

Fig. 1 — Chromatographic analyses of amylases of liver, stomach and intestine of *O. striatus* and of stomach of three species of *Mugil* [1, 2 and 3, chromatograms of liver, stomach and intestine extracts respectively of *O. striatus*. 4, 5 and 6, chromatograms of stomach extract respectively of *Mugil speigleri*, *M. dussumieri* and *M. cephalus*]

TABLE 1 — R_f VALUES OF DIFFERENT COMPONENTS OF AMYLASE IN MURREL AND MULLET

Species	Liver		Stomach		Intestine		
<i>O. striatus</i>	0.08	0.58	0.25	—	0.50	0.69	0.09
<i>M. speigleri</i>	0.31	0.77*	0.21	—	0.43	—	0.80*
<i>M. dussumieri</i>	0.22	0.33*	0.14	0.78	0.32	0.63	0.89*
<i>M. cephalus</i>	0.13	0.81*	0.21	—	0.10	—	0.84*

*Values reported earlier¹.

intestinal amylase of *O. striatus* are comparable to those of *M. dussumieri*. One of the fractions of liver has R_f values less than 0.2 in the case of *O. striatus* and this is comparable with that of *M. cephalus*. The other fractions of the liver of *O. striatus* are unique as none of the components of liver of the three species of mullets shows similar range of R_f value.

On the basis of different ranges of R_f values the amylase fractions of liver, stomach and intestine of all the four species can be grouped into four following types: A — with very low R_f values, below 0.2; B — with R_f values between 0.2 and 0.5; C — with R_f values ranging from 0.5 to 0.7; and D — with very high R_f values, above 0.7.

When the R_f values of all the components are classified as above, each extract shows one or more types as follows:

Species	Liver	Stomach	Intestine
<i>O. striatus</i>	A — C —	— B —	— B C D
<i>M. speigleri</i>	— B — D	— B —	— B — D
<i>M. dussumieri</i>	— B —	A — D	— B C D
<i>M. cephalus</i>	A — D	— B —	A — D

As seen above B and D types are common in general. On the other hand, A type is absent in all the tissues of *M. speigleri* and only in the liver of *O. striatus*. Comparatively C type is less common.

Mahalanabis and Roichaudhuri⁴ observed that the anterior stomach extracts of *O. punctatus* have two pH optima while those of posterior stomach have three. Ghanekar *et al.*⁵ observed α and β amylases

in the extracts of various organs of elasmobranchs. Similarly canine pancreatic amylase has been observed to show more than one component by electrophoretic studies⁶ or by the studies on saccharogenic and liquefying activities of the enzyme³.

The present study also indicates that the amylases of murrel and mullets have more than one component in general but it is not possible to conclude whether these components are A type amylase alone or whether they are of α and β types.

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References

1. SESHADRI, B. *J. Anim. Morph. Physiol.*, **4** (1957), 42.
2. GIRI, K. V. & PRASAD, A. L. N., *Nature, Lond.*, **167** (1951), 857.
3. WALDREN, M., *Am. J. Physiol.*, **168** (1952), 283.
4. MAHALANABIS, S. K. & ROICHAUDHURI, B. M., *Indian J. Physiol., all. Sci.*, **4** (1950), 145.
5. GHANEKAR, D. S., BAL, D. V. & KAMALA, S., *Proc. Indian Acad. Sci.*, **43B** (1956), 134.
6. KOMOROV, S. A. & GROSSBERG, A. L., *Fed. Proc.*, **10** (1951), 74.

Blood Sugar Regulation in the Crab, *Scylla serrata*: Effect of Injecting of Eye-Stalk Extract

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The effect of ablation of the eye-stalk on the blood sugar of *S. serrata* has been studied. There was an initial transient hyperglycemia followed by hypoglycemia. Crabs took 24 hr to develop hypoglycemia following eye-stalk ablation. Injection of extract from two eye-stalks into eye-stalk ablated crabs produced immediate hyperglycemia and the hyperglycemic peak was observed 2 to 4 hr after the injection. The rate of fall to normal level of blood sugar was very slow between 6 and 24 hr.

