CHAPTER 3

SEASONAL VARIATIONS IN THE METABOLITES OF THE HEPATOPANCREAS AND IN THE BLOOD GLUCOSE

In vertebrates, liver is generally believed to be the main centre for the production and distribution of intermediary metabolites (Popper and Schaffner, 1957). However, very little is known regarding the part played by the corresponding organ called the liver or hepatopancreas in the metabolism of the decapod crustaceans. George and George (1944), noted the general occurrence of fat in the hepatopancreas of certain crustaceans and also recorded some of the properties of the fat obtained from the liver of the crab Paratelphusa guerini. George and Patel (1956) studied the seasonal variations in the fat content of the liver and gonads in a marine and a freshwater decapod crustacean. The investigations of many workers (Meyerhof and Lohman, 1928; Lohman, 1935; Ball and Meyerhof, 1940; Renaud, 1949; Nayar and Parameswaran, 1955) lead to the general conclusion that in addition to lipids crustacean metabolism centres around glycogen also. That protein is utilized as the primary source of energy in starved Hemigrapsus nudus was shown by Neiland and Scheer (1953). The hepatopancreas which stores fat, glycogen and protein which are the three main metabolites involved in the intermediary metabolism should play a central role in the various metabolic processes. Paratelphusa

jacquemontii, used in the present study undergo aestivation during the dry months of the year. Certain changes are expected to occur in the organic reserves of the hepato-pancreas before and after aestivation. It is well known that many animals store large quantities of fat for utilization during hibernation and aestivation. Therefore a study of the levels of fat, water, protein and glycogen of the hepato-pancreas and the level of glucose in the blood of Paratelphusa jacquemontii, during the various seasons was undertaken to throw some light on the general metabolism of this animal..

MATERIALS AND METHODS

The common freshwater crabs Paratelphusa jacquemontii, were collected from the local rivers and animals from both the sexes were utilized for the study. The whole hepato-pancreas was dissected out, weighed and pieces were cut separately for histochemical observations and quantitative estimations. For the histochemical observations on the localization of fat, the tissue was fixed in Calcium formol and embedded in gelatine. Frozen sections of 15μ thickness were cut and stained with Sudan Black B (Baker, 1946), Nile blue sulphate (Cain, 1947), and Fettrot 7B (Pearse, 1960). Sections treated with 2:1 chloroform:methanol mixture were used as controls. For the quantitative estimations of fat content the tissue was weighed and then dried at 80°C in an air oven till constant weight was obtained and stored in

a vacuum dessicator, weighed again and subjected to Soxhlet extraction with 1:1 alcohol:ether mixture. The extracted fat was dried, weighed and the percentage of fat was calculated on dry weight basis. The percentage of water was also calculated from the same sample.

Protein content was determined by the micro-Kjeldahl steam distillation method (Hawk et al., 1954). Dried fat free tissue was used for the protein estimation.

Glycogen was estimated in the fresh tissue by anthrone method (Seifter et al., 1950).

For the estimation of glucose blood was withdrawn by means of a tuberculin syringe and a hypodermic needle through a small pinhole made in the carapace with a dissecting needle just above the pericardial cavity. The blood was transferred to a tube and 0.1 ml was used for the determination of blood sugar by the micro method of Folin and Malmros (1929).

RESULTS

Table 1 shows the results of the quantitative estimations. In the month of May specimens were not available in sufficient numbers to carry out the experiments. It was evident from the data that the chief energy reserve in the hepatopancreas was fat. A remarkable increas in the fat store in the hepatopancreas was noted towards summer. At the same time a decrease

was noted in the water, protein and glycogen contents in the same tissue. A considerable increase in the blood glucose, i.e. from 69 to 105 mg/100 ml, was also observed during this period. Soon after the aestivation in summer increased protein and water contents in the hepatopancreas and a high level of blood glucose were noted. Fat content was moderately low (17%) during this period. But there was a slight increase in the glycogen content. A sudden temporary increase in the fat store together with a decrease in the water content was seen just before the starting of the gonadal development in August, However, an increased glycogen content in the hepatopancreas and a decrease in the blood sugar level were noted towards the late gonadal development. During this period the fat content in the hepatopancreas decreased against a substantial increase in the water content. Again, towards summer a gradual decrease in the glycogen and an increase in fat were seen. As the fat content increased the water level decreased considerably.

