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Protein, RNA and DNA Levels in Liver and Brain of Starved Catfish *Clarias batrachus*

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Abstract Influence of nutritional conditions on body weight as well as weights and protein, RNA and DNA concentrations of liver and brain of the catfish *Clarias batrachus* was tested. Food deprivation resulted in a decrease in the ponderal and hepatosomatic indices as well as in protein and RNA-DNA ratio in liver. Refeeding was characterized by a reverse sequence of changes in these parameters. The brain, unlike the liver, maintained stability in its weight and biochemical composition. The data have been interpreted in detail and some biologically important relations explained.

RNA-DNA ratios of fish tissues are reported to be quite sensitive to changes in feeding levels and growth rates (Bulow 1970, 1971; Haines 1973; Buckley 1979b) and are also useful for diagnosis of nutritional status and starving condition (Buckley 1979a, 1980; Bulow et al., 1981). The assessment of the general condition of fish including that of its various internal organs, based exclusively on length-weight relationships and ponderal index of intact individuals, has several drawbacks and limitations as pointed out by Mustafa (1979) and Shams (1980). This makes a biochemical or chemical biology approach to the problem all the more important. The present study was planned to present quantitative data on the protein, RNA and DNA in liver and brain of starved and starved-refed groups of catfish *Clarias batrachus* (Linnaeus) together with indices of the condition of these two organs. For feeding trials, chopped raw slaughter house fleshy refuse was selected to emphasize the usefulness of developing low-cost nutritious food for cultivable fishes rather than incorporating the proteins of edible meat with extravagant cost. Our test species, which is highly esteemed as human food and forms substantial part of the capture fishery, and is also cultivated in some regions of the country, proved ideal for the said investigations because of its carnivorous diet.

Materials and methods

Live specimens of *Clarias batrachus* (mean length 21.8 cm and mean body weight 32.7 g) captured from local ponds at Aligarh (27°34′30″N, 78°4′26″E) were brought to the laboratory and confined in glass aquaria. Water in the aquaria was renewed daily for five months during which the fish specimens were deprived of food. Thereafter, individuals in selected numbers were taken out, recorded for length and weight and incapacitated. Brain and liver were immediately dissected out, weighed on an electrical balance sensitive up to 0.05 mg, and their known weights processed separately for the extraction and quantitative estimations of protein, RNA and DNA. The data were compared with the values of these biochemical constituents in the liver and brain of the specimens of *Clarias batrachus* of the same starved lot but fed to satiety for 10 days. The diet provided daily was in the form of raw slaughter house fleshy refuse containing about 18% protein. The unused food and wastes were siphoned off at 24 hour intervals.

Protein was assayed by the procedure of Lowry et al. (1951) and its concentration calculated with the help of a calibration curve prepared by relating the optical density to micrograms of bovine serum albumin. This technique has also been followed earlier (Mustafa and Jafri, 1977). For extraction of RNA from the tissues the method suggested by Schneider (1957) was adopted. Following the partitioning and sedimentation of protein and DNA in this extraction process, the extract in solution form was isolated and the RNA quantitated by means of colorimetric reaction of the orcinol reagent with

a pentose sugar component (Schneider, 1957). Processed yeast RNA served as the standard for comparison and evaluation of RNA values in the samples. After the extraction of DNA from the tissues according to the technique of Webb and Levy (1955) the amount of this substance was determined by the help of methodology outlined by Ashwell (1957), which is based on color reaction of cysteine-sulphuric acid reagent with the deoxy sugars. Inasmuch as the sugar moiety of DNA is known to consist exclusively of 2-deoxyribose in all of its nucleotides, this color reaction serves very well for the determination of the intact nucleic acid. The technique offers the advantage that it is considerably less affected by the presence of proteins and RNA, if any are present, and also has greater specificity compared to other methods. Highly polymerized calf thymus DNA was used for calibration. The color intensities were read on a Bausch and Lomb spectronic 20 spectrophotometer. Values of protein were expressed as mg/100 mg fresh tissue while concentrations of RNA and DNA were recorded separately as μg/100 mg fresh sample.