ALTHOUGH there is extensive literature on the hyperglycemic action of the eye-stalk hormone in crabs, the hypoglycemic principle is less investigated and its origin, nature and action are poorly understood¹. The blood sugar regulating mechanism in which both the hyper- and hypoglycemic principles are presumably involved is also not as well understood as in the vertebrates. Again, investigations pertaining to the effect of vertebrate hormones on the blood sugar of crabs and the effect of cytotoxic substances which are known to destroy the islet cells of vertebrates have been inadequately studied in crustaceans. The aim of the present work is to find out whether the eye-stalk contains any hyperglycemic factor, and if so, how far it is effective in maintaining the normal blood sugar level.

Scylla serrata in the intermoult stage, collected from Cochin backwaters, were kept starved in aquarium tanks for 5 days before the commencement of the experiment to prevent possibilities of a post-prandial

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rise in glycemia. The blood sugar was then estimated by the method of Folin and Wu as given by Hawk *et al.*². On trial this method was found to be very reliable as it gave an accuracy of about 97 per cent.

After estimating the normal blood sugar level, the eye-stalks were ablated in a number of crabs which were then divided into two sets. From one set blood was drawn for glucose assay at intervals of 1, 2, 4, 6 and 24 hr in order to get a picture of the rate of fall of glucose level, whereas the other set of crabs was kept undisturbed for 24 hr. Earlier experiments had indicated that maximum hypoglycemia occurs at about 24 hr after the removal of the eye-stalks and hence in the second set of crabs an interval of 24 hr after the eye-stalk ablation was considered a suitable duration for the estimation of blood sugar. Immediately after 24 hr, a saline extract of the two eye-stalks was prepared and injected into the body cavity. Blood was then drawn at intervals of 1, 2, 4, 6 and 24 hr for glucose assay.

The mean value of normal blood sugar of *S. serrata* was found to be 21.88 mg./100 ml. of blood and the range (2.2 × standard deviation) lying between 10.14-33.62 (n = 12). The term hypoglycemia or hyperglycemia is used whenever the values fell outside the normal range.

S. serrata, immediately after the removal of the eye-stalk, showed a transient hyperglycemia, the peak appearing in between 1 and 2 hr. The glycaemic value recorded in one crab 2 hr after eye-stalk ablation was as high as 90 mg./100 ml. of blood. Following this initial hyperglycemia, the blood sugar values rapidly fell and within 6 hr they became normal (Fig. 1). It is seen from Fig. 1 that maximum hypoglycemia occurred 24 hr after the removal of the eye-stalk and the mean blood sugar was 9.73 mg./

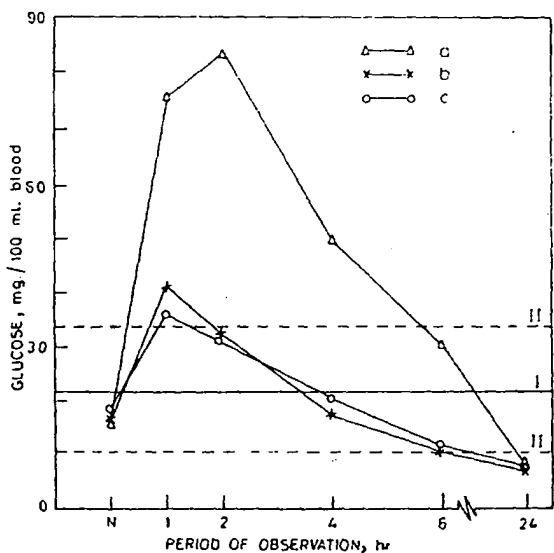


Fig. 1—Change in blood sugar concentration in *Scylla serrata* after removal of the eye-stalk [Curves a, b and c, blood glucose concentration in 3 individual crabs. N, normal blood glucose concentration. I, mean blood glucose concentration of normal crabs and II, range of blood glucose concentration, 2.2 × standard deviation]

100 ml. of blood. The rate of fall of blood sugar from 6 to 24 hr was very slow.

Twenty-four hours after the eye-stalk removal, injections of extract from two eye-stalks produced a quick hyperglycemia. The hyperglycemic peak was observed between 2 and 4 hr after the injections. The highest hyperglycemic value observed for one crab was 286.5 mg./100 ml. of blood and for another 56.5 mg./100 ml. of blood. Thereafter the blood sugar showed a gradual decline and took about 24 hr to come down to normal level (Fig. 2).

