

proximately the same degree as does ACTZ. However, based on the present biochemical results it appears that the inhibition of carbonic anhydrase isozyme C in vitro is not responsible for the decreased responsiveness to electroshock seizures in animals exposed to TET.

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## The significance of species differences in respiratory neurophysiology – the split-brainstem preparation

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**Summary.** In contrast to the generally accepted view (based upon experiments in cats), a mid-sagittal splitting of the medulla revealed the existence of 2 symmetrical, independent respiratory 'centres' in rabbits and monkeys. The role of species differences is stressed.

It is not clear whether the brainstem neurones that are responsible for the genesis and control of breathing form an integrated, extensively interconnected population, or rather, 2 symmetrical networks active in both halves of the pontomedullary complex and synchronized by decussating fibers. Also, controversy exists over whether there is crossing between the pathways descending from respiratory neurones to phrenic and intercostal motoneurones – and, if there is this crossing, to what extent and at what level it is apparent. Salmoiraghi and Burns<sup>1</sup> have shown in cats that respiratory movements are abolished by a mid-sagittal incision extending from 2 mm caudal to 2 rostral to the obex, and this observation has recently been confirmed (also in cats) by other authors<sup>2,3</sup>. On the other hand, however, a similar incision performed in rabbits by Langendorff et al.<sup>4</sup> in their classical experiments elicited independent contractions of 2 halves of the diaphragm. A similar 'desynchronisation', i.e. independent firing in both phrenic nerves was recently demonstrated (also in rabbits) in this laboratory<sup>5</sup>, thus revealing the existence of 2 symmetrical networks of respiratory neurones and their predominantly ipsilateral connections with phrenic motoneurones.

Since the above results were obtained not only in 2 different species but also under different experimental conditions (decerebrate cats<sup>1,2</sup> vs anesthetized rabbits<sup>4,5</sup> and/or a mid-sagittal incision<sup>2,5</sup> vs a mid-sagittal electrolytic lesion<sup>3</sup>), we have undertaken a comparative study with cats, monkeys and rabbits in which – under virtually identical experimental conditions of anesthesia and ventilation – we performed mid-sagittal incisions of the lower brainstem (the 'split-brainstem preparation'<sup>5</sup>) in order to answer the basic question whether species differences or

experimental conditions (or both) are responsible for the differences observed.

All animals (14 cats, 3 monkeys (*Cercopithecus callitrichus*) and 7 rabbits) were anesthetized with 0.7 vol.% halothane in an air-oxygen mixture, paralyzed with d-tubocurarine and artificially ventilated. Both vagus nerves were cut in the neck to eliminate the volume-related input from the lungs. Arterial blood pressure and end-tidal CO<sub>2</sub> concentration were continuously monitored. Arterial blood samples were frequently collected for PaO<sub>2</sub>, PaCO<sub>2</sub> and pH estimations. The electrical activity of both phrenic nerves was simultaneously recorded. An occipital craniotomy was made and the medulla exposed. After control recordings a mid-sagittal incision of the medulla was made with a segment of a razor blade fixed in the holder of a micromanipulator. The blade was lowered towards the base of the skull and this procedure was repeated until a separation of desired length was obtained. The electrical activities of the phrenic nerves, arterial blood pressure and end-tidal CO<sub>2</sub> were continuously recorded during and after surgery. Each experiment was completed by fixing the brainstem in a 10% formaldehyde solution. After 3 days serial sections were made and examined under a microscope to check the localization, extent and completeness of the separation.

Splitting the medulla from about 2 mm caudal to 7 mm rostral to the obex elicited identical effects in normocapnic (and normoxic) monkeys and rabbits: in both species the separation of the 2 halves of the medulla resulted in an immediate 'desynchronization' of inspiratory volleys, i.e. their firing at 2 different rhythms and patterns. On the other hand, in cats, a 'classical'<sup>1</sup> incision from 2 mm caudal to 2 mm rostral to the obex immediately abolished the activity in the phrenic nerves thus confirming the observa-

tion of the other authors<sup>1,2</sup>. However, hypercapnia (65 torr) restored synchronous firing in both phrenic nerves. With more rostral extensions of the incision, only severe hypercapnia combined with hypoxia (85 and 30 torr, respectively) restored the phrenic nerve activity, and the 'desynchronization' of respiratory rhythms was never observed. Splitting the brainstem did not obviously affect the blood pressure or heart rate.