Results and discussion

Specimens of *Clarias batrachus* deprived of food for about five months were in a highly emaciated state. This was evident not only by a visual examination of their physique but also by the low ponderal index (0.291 ± 0.0075 S.E.). The hepatosomatic index was 0.829 ± 0.0260 S.E. and the brain-somatic index was 0.657 ± 0.0410 S.E. In the individuals drawn from a natural population and not held in confinement for more than 12 hours the ponderal index and hepatosomatic index have been reported to be 0.704 ± 0.0127 S.E. and 1.328 ± 0.116 S.E., respectively (Shams, 1980). No data were furnished on brain-somatic index.

In the starved fish, concentrations of protein, RNA and DNA were respectively 15.7 mg/100 mg, 262.8 μg/100 mg, 97.6 μg/100 mg in the liver and 8.5 mg/100 mg, 101.3 μg/100 mg, 47.8 μg/100 mg in the brain (Fig. 1). The RNA/DNA ratio was 2.692 in the liver and 2.116 in the brain. Resumption of feeding after the prolonged fasting period resulted in gravimetric and biochemical changes in the fish which are summarized thus: the ponderal index, hepatosomatic index and brain-somatic index were altered to 0.444, 1.952, 0.398, respectively. In the liver the concentrations of protein, RNA and DNA changed to 17.6 mg/100 mg, 437.4 μg/100 mg, 93.8 μg/100 mg respectively, while in the brain these were observed to be 8.8 mg/100 mg, 113.8 μg/100 mg and 44.5 μg/100 mg. In the liver the RNA/DNA ratio increased to 4.660. This ratio in the brain remained 2.552.

Total body weight as well as hepatosomatic index increased. Absence of any noticeable difference in the weight of the brain of starved and starved-refed fish of about the same size but decline in the brainsomatic index following refeeding suggests stability in weight of this vital internal organ (brain) and instability in total body weight which is constituted largely of the muscle mass. When brain weight is constant, relative increase in intact body weight on refeeding lowers the brain-somatic index, whereas on starvation relative decrease in total body weight raises this index. This stability in brain weight shows that to keep the mental processes unimpaired, the constituents of this organ are not mobilized, even when a grave physiologic emergency is prevailing in the body wherein organic components of various other parts of the body, chiefly the musculature, are catabolised substantially. The pattern of change in the liver reveals that unlike the brain, this organ is not spared during starvation; its structural and stored reserves are drawn upon in conditions of emergency. No appreciable difference was noticeable in the protein, RNA and DNA in the brain of starved and refed specimens. A powerful stress in the form of five months of starvation evidently failed to influence these macromolecules of the nervous tissue. However, increase in the levels of protein and RNA with a consequent decrease in DNA in the liver serve to emphasize the role of diet in determining the quantitative biochemical changes. Earlier work (Bulow, 1970; Buckley, 1979b; Smigielski, 1980), furnishing data on rise in both RNA and protein concentrations of fish tissues with increasing feeding rate, focusses on RNA as template and organizer for protein biosynthesis, leaving no doubt concerning decrease in potential maximum rate of protein synthesis and limitation of cellular growth as consequences of its loss from cells. These lend support to the

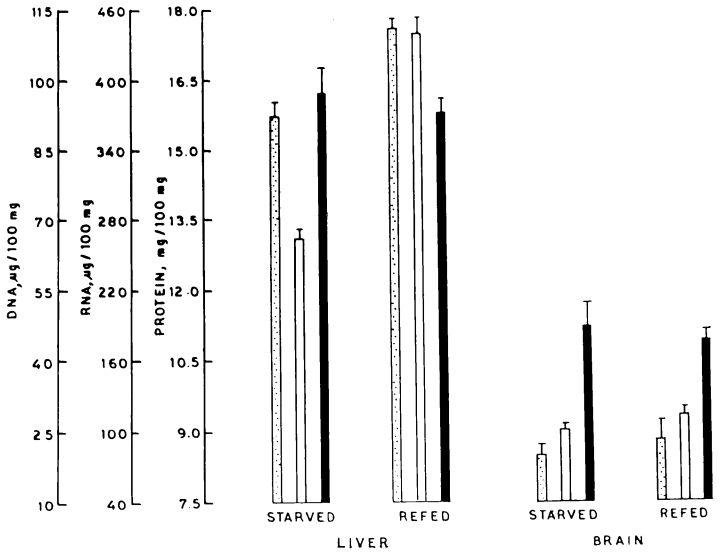

Fig. 1. Concentrations of protein (dotted bars), RNA (white bars) and DNA (black bars) in liver and brain of starved and starved-refed specimens of *Clarias bartachus* (vertical lines indicate standard error of mean of five observations).