Although the change in the blood sugar towards hyperglycemic level becomes evident in all the experimental crabs, the degree of hyperglycemia differs from one individual to the other. Similarly, the injections of the eye-stalk extract, though give rise to hyperglycemia in all the crabs, the time of appearance of maximum hyperglycemia differs in different individuals. Generally a hyperglycemic peak is observed 2 to 4 hr after the injection (Fig. 2).

The results of the present study indicate that the eye-stalk of *S. serrata* has a hyperglycemic factor and that the removal of the eye-stalk results in hypoglycemia. Scheer and Scheer³ observed that the ablation of the eye-stalk of *Panulirus* resulted in hypoglycemia, while Abramowitz *et al.*⁴ and Kleinholz *et al.*⁵ did not observe hypoglycemia in *Libinia* and in *Callinectes* respectively. These observations led Knowles and Carlisle¹ to suggest that in crabs possibly some hyperglycemic agents other than those present

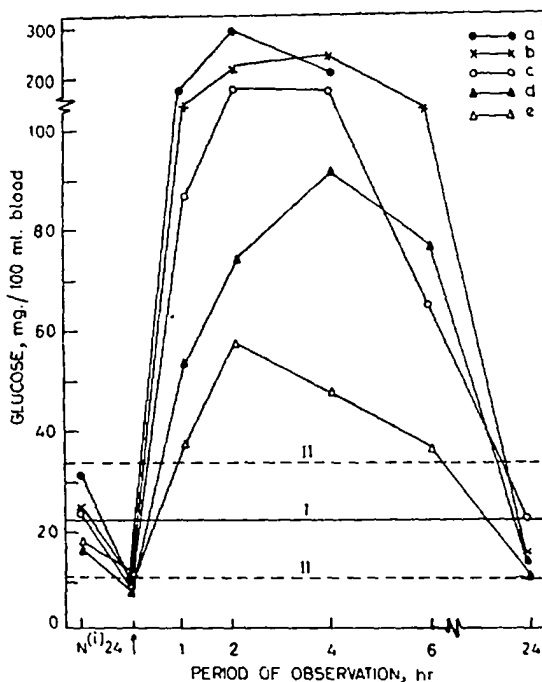


Fig. 2—Change in blood sugar concentration in eye-stalk ablated crab, *Scylla serrata*, after injecting extract of eye-stalks [Curves a, b, c, d and e, blood glucose concentration in 5 individual crabs; N, normal blood glucose concentration and (¹)24, blood glucose concentration 24 hr after eye-stalk ablation. I, mean blood glucose concentration of normal crabs and II, range of blood glucose concentration, 2.2 × standard deviation. Arrow (↑) indicates time of injecting the extract]

in the eye-stalks may be involved. The present investigation clearly suggest that in *S. serrata* the hyperglycemic factor lies nowhere else except the eye-stalk, for as has been noted earlier, the ablation of the eye-stalk readily produces hypoglycemia.

Following the eye-stalk ablation, the blood sugar value shoots up to a hyperglycemic level and in 24 hr it declines to a level of hypoglycemia. Scheer and Scheer³ observed that in *Panulirus* within the first hour of glucose injection nearly 80 to 90 per cent of it was removed from the blood and the rest disappeared more slowly and took about 6 hr more. The initial rise in glycemia following the eye-stalk ablation in *S. serrata* is presumed to be due to spontaneous release of hyperglycemic hormone. The hormone soon loses its potency which is shown by a decline in the blood sugar level.

The lowest hypoglycemic level reached in 24 hr after the eye-stalk ablation is not much below the lower limit of the normal range. This indicates that *S. serrata* tends to resist hypoglycemia. It is probable that the hypoglycemic principle in this crab is not as powerful as the eye-stalk hormone.

