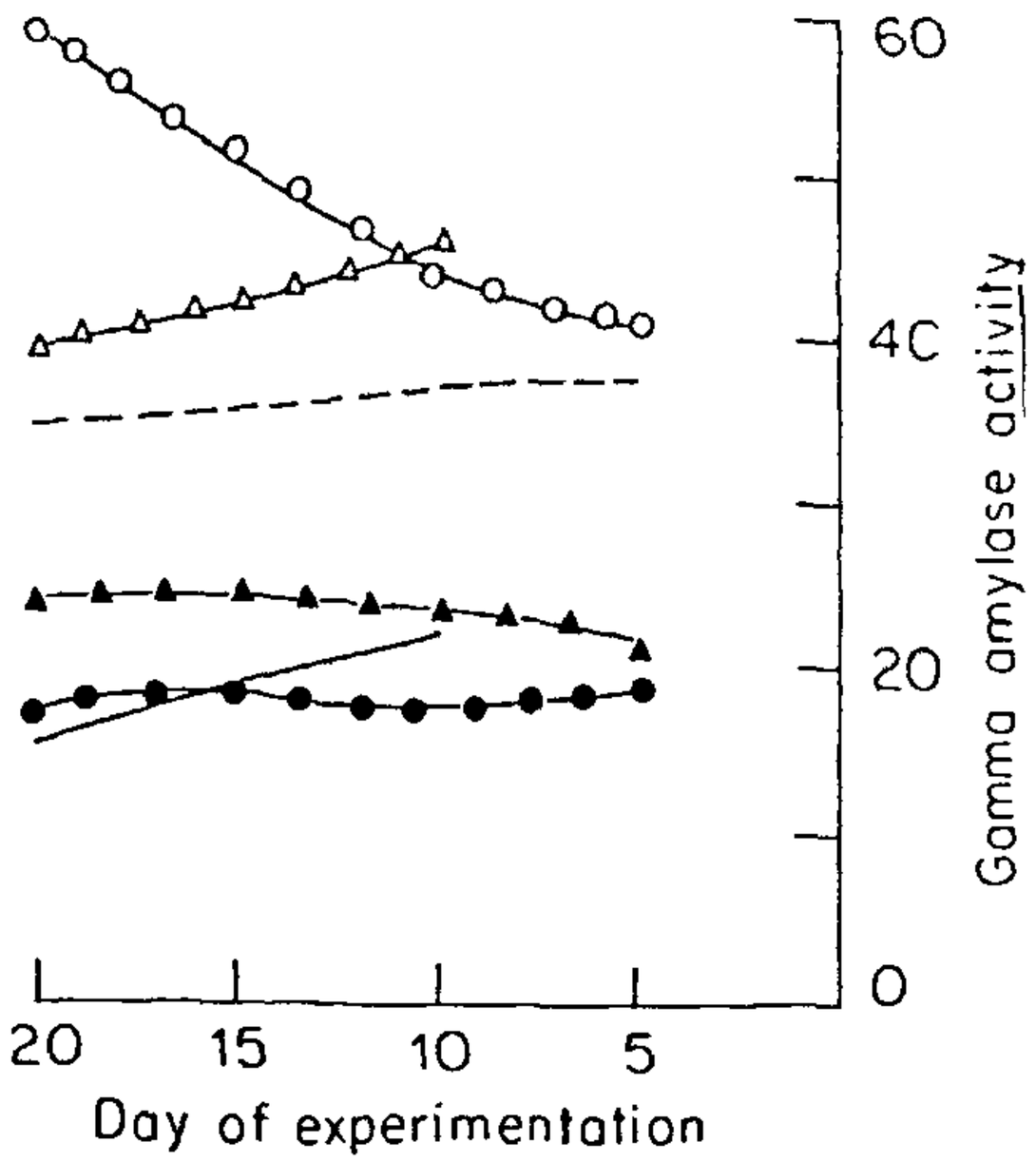

Figure 3. Gamma amylase activity of brain (control —; starved O-O-O; refed △-△-△) and muscle (control ●-●-●; starved ▲-▲-▲; refed ———) group of *H. fossilis*.