In July immediately after the aestivation period appreciable amount of fat was seen in the storage cells of the tubules in the hepatopancreas and to a smaller extent in the intertubular connective tissue (Fig. 1). Neutral fat was completely absent from the tissue during this period. Prior to the starting of spermatogenesis in August suddenly neutral fat globules started appearing in the storage cells

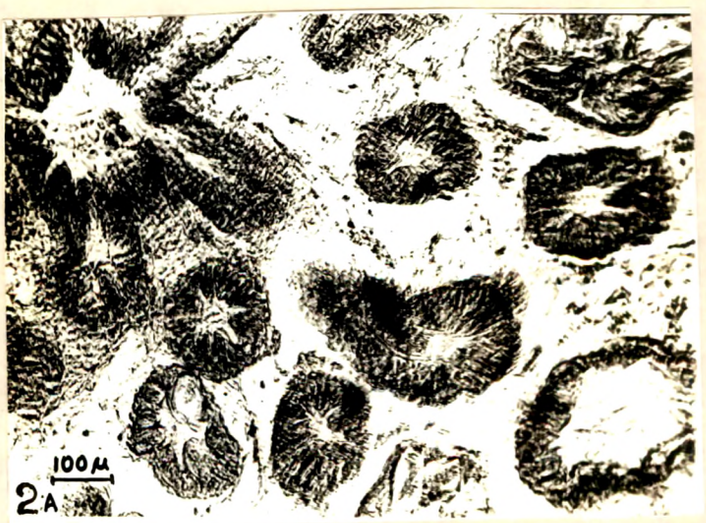
TABLE I

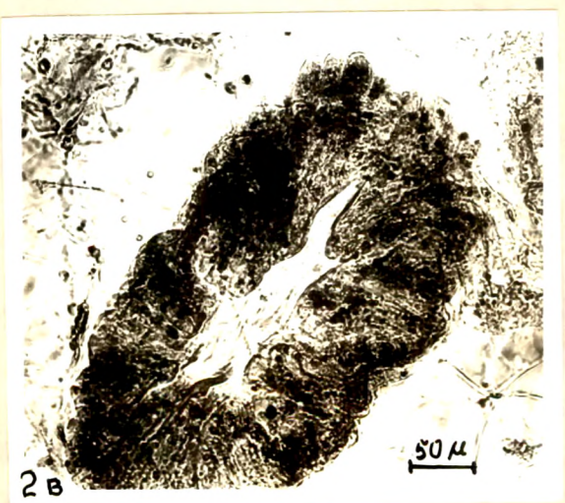
ations of blood glucose and protein, fat, glycogen and water contents of hepatopancreas of *Paratelphusa jacquemontii*

