## AN UNKNOWN METABOLIC INHIBITOR CONTAINED IN THE BRAIN OF STARVED FROG *RANA HEXADACTYLA*

K. P. RAO and his collaborators[1,2], who have investigated the effects of body fluids of "cold" and "warm" acclimated earthworm *Lampito mauritii* on tissue respiration of normal, "cold" and "warm" acclimated worms, have shown that the body fluid from "cold" acclimated worms enhanced oxygen consumption, while that of "warm" acclimated worms depressed it. Similar effects have been noted in other ectotherms, *e.g.*, the scorpion *Heterometrus fulvipes*[3] and the eel, *Anguilla anguilla*[4]. Recently, Nayeemunnisa and K. P. Rao[5] have reported similar effects of sterol fraction from the nervous tissue of "cold" and "warm" acclimated earthworms on the tissue respiration of normal worms. Coles[6] has demonstrated that extract of pleuro-pedal ganglia excised from active snail *Pila ovata* stimulated increased uptake of oxygen in the muscle slices of aestivating (starving) snails; he has also extracted a steroid factor from the ganglia and assumed that it may be responsible for stimulation of enhanced oxygen uptake by the aestivating snail. On the basis of the results obtained by his team and on the basis of related publications, K. P. Rao put forward the hypothesis that in general, where a decrease or increase in metabolism is achieved in higher metazoan ectotherms, such metabolic change is mediated through humoral factors, which are often elaborated in the neuro-humoral system[7,8]. In India, often owing to the failure or belated monsoon, ectotherms such as the frog *Rana hexalactyla* are exposed not only to the higher temperature but also to starvation stress.

It has been observed by us that oxygen consumption of brain excised from well-fed frog is 401 $\mu$l/g/h; it gradually decreases to 350 $\mu$l/g/h for that of frog starved for 40 days and remarkably to 213 $\mu$l/g/h for that of frog starved for 60 days[9]. In the light of the above-mentioned hypothesis, it is conceivable that after 40 days starvation, the frog's brain secretes and/or releases some unknown factor, which decreases oxygen consumption. With a view to study the possibility of occurrence of such a metabolic inhibiting factor, the following experiments were conducted; the present paper is a short report of the results obtained.

Brain extracts of frogs (of about 5 to 10 g), that were previously starved for a period of 60 days, were prepared by homogenizing brain (40 mg) in 5 ml of frog's Ringer[10]; after centrifuging, the supernatant was used. Brain (40 to 50 mg) or liver (about 100 mg) slices, excised from either well-fed or starved frog, were taken along with 2·0 ml of the Ringer in the main chamber of the Warburg flask and 0·1 ml (containing about 0·8 mg Ringer soluble brain substance) of the extract of starved or well-fed frog was added into the medium. Simultaneously, a control was prepared by adding 0·1 ml of starved frog's brain extract to the tissues excised from the starved frog, or well-fed frog's brain extract to the tissues of well-fed frog. Oxygen consumption of the tisues was measured following the standard manometric procedure described by Umbreit *et al.*[11].

Brain and liver of well-fed frog consumed 400·8 and 309·9 $\mu$l $O_2$/g/h, respectively (Table I; column 2); the corresponding values for those of starved (60 days) frog were 213·8 and 67·7 $\mu$l $O_2$/g/h. Oxygen uptake of well-fed frog's brain, when added with the extract of well-fed frog's brain (control), was only 261·6 $\mu$l/g/h (Table I; column 3). Similarly, starved frog's brain containing the starved frog's brain extract (control), consumed 175·3 $\mu$l $O_2$/g/h (Table I; column 4). More or less similar changes in oxygen consumption of liver of well-fed or starved frog, with the brain extract of well-fed or starved frog (controls) were also noted. The differences in the oxygen consumption between the respective normal and control may be due to the effect of protein-protein interaction.

On the other hand, there was a remarkable decrease in the oxygen uptake of brain or liver of well-fed frog, when the brain extract of starved frog was added; in the test series (Table I; column 4), brain 172·8 $\mu$l $O_2$/g/h, as against the oxygen uptake of 400·8 and 261·6 $\mu$l/g/h in the normal and control frog's brain, respectively. Such a pronounced depression in oxygen uptake was also seen in the other organ such as liver (101·2 $\mu$l $O_2$/g/h; normal: 309·9 and control: 138·0 $\mu$l/g/h). The fact that the magnitude of depression in oxygen uptake in test series are far greater than those of the control series indicates the presence of an unknown metabolic "inhibiting factor" contained in the starved frog's brain.