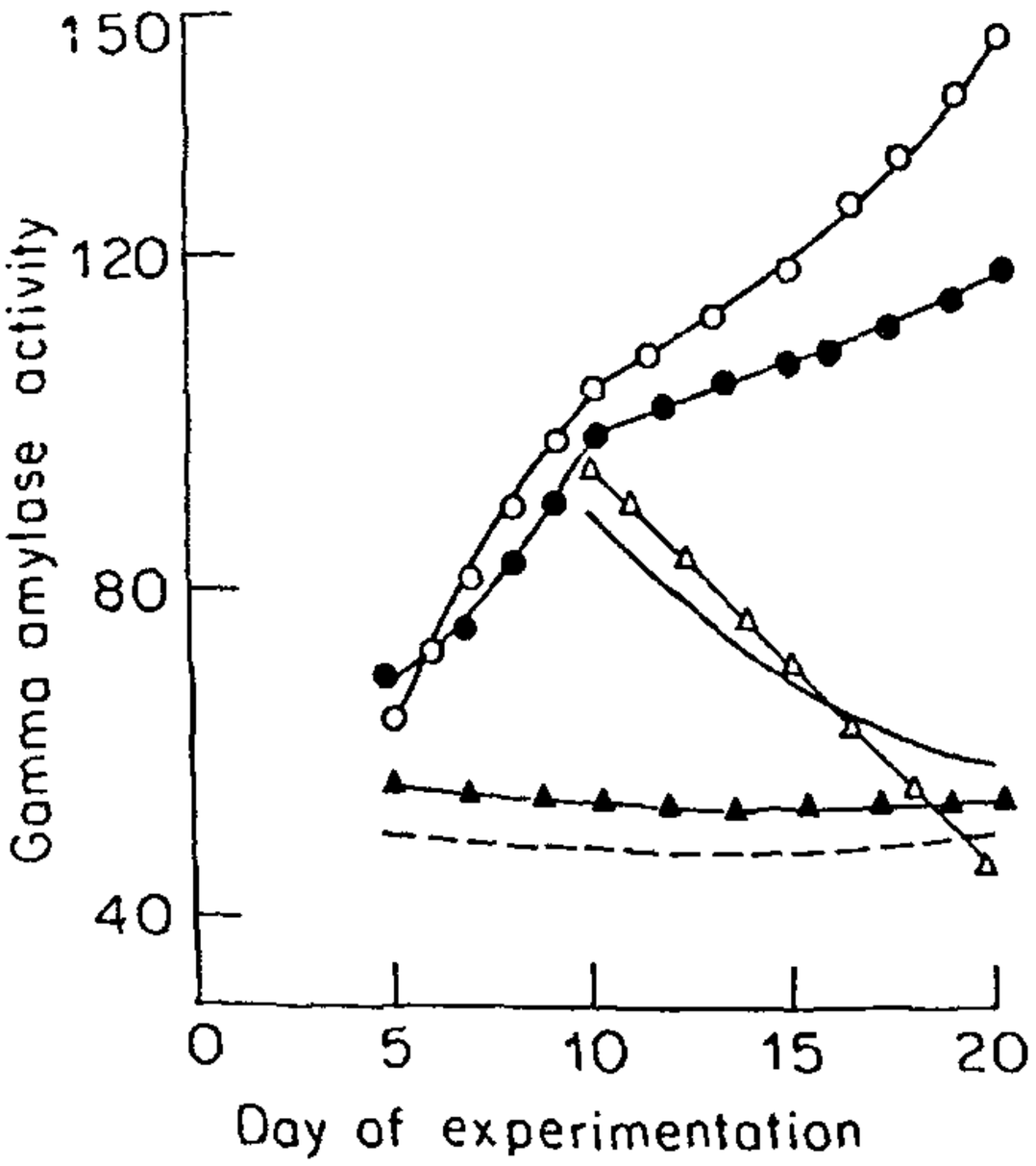

Figure 2. Gamma amylase activity of liver (control ---; starved O-O-O; refed △-△-△) and kidney (control ▲-▲-▲; starved ●-●-●; refed ———) group of *H. fossil's*.

the 20th day starved liver followed by kidney, brain and muscle over their respective controls (Figures 2 and 3). However, the enzyme activity was recorded at low level in the refed group of fish.

The results of this experiment revealed the breakdown of stored glycogen to glucose in various tissues to meet the energy demand under starvation stress[10]. But the refed group exhibited a corollary effect and the gradual fall of the enzyme activity in the refed group established the reduction of the glycogenolysis and is confirmed by the observation of Khanna and Bhatt[11].

Starvation is in many ways similar to diabetes mellitus[7] and the administration of insulin in rat reduced the activity of gamma amylase[12]. The gamma amylase is directly reponsible for the liberation of glucose from the stored glycogen[4-6] and it is inferred that the increased activity of this enzyme is one of the major causes of increasing blood sugar[1,3] under the stress of starvation.

The mechanism of gamma amylase activity is yet to be fully understood. However, the gamma amylase in liver is considered to be of lysosomal orgin[13] and this offers an explanation for glycogen breakdown in tissues[14,15]. Further, it has also been recorded that under stress, the pH of the tissues is observed in the acidic range due to the accumulation of metabolites[16,17]. Therefore, the present experiment is only suggestive of

the fact that the gamma amylase activity under starvation stress was enhanced to produce glucose directly from stored glycogen as an alternate pathway of carbohydrate metabolism.

