

both the direct oxytocic effect and kininogenase activity of the urine. 5. Soyabean antitrypsin also inhibits at a high concentration (200–500 μg for 0.1 ml of urine) this kininogenase activity, but ovomucoid (Sigma Chemicals Co.) does not. 6. Pepstatin, a polypeptide which inhibits renin but not kallikrein activity, does not hinder kininogenase effects. The kallikrein activity of the urine produced during the perfusion period was 10–30-fold lower compared with the urine voided by the rat before the experiment. During the experiment, O_2 consumption and vascular resistance were not significantly changed, but a progressive decrease in kallikrein concentration was observed in the formed urine. On this question further studies will be required to establish whether some biochemical impairment or other factors are involved. The small amount of urine collected (not greater than 0.6 to 1.8 ml in the experimental period) limited the possibility of a chemical purification of the

enzyme. The results tend to support the assumption that kallikrein found in the normal urine is also produced by the kidney and is not an enzyme cleared from the circulating blood.

Résumé. L'urine produite par les reins isolés du rat perfusés à pression normale pendant 60–180 min avec des liquides oxygénés ayant en suspension des globules rouges contient une kininogénase qui a les mêmes propriétés que la kallikréine de l'urine produite en conditions physiologiques.

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Effect of Starvation on Blood Glucose and Nonprotein Nitrogen Levels of the Fish *Clarias batrachus*

Starvation effects the normal body metabolism and prolonged starvation may even cause death of the animal. A decline in various body constituents of fish, following experimental starvation, have been reported by various authors^{1–3}. Studies, following starvation, on the blood glucose and nonprotein nitrogen (NPN) levels, etc. have also been made^{4–10}. This paper deals with the results obtained for the fresh-water cat fish *Clarias batrachus*, following starvation up to 150 days.

Materials and methods. Normal, well fed *Clarias batrachus*, fully acclimatized to laboratory conditions were used. Right from the beginning of the experiment the starving fish were kept in separate aquaria and were not given any food for the entire period of starvation. Even aquatic plants were removed and nothing except pebbles and bed of river-sand was left in the aquaria. Water was changed twice a week throughout the experimentation period and this possibly removed even the naturally developing micro-fauna and flora and also accumulating toxic waste products of the fish. Control fish were kept in separate aquaria and were given minced goat liver, earthworms and snails on alternate days.

The experiment was started in the month of November and concluded in the month of April. The starvation was prolonged up to 150 days and observations were made on 1st, 10th, 30th, 90th and 150th day of starvation. On the first day only 5 fish of the control batch were examined. In subsequent periods, 5 fish of control group were also examined along with the starved fish, to counter the effects of seasonal variations, etc. Thus 25 fish of the control group and 36 starved fish were examined in this experiment.

For taking blood, the fish was carefully taken out of the aquarium with the help of a small hand net, immersed in a jar containing 1.5% paraldehyde solution. It took about 2 min to make a fish senseless. Immediately the fish was taken out, wiped dry with a turkish towel and put on a dissection tray. Its caudal vein exposed just behind the anal region and blood drawn in a syringe fitted with a 20 gauze needle. Exactly 1 ml blood was deproteinized using zinc hydroxide-barium-sulphate procedure (OSER¹¹). Blood glucose and NPN determinations were made following the Nelson-Somogyi and Folin-Wu methods, respectively (OSER¹¹). After the blood was drawn, gut contents and viscera of both the control and starved group of fish were examined. No mortality of these fishes occurred during the entire period of starvation.

¹ Y. CREACH and A. SERFATY, C. r. Seanc. Soc. Biol., Paris 159, 483 (1965).

² I. ROBERTSON, R. M. LOVE and W. P. COWIE, J. Sci. Fd. Agric. 18, 563 (1967).

³ Y. CREACH, in D. Sc. thesis presented at the University of Paul Sabatier, Toulouse.

⁴ A. KIERMEIR, Z. vergl. Physiol. 27, 460 (1939).