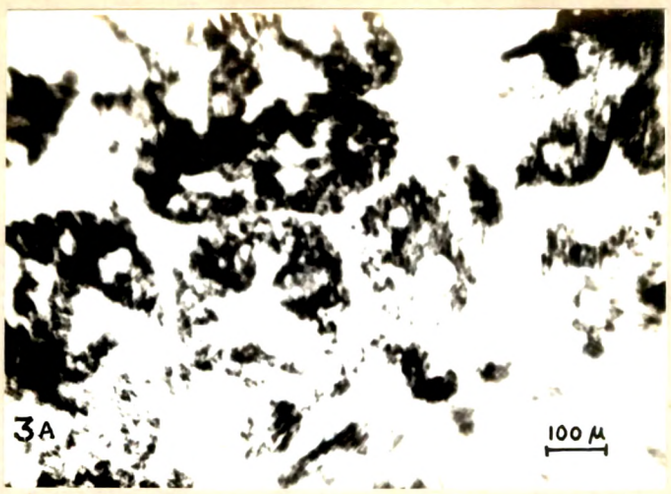
Body weight in grams	Protein % dry wt. (fat free)	Water % wet wt.	Fat % dry wt.	Glycogen % wet wt.	Blood glucose mg/100 ml.
44.00(6)[1] (42.50-46.00)[2]		61.273(4)[1] (60.252-63.503)[2]	19.764(4)[1] (18.545-20.984)[2]	0.328(4)[1] (0.223-0.583)[2]	108.242(3)[1] (99.282-112.852)[2]
32.50(6) (30.00-34.50)		63.453(4) (61.223-65.135)	18.754(4) (16.913-20.995)	0.308(4) (0.258-0.579)	107.533(3) (101.182-112.183)
45.00(7) (43.50-47.00)	18.292(5) (17.187-20.307)	62.253(6) (60.187-63.555)	17.585(6) (16.188-19.733)	1.972(5) (1.522-2.598)	152.674(5) (149.182-160.752)
33.50(7) (31.00-35.00)	16.288(5) (14.253-18.628)	63.311(6) (61.917-65.214)	17.991(6) (15.183-19.884)	2.412(5) (2.015-3.288)	116.273(5) (112.164-122.002)
52.30(6) (50.00-54.50)	19.947(6) (18.723-21.733)	42.112(5) (40.128-44.001)	36.441(5) (33.833-38.123)	1.773(5) (1.229-2.833)	73.711(4) (68.125- 78.813)
38.25(6) (37.00-40.75)	16.106(6) (15.642-17.184)	48.781(5) (47.125-50.183)	33.613(5) (31.166-36.822)	1.524(5) (1.013-1.997)	72.165(4) (70.064- 74.183)
52.60(6) (50.75-54.00)	17.152(6) (15.942-19.252)	38.229(7) (36.223-39.919)	41.067(7) (40.112-43.204)	3.552(7) (3.001-4.522)	66.702(4) (64.007- 70.344)
48.75(6) (46.00.50.25)	13.967(5) (11.945-15.715)	40.015(7) (37.939-42.112)	43.065(7) (42.182-45.882)	2.953(7) (2.223-3.883)	67.013(4) (66.197- 70.593)

TABLE I (Contd.)

Sex	Body weight in grams	Protein % dry wt. (fat free)	Water % wet wt.	Fat % dry wt.	Glycogen % wet wt.	Blood glucose mg/100 ml.
Male	45.00(8)[1] (43.50-46.50)[2]	16.065(6)[1] (14.205-18.013)[2]	47.578(6)[1] (45.913-48.912)[2]	35.807(6)[1] (33.652-37.118)[2]	0.552(6)[1] (0.283-0.812)[2]	82.744(5)[1] (78.181- 84.124)[2]
Female	46.20(8) (44.00-48.50)	13.432(6) (12.216-15.539)	58.573(6) (56.156-60.183)	27.321(6) (26.113-29.358)	0.674(6) (0.413-0.908)	72.862(5) (68.228- 76.142)
Male	48.50(6) (46.00-49.50)	15.752(6) (13.272-17.488)	38.275(6) (35.227-40.182)	44.838(6) (42.338-47.118)	1.135(5) (0.917-1.543)	94.329(5) (91.183-97.117)
Female	40.50(6) (39.00-42.00)	13.207(6) (11.182-15.117)	37.789(6) (35.117-39.339)	47.604(6) (45.224-49.828)	1.422(5) (1.012-1.934)	86.028(5) (84.208- 89.123)
Male	50.70(6) (48.50-52.00)	15.514(6) (13.122-16.918)	32.713(5) (30.234-34.919)	50.947(5) (47.917-52.184)	0.826(5) (0.399-1.332)	92.783(5) (90.113- 94.882)
Female	44.50(6) (42.00-46.00)	12.744(6) (10.144-13.989)	33.922(5) (30.912-36.002)	52.467(5) (50.114-55.015)	0.867(5) (0.572-1.048)	87.039(5) (82.924- 90.284)
Male	58.00(5) (56.00-60.50)	13.352(5) (10.934-15.535)	30.661(4) (27.933-33.671)	55.072(4) (52.912-58.184)	0.915(4) (0.625-1.639)	69.587(4) (65.854- 72.586)