We conclude that the respiratory centers in monkeys and rabbits are organized in 2 symmetrical and relatively independent networks of respiratory neurones whose synchronized activity (but not the activity itself!) depends upon neural connections crossing in the medulla. In these 2 species fibers from respiratory neurones to phrenic motoneurones appear to descend predominantly ipsilaterally.

On the other hand, breathing in cats seems to be controlled by a more integrated brainstem structure in which the decussating connections play a vital part. It is not clear why the cat should differ to such an extent from 2 species that are so remote from each other as are monkeys and rabbits.

Species differences in, e.g., the strength and even pattern of vagal respiratory reflexes are not uncommon<sup>6</sup> but – as far as we know – qualitative differences in the central organization of respiratory centers were never suspected.

Since the cat has recently become the most frequently used laboratory animal, one of the implications of our results is that one can hardly accept the universal validity of physiological concepts and models unless they are confirmed in more than one species of experimental animal.

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## Effect of starvation on serum acid phosphatase levels of freshwater catfish *Clarias batrachus*

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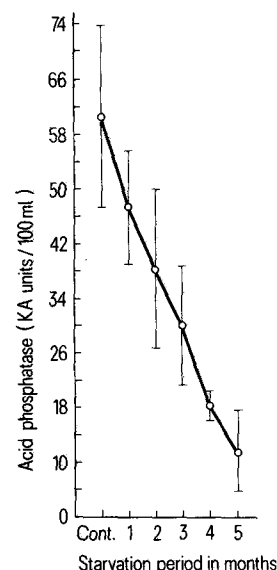
**Summary.** Starvation resulted in a regular and continuous decrease in the serum acid phosphatase levels of the freshwater catfish *Clarias batrachus*. The levels were found to be inversely proportional to the length of starvation. A maximal loss of 80.6% was noted at the end of the 5th month of starvation.

Starvation is one of the numerous adverse situations which fish have to face during some parts of the year. Acid phosphatase is one of several phosphatases; it catalyses the liberation of inorganic phosphate from monophosphate esters in acidic medium. Starvation causes a marked degree of change in various energy-yielding and other metabolic processes in the fish body. The effects of experimental starvation have been studied on various body and blood constituents of fishes by several investigators<sup>2-7</sup> in different parts of the world, but such studies on Indian freshwater fishes, particularly on blood enzymes, are scanty. This paper deals with the results obtained from the freshwater catfish *Clarias batrachus* following experimental starvation lasting up to 5 months.

**Materials and methods.** The methods for collection, transportation and maintenance of fish have been described earlier<sup>2,8</sup>. Only healthy fishes in the weight range 250–300 g were selected for the experiment. They were treated with KMnO<sub>4</sub> (2 mg/l) solution to remove external parasites, developing bacteria and fungi etc. The aquaria in which fish were kept under starvation were practically devoid of any fauna and flora, whereas the control fish were given minced goat-liver, snails and earthworms alternately, besides the naturally-growing fauna and flora. At the end of every month 4 starved and 4 control fish were taken out for the experiment. Blood was collected in a clean vial by severance of the caudal end, allowed to clot at room temperature for 10 min, then centrifuged, and the clear serum was decanted into another tube. The procedure of King and Wootton<sup>9</sup> was followed to determine serum acid phosphatase levels, using a Bausch and Lomb spectronic-20 colorimeter at 650 nm.

**Results.** The results obtained on serum acid phosphatase levels of the control and starved fishes are shown in the

figure. Starvation resulted in a regular and continuous decrease in the enzyme level. After the 1 month of starvation the enzyme level fell to  $47.4 \pm 8.3$  KA units/100 ml from the control value of  $60.5 \pm 13.2$  KA units/100 ml, i.e. a loss of 21.7%. It decreased by another 37.0% after 2 months and 50.4% after 3 months. At the ends of the 4th and 5th months of starvation, the enzyme levels were 30.3% and 19.4% of control, respectively. Thus, acid phosphatase level was found to be inversely proportional to the starvation



Effect of starvation on serum acid phosphatase levels of *Clarias batrachus*.