⁵ A. M. PHILLIPS, F. E. LOVELACE, D. R. BROCKWAY and G. C. BALZER, Fish. Res. Bull., N.Y. 16, 46 (1953).

⁶ A. E. I. AL-GAUHARI, Z. vergl. Physiol. 41, 26 (1958).

⁷ T. SANO, J. Tokyo Univ. Fish. 48, 99 (1962).

⁸ T. SANO, J. Tokyo Univ. Fish. 48, 105 (1962).

⁹ V. V. KOSMINA, J. Hydrobiol. 2, 74 (1966).

¹⁰ L. TASHMINA and G. F. CAHILL, Gen. comp. Endocr. 11, 262 (1968).

¹¹ B. L. OSER, *Hawk's Physiological Chemistry*, 14th edn. (McGraw-Hill Publ., New York 1965).

Effect of starvation on blood glucose and NPN levels of the fish *Clarias batrachus*

Status and No. of observations	Glucose and standard deviation (mg/100 ml)	NPN and standard deviation (mg/100 ml)
Control (25)	63.3 \pm 11.7	38.9 \pm 7.7
Post starvation		
10th day (8)	59.8 \pm 13.2	35.6 \pm 6.0
30th day (8)	53.5 \pm 11.7	31.1 \pm 7.7
90th day (10)	44.3 \pm 12.5	27.3 \pm 7.3
150th day (10)	32.0 \pm 9.1	20.5 \pm 4.8

Results and observations. The normal blood glucose level in the control fish was found to be 63.3 ± 11.7 mg/100 ml (Table). The level showed a fall as the starvation commenced. A direct correlation was found to exist between the blood glucose level and period of starvation, i.e. the level went on falling as the period of starvation was prolonged. Though the changes in blood glucose and NPN level (38.9 ± 7.7 mg/100 ml) in the control group fish were statistically not significant by the end of the 10th day of starvation ($P > 0.5$), nevertheless a fall in the two values was observed. This depletion of the 2 constituents of the blood became highly significant on the 30th day ($P < 0.01$) of starvation. The values of glucose and NPN levels in the blood of starving *Clarias batrachus*, on the 150th day, the last day of the experiment, were 32.07 ± 9.1 mg/100 ml and 20.5 ± 4.8 mg/100 ml respectively. It was calculated that a fall of 49.3% and 47.3% had occurred in the blood glucose and NPN levels respectively, by the end of the 150th day compared with the control values.

Discussion. A perusal of the results obtained (Table) clearly shows that marked depletion in the blood glucose and NPN levels occurred throughout the period of 149 days of starvation. AL-GAUHARI⁶ did not observe any change in blood glucose level of the fish *Clarias lazera*, even after 4, 5, 6 and 7 months of fasting period. However, HANNA¹² noted a fall of 60% in the glucose level of the same species after 7 months of starvation, although the value did not change during the first 4 months. PHILLIPS et al.⁵, on the contrary, observed a decrease in blood glucose level of the fish *Salvelinus fontinalis* in the first 3 days, after which the level was almost steady. Similarly the blood glucose level of *Gadus morhua* declined from 108 mg/100 ml to 72 mg/100 ml in the first 37 days but remained at this level after 51 days at 6.5°C ¹³. Surprisingly, the blood glucose level appeared unaffected, even after 3 weeks of starvation in *Myxine glutinosa*¹⁴. KIERMEIR⁴ observed that several species of fresh water teleosts, both active and sluggish, maintained their normal blood glucose levels during long periods of starvation. Interestingly, the active fish showed a very gradual decrease, while the sluggish ones exhibited a remarkable constancy.

BENTLEY and FOLLETT¹⁵ observed a definite fall in the blood glucose level of the lamprey after 5 months of starvation. SUNDARARAJ et al.¹⁶ noticed a definite and continuous fall in the blood glucose level of the Clupeoid fish *Notopterus notopterus*, but only after 48 h of starva-

tion, during which a hyperglycemic peak was found after 24 h, while HOCHCHKA and SINCLAIR¹⁷ did not notice any change in the blood glucose level of *Salmo gairdnerii*, even after 14 days of starvation, though its liver glycogen had fallen considerably.