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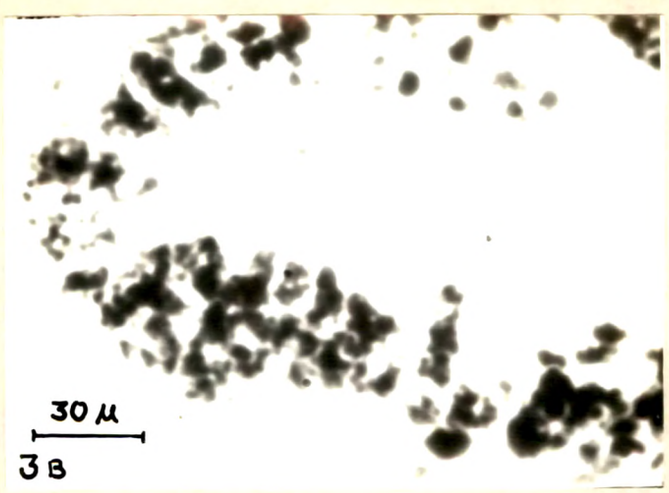
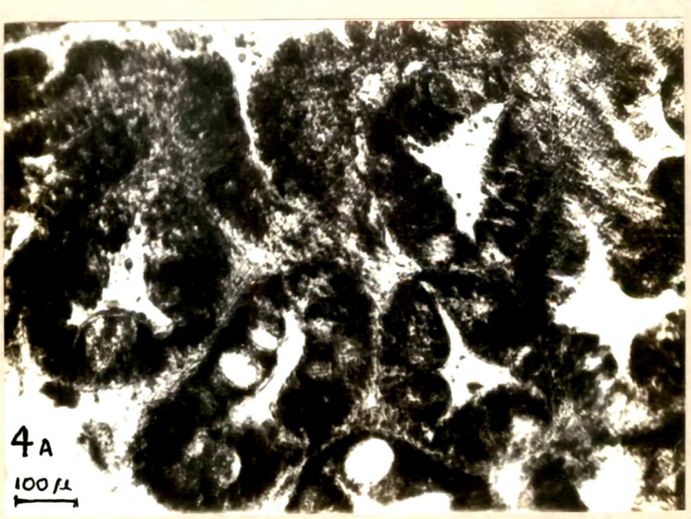
100 M


30 M
3B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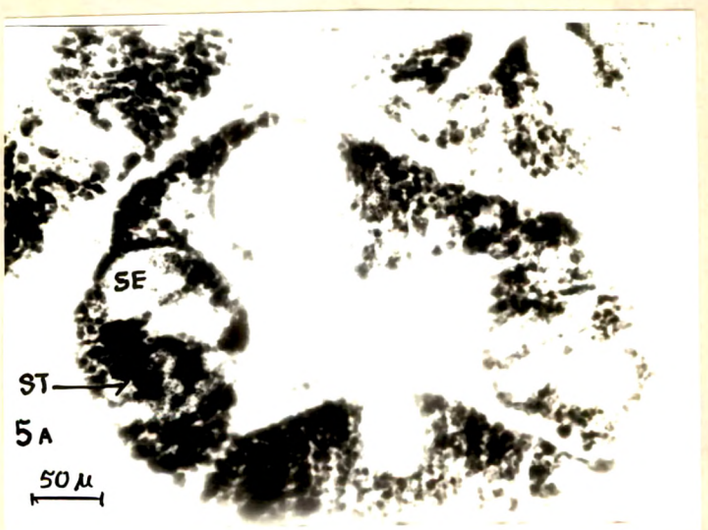
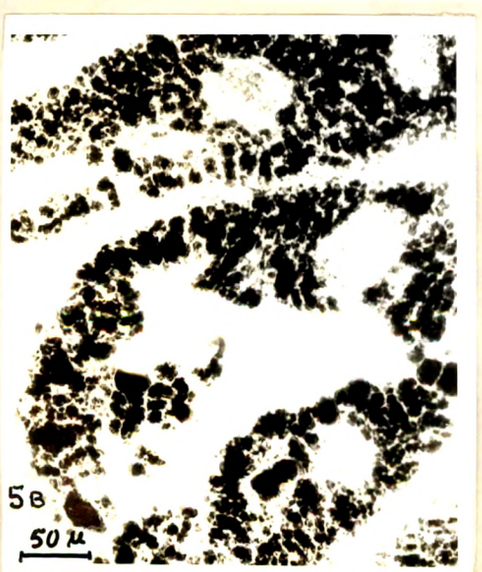
100 M


