XLI. THE RELATIONSHIP OF LECITHIN TO THE GROWTH CYCLE IN CRUSTACEA.

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THE period leading up to moulting in Crustacea is characterised by certain metabolic and anatomical changes, some of which we have dealt with in previous papers [Paul, 1914; Paul and Sharpe, 1916]. Briefly, these are as follows. There is a marked increase of fat, glycogen, and calcium in the liver, the last reaching as much as 20 % of the dried weight of the organ. At the same time mitosis occurs rapidly in the cells of the integument and other tissues of the body, but no increase in the size of the animal takes place till the rigid shell is cast and rapid absorption of water makes the animal increase in size.

When the moult is complete, an exodus of stored material takes place from the liver; the fat is broken down to glycerol and lower fatty acids before it enters the blood, and calcium is carried off as soaps of lower fatty acids to be deposited in the integument as calcium carbonate. Cell proliferation in the body then ceases, but increase in the size of the cells is marked.

After a period of rest the crab again stores material in the liver in preparation for the next moult. It has been found that as the period of moulting approaches the liver increases in weight relatively to the body. We have therefore employed the term *hepatic factor* to denote this relationship, and have applied it to the figure obtained by dividing body weight by weight of fresh liver. Shortly after moulting this factor may be 10 or 12, but as the storage goes on it may fall to five or even lower [Paul, 1915]. We therefore use the hepatic factor to indicate proximity to moulting.

Fat is present in the liver in quantities varying from 20 to 30 % of the total gland. In this fat we found phospholipins. The question arose as to the relationship of these substances to the metabolic cycle, and we have examined many individuals of the Decapod Crustacea to determine what it is.

The results of four of these examinations carried out on crabs (*Cancer pagurus*) at different periods between the moults are to be found in the table and graph hereunder.

METHODS.

The legs were removed by the production of autotomy [Paul, 1915] at the breaking-planes, and the weight of the body was then taken. The fresh liver was removed and put into ether-alcohol mixture (50 %). The fats were extracted by repeated washings with ether and hot alcohol and weighed. The fat was then ashed and the total amount of phosphorus present was determined by the ordinary molybdate method. Using the formula $C_{43}H_{85}O_8NPH$ [MacLean, 1918] this phosphorus was calculated to lecithin.

We consider the above method more exact than that in which acetone is employed to precipitate the phospholipins as such, and the calculation has been made to *true lecithin* [MacLean, 1918] in all cases, for purposes of comparison only.

RESULTS.

We find that the amount of fat in the liver increases both in total quantity and relatively to the weight of the body and liver as the animal approaches the moult.

It is also made clear that the amount of phospholipin in the liver (calculated to lecithin) diminishes both in total quantity and relatively to the weight of the body and liver as the animal approaches the moult.

CONCLUSIONS.

The relationship of phospholipin metabolism to developmental changes has been the subject of varied researches.

Noël Paton has shown that lecithin accumulates in the ovary of the salmon at the expense of the inorganic phosphorus of the muscle [1898]. The same author has indicated the increased percentage of lecithin in liver fats of mammals during starvation, and has associated it with the storage of phosphorus for nuclein formation [1896]. Hoppe-Seyler has demonstrated the high lecithin content of embryonic tissues [1887] and Plimmer and Scott have indicated the marked diminution of lecithin in hens' eggs during development of the embryo [1909]. More recently Brailsford Robertson, studying the development of certain sea-urchin eggs, has shown that there is a marked decrease of lecithin whilst nucleoprotein is increasing in the egg [Robertson and Wasteneys, 1913]. The same author, working with Burnett, has also described decrease in the rate of growth of carcinoma in rats, which he ascribes to the injection of lecithin into the tumor [1913]. Again, Brailsford Robertson has indicated by experiments on developing echinoderm eggs that lecithin is the possible auto-catalyst of growth [1913] and Halliburton [1909] has pointed out that the formation of choline or choline soaps by the splitting up of lecithin or other phospholipins may cause that alteration of surface tension around the nucleus which is the prelude to division.

The present work therefore falls into line with previous researches. It shows that the phospholipin of crustacean livers decreases as cell proliferation advances, and the nuclei, though not the cell bodies, become larger. Since the integument is composed of calcium carbonate, the phosphorus cannot be associated with integument formation, comparable to calcium phosphate deposition in vertebrate bone. It is strongly probable, therefore, that at least some, if not all, of the phosphorus of the nucleoprotein molecule is derived from the phospholipin molecule.

Crab	A				B				Hepatic factor
	Weight without legs	Weight of fresh liver	Fat in liver	Phospholipin as lecithin	Liver fat % of crab	Fat % of liver	Lecithin % of crab	Lecithin % of liver	
1	283	53	10.95	0.077	3.8	20.6	0.03	0.7	5+
2	382	45.5	10.07	0.2179	2.6	22.1	0.057	2.1	8.39
3	339	38.7	11.01	0.1892	3.2	28.4	0.055	1.7	8.7
4	283	28.4	3.53	0.2091	1.2	12.4	0.07	5.9	10 -

A = Quantitative results in grams.

B = Percentage results. These are graphically represented in relation to hepatic variation.

Graph of percentage results. (Section B of Table.)

- (1) Hepatic phospholipin percentage of crab.
 (2) Phospholipin percentage of fresh liver.
 (3) Fat percentage of fresh liver.
 (4) Hepatic fat percentage of crab.

On the Y-axis (1) Five small divisions = 0.01 %.

- (2) " " = 1 %.
 (3) " " = 5 %.
 (4) " " = 1 %.

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REFERENCES.

- Halliburton (1909). *Science Progress*, **14**, 197.
Hoppe-Seyler (1887). *Physiol. Chemie*, Berlin.
MacLean (1918). *Monograph on Lecithin*, Longmans.
Noël Paton (1896). *J. Physiol.* **19**, 167.
—— (1898). *J. Physiol.* **22**, 333.
Paul (1914). *Proc. Roy. Soc. Edin.* **35**, 88.
—— (1915). *Proc. Roy. Soc. Edin.* **35**, 232.
Paul and Sharpe (1916). *J. Physiol.* **50**, 183.
Plimmer and Scott (1909). *J. Physiol.* **38**, 247.
Robertson (1913). *Arch. Entw. Mech.* **37**, 497.
Robertson and Burnett (1913). *J. Exp. Med.* **17**, 344.
Robertson and Wasteneys (1913). *Arch. Entw. Mech.* **37**, 485.