Studies on blood NPN level are not many⁸, a slight rise in serum NPN level following 3–4 days of starvation was found in the eel *Anguilla japonica*. However, the present study shows that there was a parallel fall in blood glucose and NPN levels of the fish *Clarias batrachus* during starvation, the fall appeared to be very prominent as compared with the control values.

Conclusions. From the foregoing discussions it is evident that contradictory results have been obtained by various authors, on the blood glucose and NPN levels of fishes. It appears strange that many workers did not find any change in the blood glucose level of several species, even after quite long periods of starvation, since starved animal should ordinarily show little or more depletion in its blood glucose level. However, the present observations, like those of many workers, show that a definite fall in the blood glucose and NPN values, from the control levels of 63.3 ± 11.7 mg/100 ml and 38.9 ± 7.7 mg/100 ml to 32.0 ± 9.1 mg/100 ml and 20.5 ± 4.8 mg/100 ml respectively occurred by 150th day of starvation.

Zusammenfassung. Untersuchungen über das Verhalten des Blutzuckers nach längerem Nahrungsentzug bei Fischen.

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¹² M. Y. HANNA, Z. vergl. Physiol. 45, 315 (1962).

¹³ S. K. KAMRA, J. Fish. Res. Bd. Can. 23, 975 (1966).

¹⁴ S. FALKMER and A. J. MATTY, Gen. comp. Endocr. 6, 334 (1966).

¹⁵ J. BENTLEY and B. K. FOLLETT, Life Sci. 4, 2003 (1965).

¹⁶ B. I. SUNDARARAJ, M. KUMAR, P. V. NARSIMHAN, M. R. N. PRASAD, T. A. VENKITASUBRAMANIAN and J. MALETHY, Indian J. exp. Biol. 4, 1 (1966).

¹⁷ P. W. HOCHCHKA and A. C. SINCLAIR, J. Fish. Res. Bd. Can. 23, 975 (1962).

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The Ability of Cod (*Gadus morhua*) to Orient Towards a Sound Source

The acoustic perception of the direction of a sound wave (sound localization) in fish is theoretically problematic^{1–3}, but there is quite a lot of evidence that several sharks⁴ and some teleosts^{5–8} possess this sensory ability. However, for several reasons, either incomplete experimental designs or inadequate sound fields caused by improper boundary conditions, almost none of these experiments provided a really decisive proof for acoustic localization at several meters from the sound source, e.g. no specific alternative explanations have been investigated⁹.

The present experiments¹⁰ meet the requirements to answer the following questions: Is cod able to determine the direction of a sound? If so, is the labyrinthine system involved or the lateral-line system (hypothesis of VAN BERGEIJK¹)?

Under a raft in the middle of an almost circular fjord (local depth 35 m; 'diameter range' 300–400 m) a round netting cage, altitude 17 cm, was suspended at a depth of

¹ W. A. VAN BERGEIJK, in *Marine Bio-Acoustics* (Ed. W. N. TAVOLGA, Pergamon Press, New York 1964).

² E. SCHWARTZ, Fortschr. Zool. 21, 121 (1973).

³ A. N. POPPER and R. R. FAY, J. acoust. Soc. Am. 53, 1515 (1973).

⁴ D. R. NELSON, Diss. Univ. of Miami, USA (1965).

⁵ K. OLSEN, Working Group for fishing Technology, 8th IF meeting (Lowestoft, England 1969, mimeo), p. 10.

⁶ K. OLSEN, Coun. Meet. Int. Coun. Explor. Sea (1969; mimeo B20).

⁷ A. SCHUIJF, J. W. BARETTA and J. T. WILDSCHUT, Neth. J. Zool. 22, 81 (1972).

⁸ A. N. POPPER, M. SALMON and A. PARVULESCU, Animal Beh. 21, 86 (1973).

⁹ In experiments where *unconditioned* oriented responses to sound are studied NELSON⁴ (partially), OLSEN⁶ and POPPER et al.⁸; this is probably irrelevant.

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