present results. Starvation evidently causes decrease in protein and RNA. The energy harnessed from protein is used for the various maintenance requirements. Protein catabolism right from the commencement of starvation has also been seen to occur in larval plaice *Pleuronectes platessa* (Ehrlich, 1974a), herring *Clupea harengus* (Ehrlich, 1974b) and winter flounder *Pseudopleuronectes americanus* (Buckley, 1980). The fact that RNA content of cells is determined by protein content of diet (Brachet, 1955; Leslie, 1955; Mustafa and Jafri, 1977), the supply of protein-free diet or a complete cessation of feeding activity effecting a decrease of RNA is more than likely. Bouche et al. (1970), too, have reported the loss of protein and RNA from the liver of common carp *Cyprinus carpio* subjected to starvation. The work of Mustafa and Jafri (1977) on growth and feeding relations in protein and RNA turnover in *Channa punctatus* substantiates the present findings. Buckley (1979b) further elaborated that a decrease in total RNA content of starved fish liver is not due entirely to a net breakdown of messenger RNA, which forms only a small proportion of the total RNA, but other types of RNAs, including ribosomal RNA, are also called in during the biochemical constituents' breakdown frenzy in starving fish. Hayashi and Kaymievowski (1972) examined the liver of starved rats and noticed reductions in total ribosomal as well as non-ribosomal RNAs but no alteration in the DNA.

Unlike protein and RNA which are labile, the DNA maintains a remarkable stability. Its amount in individual cells is generally conserved during starvation of fish (Hayashi and Kaymievowski, 1972; Smigielski, 1980). The slightly higher amount of DNA/unit weight of tissue samples of the starved fish compared to starved-refed specimens is evidently the outcome of a larger number of cells contained in unit weight of the tissue sample of starving individuals. It is well established that when a fish is starved, the intracellular constituents like protein, fat, and carbohydrates are catabolized, the cytoplasmic volume reduced and the individual weights of cells decreased so that a larger number of cells could make up a given weight of tissue sample, hence giving the greater content of DNA/unit weight of tissue. Following refeeding a replenishment of lost cytoplasmic nutrients takes place, the volume of cytoplasm increases, cells gain weight and thus a relatively smaller number of cells can contribute to an equal weight of tissue sample, which in turn

manifests in decreasing the concentration of DNA in a unit weight of tissue sample. It is, therefore, obvious that the apparent increase in DNA concentration with starvation is the side effect of the depletion of cells rather than a consequence of the actual synthesis of this substance. Such a metabolic stability of DNA is indeed an adaptation on the part of the living matter to keep its biological heritage intact if it has to minimize the participation of the genetic material in wasteful equilibria involving the breaking down and rebuilding of so many cellular constituents (Hotchkiss, 1955).

From the foregoing discussion it is fairly certain that protein and RNA are labile whereas DNA is stable and is an index of cell number; change in protein-DNA ratio or RNA-DNA ratio must thus invariably imply changes in the amount of protein or RNA respectively in the cells. Individuals with high RNA-DNA ratios can be imagined to actively synthesize and accumulate protein and grow faster than the ones with low ratios and stunted growth. Buckley (1979b) expressed the view that though the RNA-DNA ratios reflect the past feeding levels of fish, these are, in fact, to some extent determined by the pre-sampling nutritional status itself, but it cannot be said with greater certainty whether the effect of nutritional success on growth rate is actually mediated or simply accompanied by a change in the RNA-DNA ratio. Published information available to date suggests higher sensitivity of liver RNA-DNA ratio to change in ration size and body weight compared to such ratios in other tissues of the body. In the present investigation on *Clarias batrachus* no appreciable change was found to occur in the RNA-DNA ratio in the brain where protein and nucleic acids remained almost unaltered by starvation or refeeding, when significant changes occurred in the liver.

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飢餓状態下の *Clarias batrachus* の肝と脳の蛋白，RNA と DNA 量

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ヒレナマズ *Clarias batrachus* の体重，肝と脳の重量，蛋白，RNA および DNA 濃度への栄養状態の影響を調べた．飢餓状態におくと体重と比肝重および肝の蛋白と RNA/DNA 比の減少を来し，再投餌するとこれらの値は増大した．肝と異なり，脳の重量とこれらの生化学的成分量は変動しなかった．これらの結果を詳細に述べ，生物学的に重要な関連事項について論じた．