The histophysiology of the saccus vasculosus of *Notopterus chitala* (Teleostei)

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With one plate (fig. 2)

Summary

The apical protrusions of the coronet cells of the saccus vasculosus in *Notopterus chitala* contain glycogen which passes through the hairs into the globules. In spite of the availability of normal blood-glucose levels in fed controls, only a few cells in the loculi show glycogen; thus there are individual differences in the physiological activity of the cells. Specific variations in the distribution of glycogen in the different parts of the coronet cells were observed under conditions of starvation and glucose administration. Starvation for 48 h resulted in depletion of glycogen unidirectionally from the apical protrusion towards the globules. Fish starved for 46, 44, and 42 h were injected intraperitoneally 20% glucose (2 g of glucose per kg of body-weight) and autopsied 2, 4, and 6 h respectively after glucose administration. Comparison of the results obtained with starved fish injected with glucose suggests the gradual conversion of blood-glucose into glycogen. This conversion appears to begin at the base of the cell-body and to move towards the apical protrusion. The saccus vasculosus in *Notopterus chitala* is possibly a source of glucose for the cerebrospinal fluid.

Introduction

THE morphology and histochemistry of the saccus vasculosus in the Indian fish, *Notopterus chitala*, have been described by Sundararaj and Prasad (1963*b*). They have shown that the apical protrusions and the globules of the coronet cells contain glycogen. Further experiments were therefore designed to determine the effects of starvation and glucose administration on the origin and fate of glycogen in these cells. A preliminary report has appeared (Sundararaj and Prasad, 1963*a*).

Material and methods

Specimens of *N. chitala* (Ham.) were collected from the backwaters of the river Jamuna near Delhi in the early spring of 1962. The fish were acclimatized to laboratory conditions and divided into 5 groups. The first group of 3 fish, which served as fed controls, was kept in a glass aquarium containing tap-water and provided with *Gambusia* and *Hydrilla*, on which they normally feed. The second group of 3 fish was starved for 48 h by keeping them in a clean glass aquarium containing filtered tap water. The water in the aquarium was changed every 12 h to ensure the removal of faecal matter. Groups 3, 4, and 5 of 2 fish each were similarly starved for 46, 44, and 42 h respectively,

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at the end of which they were injected intraperitoneally with 20% glucose (2 g of glucose per kg of body-weight). The experiment was terminated at the end of 48 h and the fish were killed by decapitation. The saccus vasculosus was immediately exposed and fixed without delay in Helly's fluid. After a few minutes the saccus vasculosus along with the rest of the brain was carefully separated from the cranium and left in Helly's fluid for 18 h. Paraffin sections were cut at 5 μ and stained to demonstrate glycogen by the periodic

TABLE 1

Reaction of coronet cells to PAS test

Experiment	*PAS reaction in coronet cells*		
	cell-body	*apical protrusion*	*globule*
Group 1 (fed control)	+	+++	+
Group 2 (starved for 48 h)	+	+	+++
Group 3 (starved for 46 h and injected with glucose 2 h before autopsy) . . .	+++	+	+ or o
Group 4 (starved for 44 h and injected with glucose 4 h before autopsy) . . .	++	++	+ or o
Group 5 (starved for 42 h and injected with glucose 6 h before autopsy) . . .	+	+++	+

Key. +++ = strong reaction; ++ = moderate reaction; + = weak reaction; o = no reaction.

acid / Schiff (PAS) method of McManus (Pearse, 1961). The number of coronet cells showing a PAS-positive reaction in the apical protrusions was taken as the index of glycogen storage in the coronet cells. A count of cells actively storing glycogen was made in every 10th section and expressed as the percentage of the total number of cells counted.

Observations

The saccus vasculosus of *N. chitala* is situated on the ventral side of the brain, just behind the pituitary gland. It is made up of a number of loculi surrounded by blood sinusoids. The loculi are lined by a single layer of pear-shaped coronet cells, each possessing an apical protrusion fringed with a number of fine hairs; each hair ends in a globule (Sundararaj and Prasad, 1963*b*).

The results of experiments are summarized in table 1 and in fig. 1.

The fed controls (group 1) showed the characteristic granular glycogen deposits in the apical protrusions, but only occasionally in the globules (fig. 2, A). The cell-body showed a faint PAS-positive reaction. The term 'PAS-positive reaction' used in this paper refers specifically to glycogen (Sundararaj and Prasad, 1963*b*). In spite of the availability of normal blood-glucose levels, not all the coronet cells in a loculus show glycogen in the apical protrusions.

This indicates that there are individual differences in the physiological activity of the cells.

In group 2 (starved for 48 h) glycogen was depleted from the apical protrusions of most of the coronet cells, but it was clearly demonstrable in the globules (fig. 2, B). The cell-body showed only a faint PAS-positive reaction.