S. serrata shows high hyperglycemic values after the injection of the eye-stalk extracts. The degree of hyperglycemia is sometimes as high as 286.5 mg./100 ml. of blood. Abramowitz *et al.*⁴ have also observed that the injections of the eye-stalk extracts of *Callinectes* or *Uca* into *Callinectes* led to hyperglycemic conditions. Similarly injection of the eye-stalk extract from the same or other species provoked hyperglycemia both in intact *Panulirus* or in those lacking the eye-stalks or the sinus gland⁵. Contrary to this are the observations of Rangnekar *et al.*⁶ where the injections of the eye-stalk extracts into eye-stalk ablated *Paratelphusa* produced hypoglycemia, while the removal of the eye-stalk resulted in hyperglycemia. The conspicuous increase in glycemia after the eye-stalk extract injections positively indicates that in *S. serrata* the hyperglycemic factor is much more powerful than the hypoglycemic factor produced elsewhere. A detailed physiological investigation of the hypoglycemic principle might solve the problem as to how far it is important in maintaining the homeostasis of the blood sugar in normal crabs.

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References

1. KNOWLES, F. G. W. & CARLISLE, D. B., *Biol. Rev.*, **31** (1956), 396.
2. HAWK, P. B., OSER, B. L. & SUMMERSON, W. H., *Practical physiological chemistry* (McGraw-Hill Book Co. Inc., New York), 1954.
3. SCHEER, B. T. & SCHEER, M. A. R., *Physiol. Comp.*, **2** (1951), 198.
4. ABRAMOWITZ, A. A., HISAW, F. L. & PAPANDREA, D. N., *Biol. Bull. mar. biol. Lab., Woodshole*, **86** (1944), 1.
5. KLEINHOLZ, L. H., HAVEL, V. J. & REICHAERT, R., *Biol. Bull. mar. biol. Lab., Woodshole*, **99** (1950), 454.
6. RANGNEKAR, P. V., SARNIS, P. B. & NIRMAL, H. B., *J. Anim. Morph. Physiol.*, **8** (1961), 137.

Levels of Oxidative Enzyme Activity during Recovery of Fatigued Frog Muscle

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The activity of succinate, malate and lactate dehydrogenases and pyruvate oxidase has been studied in fatigued and recovering frog muscle. The activity of all the enzymes decreased on fatigue. During recovery of the muscle from fatigue, the enzymes showed increased activity reaching the activity of unfatigued muscle (control) after the 15th min. It is suggested that the decrease in activity of the enzyme during fatigue and increase in enzyme activity during recovery may be dependent on the change in the charge density during muscle activity.

IT is known that muscles undergo fatigue on repeated stimulation, losing the capacity to contract further but the substrates necessary for the energy supply are not broken down completely¹ suggesting that the onset of fatigue is not due to the depletion of the energy reserve. It is likely that certain other factors, such as the inactivation of the enzymes connected with muscle metabolism, are probably involved in the catabolic process. In the present study an attempt is made to investigate this aspect.

Adult frog (*Rana hexadactyla*) were double pithed, and the gastrocnemius muscles from both the legs were isolated with least injury. The muscles were washed 3-4 times in amphibian Ringer's medium² and then allowed to stand for 10 min. in Ringer's medium to recover from shock effects. One of the two muscles was used as the experimental and the other as the control muscle. The experimental muscle was placed in 10 ml. amphibian Ringer's medium and subjected to repeated stimuli of 6 V. d.c. at a pulse frequency of 100/min. using an electronic stimulator. The set-up was arranged in a glass chamber flushed with nitrogen to provide anaerobic environment. After the onset of fatigue, the experimental muscles removed from the medium were allowed to stand in air at room temperature for different periods of time, 0, 5, 10, 15 and 20 min., and chilled to 0°C. These were termed as muscles recovered at 0, 5, 10, 15 and 20 min. The control muscles were treated similarly but not stimulated.

The control and the experimental muscles were separately homogenized to 10 per cent (wt/vol.) in sodium-potassium phosphate buffer (0.1M; pH 7) at 0°C. using pestle and mortar. The activities of the enzymes, succinate dehydrogenase [EC 1.3.99.1, succinate: (acceptor) oxidoreductase], malate dehydrogenase (EC 1.1.1.37, L-malate: NAD oxidoreductase), lactate dehydrogenase (EC 1.1.1.27, L-lactate: NAD oxidoreductase) and pyruvate oxidase [EC 1.2.3.3, pyruvate: oxygen oxidoreductase (phosphorylating)], were measured adopting the method of Srikanthan and Krishnamurti³. The incubation mixture contained 1 ml. of 10 per cent (wt/vol.) homogenate, 1 ml. of sodium-potassium phosphate buffer (0.1M; pH 7), 0.5 ml. (0.1M) substrate (sodium