Oxygen consumption of starved frog's brain with the brain extract prepared from starved frog (Table I; column 4) too was only 175·3 $\mu$l/g/h a value, which well compares with that (172·8 $\mu$l/g/h) obtained for that of well-fed frog's brain added with starved frog's brain extract. Assuming the presence of metabolic inhibiting factor in the starved frog's brain, the control series containing (40 mg substance) starved frog's brain and the added (0·8 mg) brain extract prepared from the starved frog should have had about 50 times greater concentration of the metabolic inhibiting factor in comparison to that of the test series containing

(40 mg) well-fed frog's brain and the added (0·8 mg) brain extract of the starved frog's brain. However, in both the cases, the oxygen consumption was more or less the same (about 175 µl/g/h). This suggests that it is the very presence of the inhibiting factor that reduces metabolic level. Probably a greater or lesser concentration of the factor has little to do with the intensity of inhibiting effect, beyond the threshold level.

TABLE I

*Effects of brain extracts prepared from well-fed or starved frog on the tissue respiration of brain and liver excised from well-fed or starved frog*

| Tissue | Oxygen consumption (µl/g/h) Normal, untreated | treated with well-fed frog's brain extract | Treated with starved frog's brain extract |
|---|---|---|---|
| I Well-fed frog's | | | |
| (*a*) Brain | 400·8 ± 14·8 | 261·6 ± 11·9 | 172·8 ± 6·4 |
| (*b*) Liver | 309·9 ± 12·4 | 138·0 ± 3·2 | 101·2 ± 9·3 |
| II Starved frog's | | | |
| (*a*) Brain | 213·8 ± 4·5 | 389·3 ± 8·1 | 175·3 ± 5·6 |
| (*b*) Liver | 67·7 ± 3·8 | 173·4 ± 6·4 | 69·0 ± 10·5 |

Liver of well-fed frog, containing only the (0·8 mg) brain extract of starved frog, consumed 101·2 µl $O_2$/g/h, while that of starved frog's liver exposed to the brain extract of starved frog consumed even lesser quantity of oxygen (69·0 µl/g/h; Table I; column 4) suggesting that a relatively greater concentration of the starved frog's brain extract is required to lower the oxygen uptake of the liver to its minimum level.

Apart from the assumption of the presence of the metabolic inhibiting factor in brain extracts of starved frogs, there seems to be a "stimulating factor" in the well-fed frog's brain extract, which enhanced the oxygen consumption of the brain and liver of the starved frog, when added *in vitro*. Oxygen uptake of the brain and liver of the starved frogs increased to 389·3 and 173·4 µl/g/h (Table I; column 3), respectively, when the brain extract of the well-fed frog was added, as against the control (175·3 and 69·0 µl $O_2$/g/h for brain and liver, respectively; Table I; column 4) and normal values of the brain (213·8 µl $O_2$/g/h) and liver (67·7 µl $O_2$/g/h) of the starved frog[12].

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## THE RELATIVE ABUNDANCE OF DANAID BUTTERFLIES IN NATURAL POPULATIONS OF SRINIKETAN, WEST BENGAL

SOME species of Danaid butterflies like the plain tiger *Danaus chrysippus* and the common tiger *D. plexippus* are frequently observed in the fields and gardens of Sriniketan in the district of Birbhum, West Bengal. They are extremely distasteful because of their habit of feeding on toxic food plants[1]. *D. chrysippus* is known to serve as model for a palatable Nymphaline species *Hypolimnus misippus*[2]. The latter, therefore, exhibits Batesian mimicry.

There is experimental evidence[3] that the mimetic selective advantage wanes as the mimics become more common than the models. This is because of the fact that with the rise in frequency of the mimic the predator will begin to associate the mimetic form with tastefulness rather than with the unpalatability of the model. It is, therefore, interesting to find out the relative abundance of models and mimics in the natural populations of butterflies. The present investigation attempts to determine the relative abundance of *D. chrysippus*,