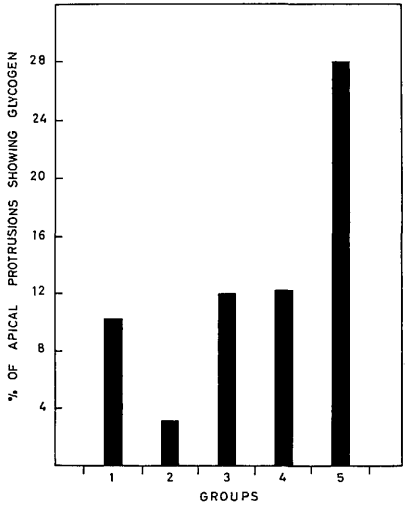

FIG. 1. Diagram showing what percentage of apical protrusions contain glycogen in the 5 groups of fishes described in the text. The diagram is based on the following figures:

	No. of apical protrusions examined	*No. of apical protrusions containing glycogen*
Group 1 (fed controls)	13,835	1,416
Group 2 (starved 48 h)	14,817	457
Group 3 (starved 46 h, killed 2 h after glucose injection)	15,578	1,867
Group 4 (starved 44 h, killed 4 h after glucose injection)	9,261	1,285
Group 5 (starved 42 h, killed 6 h after glucose injection)	9,205	1,841

In group 3 (starved for 46 h and killed 2 h after glucose injection) the cell-body showed a generalized PAS-positive reaction, with a clear gradient of staining intensity increasing from the base of the cell towards the apical protrusion (fig. 2, C). Characteristic glycogen granules were seen in the apical protrusions (fig. 2, C), while most of the globules were free from glycogen.

In group 4 (starved for 44 h and killed 4 h after glucose injection) a large number of cells showed granular glycogen deposits in the apical protrusions

while the PAS-positive reaction in the cell-body was not as intense as that in group 3.

In group 5 (starved for 42 h and killed 6 h after glucose injection) glycogen was noticed in a larger number of apical protrusions than in groups 3 and 4 (fig. 2, D). Continued transformation of blood-glucose does not appear to occur in coronet cells of glucose-injected fish whose apical protrusions are loaded with glycogen granules. This is indicated by the absence of a generalized PAS-positive reaction in the cell-body of such cells.

Discussion

The data indicate that starvation resulted in the depletion of glycogen from all parts of the coronet cells except the globules. This suggests that the glycogen in the apical protrusions passes into the globules along the hairs, and that it is stored in the globules before utilization. In making this suggestion we are envisaging a transport of blood-glucose only towards the apical protrusion and globules, and not in the opposite direction.

A comparison of the results with groups 3, 4, and 5 demonstrates clearly the sequence in time of the transformation of blood-glucose into the glycogen granules of the apical protrusions. Within 2 to 6 h after the injection of glucose, glycogen is presumably formed and polymerized into a granular form of high molecular weight as it moves through the cell towards the apical protrusion. The intensity of the PAS-reaction may be taken as an indication of the degree of polymerization of the glycogen. The forces responsible for the movement of glycogen from the base of the cell-body towards the apical protrusion are, however, not clear.

These observations have a bearing on the function of the saccus vasculosus. It has been suggested that the coronet cells are sensory and are used by the fish for estimating changes in oxygen pressure (Dammerman, 1910). They have also been regarded as secretory cells (Bargmann, 1954; Bargmann and Knoop, 1955, 1961; Kamer and others, 1960; Stahl and Seite, 1960; Jansen and Kamer, 1961; Zwillenberg, 1961). Kamer and his colleagues have demonstrated glycogen in the apical protrusions of the coronet cells of the rainbow trout, *Salmo irideus*, and suggested that the glycogen stored in the apical protrusions passes into the globules where it is 'used up in the formation of an acid mucopolysaccharide, which was first stored in the globules and ultimately secreted into the ventricle'. Jansen and Kamer (1961), however, believe that the synthesis of the acid mucopolysaccharide is initiated in the

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FIG. 2 (plate). Sagittal sections of the saccus vasculosus.

A, group 1 (fed control). Note the intense PAS reaction in the apical protrusions of some of the coronet cells. Helly; PAS/haematoxylin.

B, group 2 (starved 48 h). Note the presence of glycogen in the globules (arrows): it has been partly depleted from the apical protrusions. Helly, PAS.

C, group 3 (starved 46 h, killed 2 h after glucose injection). Note the gradient in intensity of staining from the base of the cell towards the apical protrusion. Helly, PAS.

D, Group 5 (starved 42 h, killed 6 h after glucose injection). Most of the apical protrusions are loaded with glycogen. Helly; PAS/haematoxylin.