4B
30 μ


SE
ST
5A
50 μ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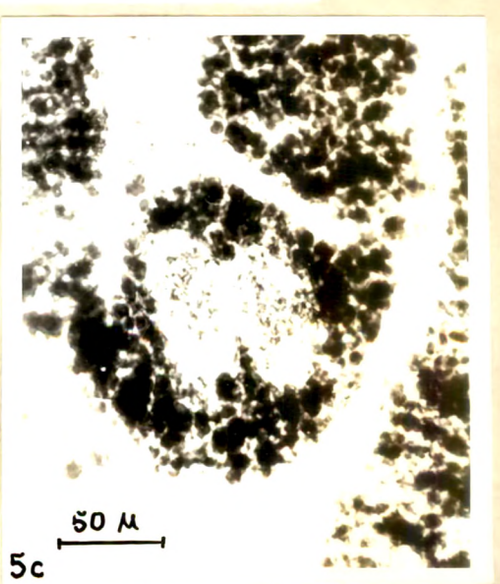
50 μ
5C

EXPLANATION TO THE FIGURES

Fig. 1. T. S. of hepatopancreas (July) stained with Sudan Black B showing the presence of the acidic lipids.

Fig. 2A. T. S. of hepatopancreas (prior to spermatogenesis) stained with Fettrot 7B showing neutral fat in the tubules.

Fig. 2B. One single tubule of Fig. 2A magnified.

Fig. 3A. T. S. of hepatopancreas (late spermatogenesis) stained with Sudan Black B showing decreased amount of fat.

Fig. 3B. Part of Fig. 3A magnified.

Fig. 4A. T. S. of hepatopancreas (towards summer - March/April) stained with Fettrot 7B showing increased neutral fat in the tubules.

Fig. 4B. Part of Fig. 4A magnified showing neutral fat containing tubule.

Fig.5A&B. T.S. of hepatopancreas stained with Nile blue sulphate showing the presence of more secretory cells in the tubules. Note the high neutral fat content if the narrow storage cells. SE - Secretory cells. ST - storage cells.

Fig. 5C. T.S. of hepatopancreas stained with Nile blue sulphate showing acidic lipids in the secretory cells and neutral fat in the storage cells.

of the tubules and gradually the tubules were filled with neutral fat droplets (Fig. 2). But the intertubular connective tissue showed negative reaction to the neutral fat stain. Towards the late spermatogenesis a remarkable decrease in the tubular fat and connective tissue fat was seen (Fig. 3). The same was the case with female hepatopancreas also. Towards the maturation of ova a total decrease in the fat content of the hepatopancreas was noted. Again an increase in the fat content was noted towards summer. Neutral fat globules accumulated in the narrow cells of the tubules (Fig 4). Connective tissue also showed the presence of large amount of acidic fat. At this stage the digestive tubules contained more secretory cells when compared with those of the previous months. The material inside these large vacuolated secretory cells stained blue with Nile blue sulphate which was not seen in the control sections. The elongated narrow storage cells stained pink with Nile blue sulphate showing the presence of acidic lipid in the secretory cells and neutral fat in the storage cells (Fig. 5). In the secondary secretion duct also large number of acidic fat droplets were seen near the basement membrane of the epithelial cells.

DISCUSSION

Both quantitative and histochemical studies showed that the increase of the fat content in the hepatopancreas

takes place in two seasons; January to April and August to September. These animals aestivate from the end of April to the end of June. That the animal utilizes stored fat during the dormant state is indicated by the remarkable reduction of fat soon after aestivation. The tubules of the hepatopancreas were filled with neutral fat before aestivation, whereas after aestivation it was seen to be replaced with acidic fat. During the aestivation time the animals stop feeding and the various metabolic requirements are met through the oxidation stored lipids. Again, July to September is an active feeding period, and is accompanied by the storage of fat in the hepatopancreas. This stored fat does not remain in the hepatopancreas, but gets depleted during the period from October to November. It is during this period that the gonads develop and both sperms and ova become fully mature. The storage of fat in certain animals for utilization in the production of eggs and sperms is well known. It therefore appears that stored fat in the hepatopancreas might be used for the formation of sperms and eggs. Prior to the period of aestivation and gonadal development the water content decreased considerably, making possible the storage of fat in the cells of the tubules.