1. Wheelan, W. J. and Cameron, M. P., in *Control of Glycogen Metabolism*, Ciba Foundation Symposium, J. and A. Churchill Ltd., London, 1964, p. 176.
2. Torres, H. and Olivaria, J., *Acta. Physiol. Lat. Am.*, 1961, **2**, 95.
3. Hers, H. G., *Biochem. J.*, 1963, **2**, 86.
4. Rosenfield, E. L., *Izd. Akad. USSR*, 1962, p. 99.
5. Rosenfield, E. L. and Papova, V., *Bull. Soc. Chem. (Paris)*, 1962, **44**, 129.
6. Rosenfield, E. L., in *Control of Glycogen Metabolism* (eds. Wheelan and Cameron), Ciba Foundation Symposium, J. and A. Churchill Ltd., London, 1964, p. 184.
7. Unger, H., *Metabolism*, 1974, **23**, 541.
8. Oser, B. L., (ed.) in *Hawk's Physiological Chemistry*, McGraw Hill Pub., New Delhi, 1965, p. 1154.
9. Lowry, O. H., Rosenbrough, N. J., Furr, A. L. and Randall, R. F., *J. Biol. Chem.*, 1951, **193**, 265.
10. Sharma, D. K. and Sengupta, S., in *Proc. 2nd Internatl. Conf. Life Sci.*, 1988, (in press).
11. Khanna, S. S. and Bhatt, P. D., *Proc. Natl. Acad. Sci., India*, 1972, **42**, 415.
12. Sarma, J. K., Ph D thesis, Gauhati University, 1977.
13. Murat, J. C., *Comp. Biochem. Physiol.*, 1976, **55**, 461.
14. Sarkar, C. R. and Goswami, P., *J. Ind. Med. Assoc.*, 1973, **61**, 166.
15. Sarkar, C. R. and Goswami, P., *Indian Med. Gaz.*, 1975, **14**, 489.
16. Jafri, A. K. and Shaffi, S. A., *Broteria*, 1975, **44**, 59.
17. Dhillon, S. S. and Gupta, A. K., *Water, Air, Soil Pollut.*, 1983, **20**, 63.

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Thyroid hormone stimulates progesterone release from the ovary of a fish, *Anabas testudineus*

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Release of steroid hormone from the ovary is so far believed to be regulated solely by gonadotropic hormones. Thyroid hormone has been implicated in the reproduction of vertebrates but its role is rather indirect. Addition of thyroid hormone, triiodothyronine (T_3), to perch oocyte culture in vitro surprisingly resulted in a dose-dependent increase in progesterone release into the medium. To examine the specificity of this stimulation, perch were treated with thiourea, an antithyroid drug, for 7 days and oocytes from them when cultured in the presence of T_3 did not show increase of progesterone release over the control. Addition of cycloheximide with T_3 into the oocyte culture completely inhibited T_3 stimulation of progesterone release suggesting that the effect of T_3 on stimulated progesterone release is not direct and it possibly involves a protein(s) mediator. To reinforce this contention, control and T_3-treated oocytes were homogenized, fractionated and a $2 \times 10^5 g$ supernatant (200 K sup) was added to oocyte culture. 200 K significantly stimulated progesterone release and this property could be destroyed by trypsin digestion. Such an increase in progesterone release was not effected by 200 K sup from control oocytes. Results indicate that T_3-stimulated progesterone release from perch oocytes via the induction of proteinaceous factor.

STEROID hormone synthesis and release from the gonad of vertebrates including human being is known to be regulated by brain-pituitary axis. Hypothalamus of the brain releases a decapeptide hormone, gonadotropin-releasing hormone, which stimulates the release of gonadotropins from the pituitary. Gonadotropins then act on the target tissue i.e. ovary and testis, cause steroid hormones synthesis and release which in turn control the development and maturation of gonad. This is a well-established concept and till date no other hormones have been reported to have a direct effect on the gonadal steroid hormone synthesis or release. Thyroid hormone has long been implicated in the reproduction of vertebrates[1-5] and it has been shown to influence both ovarian and testicular functions in mammals[6-10]. These reports indicate an influence of thyroid hormone on gonadal activity but how it does so remains unclear. Recently we have reported thyroid hormone receptor in the nuclear preparation from perch ovary[11] and human corpus luteum[12]. These reports imply a direct influence of thyroid hormone in the reproduction. In this communication we report biological relevance of thyroid hormone receptor in the ovarian nuclei of perch.

Ovaries from the perch (*Anabas testudineus*) belonging to the prespawning stage of the annual reproductive cycle (April-May) were dissected out, oocytes isolated and cultured *in vitro* for 4 hours at 30°C by following the methods reported earlier[13]. Equal amounts of (25 mg) oocytes were placed in a sterile beaker containing ice-cold Earle's minimum essential medium (MEM) gassed with 95% O_2 and 5% CO_2. Viability of oocytes was examined by Trypan blue dye exclusion method and at the end of 4 hour more than 90% of oocytes were found viable. We have used triiodothyronine (T_3) as thyroid hormone since this is biologically more active than thyroxine (T_4). Progesterone released into the culture medium was extracted and estimated by specific progesterone radioimmunoassay. Progesterone antibody was a kind gift from Dr G. Niswinder, University of Colarado, USA.

Addition of T_3 in two different doses, i.e. 200 and