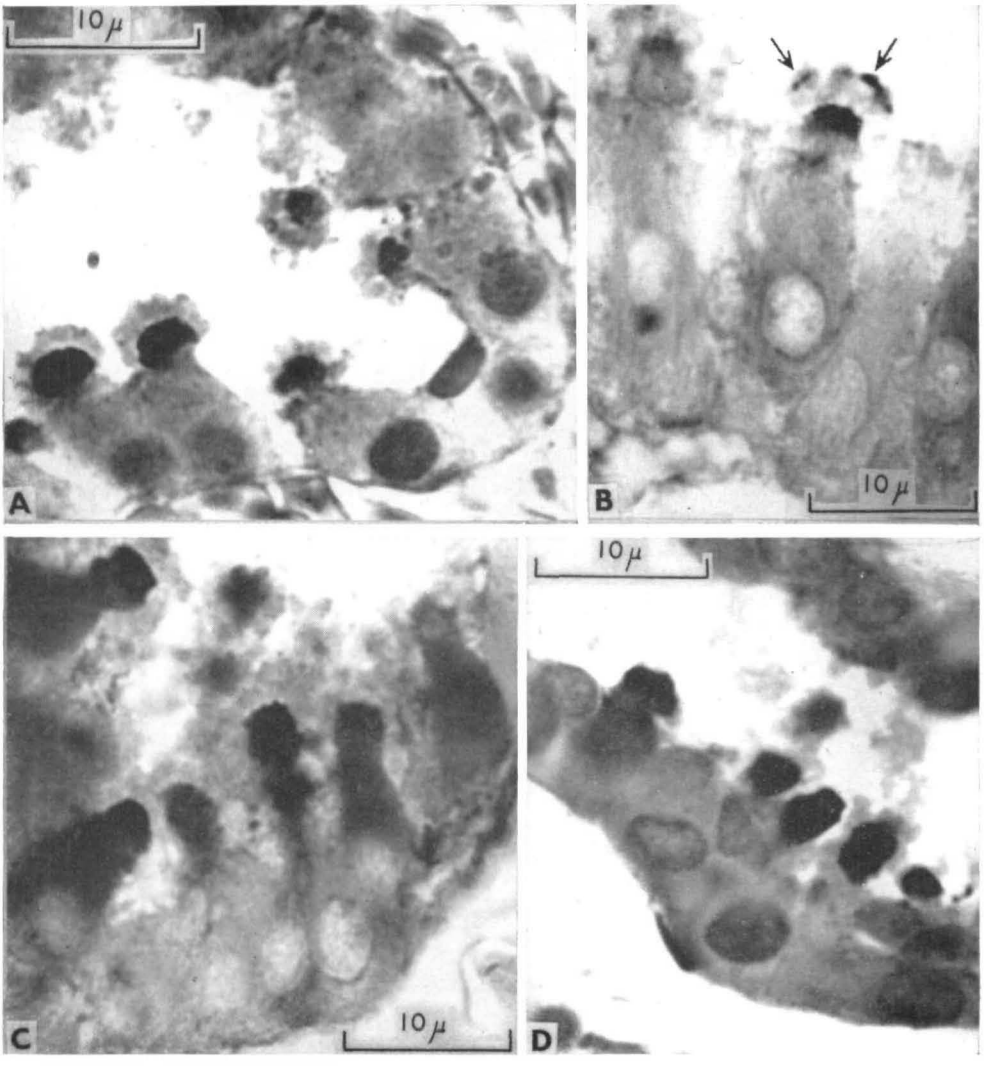

FIG. 2

B. I. SUNDARARAJ and M. R. N. PRASAD

cell-body and the formed product is transported to the basal corpuscles; the material moves to the globules along the hairs; and is stored in the globules before it is secreted into the cavity of the organ. While supporting the secretory hypothesis, we differ from the views of Kamer and his colleagues (1960), Stahl and Seite (1960), Jansen and Kamer (1961), and Zwillenberg (1961) in regard to the product formed in the cell-body and released from the globules into the lumen of the saccus vasculosus.

On the basis of our observations on the histophysiology of the saccus vasculosus in *N. chitala*, we conclude that the apical protrusions contain glycogen. Glycogen in the apical protrusions reaches the globules by passage along the hairs, and is presumably released in the form of glucose into the cerebro-spinal fluid.

Examination of the serial sections of the brain of *N. chitala* indicates that the choroid plexus is poorly developed, while the saccus vasculosus is comparatively large. Further work is in progress to determine the correlation, if any, between the degree of development of the saccus vasculosus and of the choroid plexus in a number of teleostean fishes.

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References

BARGMANN, W., 1954. Z. Zellforsch., **40**, 49.
—— and KNOOP, A., 1955. Ibid., **43**, 184.
—— 1961. Ibid., **55**, 577.
DAMMERMAN, K. W., 1910. Z. wiss. Zool., **96**, 654.
JANSEN, W. F., and KAMER, J. C. VAN DE, 1961. Z. Zellforsch., **55**, 370.
KAMER, J. C. VAN DE, BODDINGIUS, J., and BOENDER, J., 1960. Ibid., **52**, 494.
PEARSE, A. G. E., 1961. *Histochemistry, theoretical and applied*, Boston (Little, Brown).
STAHL, A., and SEITE, R., 1960. C.R. Soc. Biol. Paris, **154**, 1020.
SUNDARARAJ, B. I., and PRASAD, M. R. N., 1963*a*. Naturwiss., **50**, 362.
—— 1963*b*. Quart. J. micr. Sci. (in press).
ZWILLENBERG, L. O., 1961. Z. Zellforsch., **54**, 437.