Renaud (1949), reported that during the moult cycle of Cancer, fat appears as droplets within the storage cells of the hepatopancreas at the C4 stage of the moulting , and

is demonstrable also in the secretory cells during D1 and D2 stages. Since Cancer is no longer feeding during the later stage (Drach, 1939) the presence of lipid in the secretory cells may show that they are the sites of fatty acid utilization (Passano, 1960). In the present investigation also appreciable amount of acidic fat staining blue with Nile blue sulphate was seen in the numerous secretory cells of the digestive tubules. Further the presence of numerous fat droplets in the secondary secretion duct indicates the possibility of fatty acids being utilized by the secretory cells and transported through the secondary secretion duct to the other organs during the non-feeding time.

Passano (1960) suggested that much of the lipid reserve is converted into glycogen and then to glucose for utilization in chitin synthesis. Renaud (1949) reported that the hepatopancreas carbohydrate consists primarily of glycogen resulting from the proecdysis mobilization of the lipid reserves. Pathways of carbohydrate metabolism in lobster hepatopancreas was studied by Hochachka et al.(1962). But even though the role of glycogen in decapod intermediary metabolism is not yet clearly understood, it is unquestionably an intermediate in chitin formation(Renaud, 1949; Verne, 1924). Tissue glycogen is converted to glucose which in the course of chitin formation is converted to glucosamine and acetyl glucosame. The latter is then polymerized to form chitin (Passano, 1960). Glucose derived from the tissue glycogen is transported through the circulating blood to the

sites of chitin formation. In addition to this Hu (1958) reported that glucose is oxidized by the crab Hemigrapsus nudus. In the present investigation a slight increase in the blood sugar was noted during the aestivation period which was followed by moulting. The blood sugar value was still higher immediately after aestivation or during the breeding season. Thus the blood sugar functions as a transport substance between the glycogen content of the hepatopancreas and other tissues to the sites of chitin formation and it also serves as a substrate for the metabolism, thereby supplying energy to the animal during the breeding season.

The increase in the glycogen levels is of special interest, since it has been suggested that the increased storage of glycogen may lead to its conversion to fat (Naik, 1963). In the present study an increase in the glycogen content of the hepatopancreas was noted during the early stage of gonadal development, with a slight decrease in the fat levels. This suggests that some fat may be utilized for the production of the reproductive elements. The glycogen may be converted to fat later as fat is used up.

Although the knowledge on protein storage and utilization is meagre, protein seems to be an essential component of the organic reserves as it is accumulated in the hepatopancreas. Neiland and Scheer (1953) reported that Hemigrapsus nudus utilized protein in preference to either lipids or carbohydrates

during a starvation period of 23 days. But the methods used in their investigation have been questioned (Vonk,1960) and it was proved that fatty acids are the most likely substrates utilized during starvation in these animals. However, in the present study an increased protein content was observed in July soon after the aestivation period, when the fat content was very low. Thereafter towards summer the fat content increased with a remarkable fall in protein and water levels. This is striking because it is generally stated that increased fat level is associated with a decrease in water content making no reference to the level of protein. Naik (1963), had suggested two possibilities for the reduction in protein in the liver of the migratory starling, Sturnus roseus - conversion of some proteins to fat, a decline in the protein synthesis due to the increased fat synthesis. Therefore the decreased protein levels in the hepatopancreas could be explained on the basis of these suggestions.

It should be emphasized that the metabolism of these animals centres mainly around the hepatopancreas. This organ could be compared, in some respects to the vertebrate liver, and to the vertebrate pancreas with respect to its enzyme secreting function. Moreover, it plays a prominent role in the primary food absorption carried out in other invertebrates by the small intestine (Vonk, 1964).