The Effect of Sacculina upon the Fat Metabolism of its Host.

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With 2 Text-figures.

CONTENTS.

I. INTRODUCTION.

THE observations recorded in this paper were carried out in order to test a theory put forward by Geoffrey Smith (1), to explain the effect of the Rhizocephalan parasite Sacculina neglecta upon the sexual physiology of its host, the spider-crab, Inachus mauritanicus.

The effect of the Sacculina (2), stated briefly, is to cause the atrophy, partial or complete, of the generative organs, and to influence the external secondary sexual characters in such a way that the male tends to assume all the characters of the mature female; while immature females prematurely acquire the adult characters of that sex.

The theory suggested to account for this phenomenon was, that the Sacculina forced its host to elaborate a yolk-forming substance similar to that formed in a normal adult female

and stored in the ovary at sexual maturity, and that the presence of this substance circulating in the blood acted as a stimulus for the production of the secondary sexual characters.

The chief evidence upon which this theory was founded was that the roots of the Sacculina can be seen taking up from the blood of the crab a fatty pigmented yolk material of the same micro-chemical nature as that which is normally deposited in the ovary of the mature female crab. Mr. Smith adduced no evidence at the time to define the origin of this substance, but the chance perusal by him of a paper by Heim (**3**) on the blood of Decapod Crustacea suggested a means of procuring such evidence. Heim had observed that the freshly drawn blood of certain Decapoda (Maia, Homarus, Portunus and Astacus), is periodically charged with a pink or a yellow colouring matter (lipochrome). He came to the conclusion that this colouring matter is present only in the blood of the female, at the time when the ovary is maturing. He suggests that the lipochrome is formed in the liver and transferred thence by the blood to the ovary. Since lipochromes in general are soluble in fatty substances and are invariably found associated with them, it is obvious that Heim's observations have an important bearing upon the theory recapitulated above. It shows that at the period of sexual maturity in the female there is circulating in the blood a fatty material which is probably transferred thence to the ovary. It seemed to suggest that if the theory as to the effect of the Sacculina were true, we might possibly find such a lipochrome in the blood of sacculinised crabs of both sexes. The evidence I have collected as a result of these investigations, on the whole, seems to support Mr. Smith's theory, though the occurrence of the lipochrome in Inachus does not follow the simple and diagrammatic rule laid down by Heim for the animals upon which he worked.

The subject was suggested to me by Mr. Smith, and I wish to take this opportunity of expressing my gratitude

and indebtedness to him for his continued advice and criticism both as to practical methods and theoretical interpretation. The work was done during my tenure of the Oxford University Biological Scholarship at the Zoological Station at Naples, from November to May, 1910–1911.

My thanks are due to the staff of that institution (and in particular to Dr. Paul Mayer and Dr. Cerruti), for the ready way in which material was procured and my work facilitated.

Lastly, I must thank the Trustees of the British Museum for leave of absence from my official duties.

II. The Effect of Sacculina upon the Lipochrome and Fat Supply of Inachus.

The blood of Inachus will be found according to my experience to be colourless or to exhibit one of two tints—a pink and a yellow. The latter are represented each in various degrees of intensity, and often seem to pass insensibly into each other; the ultimate terms, however, of such a series are strongly characterised and clearly distinguishable.

It will be convenient for the present to regard these two pigments as a single body—a lipochrome; for, whatever differences may exist between them they both exhibit a characteristic property of lipochromes, namely, solubility in fat or fatty substances, and for our immediate purposes this is their most important feature. By reason, then, of this solubility in fat we may regard lipochrome as a clue to the presence of fat in a given medium. The medium, in this present case, is the blood of Inachus, and, according as lipochrome is present, I consider fat to be similarly present. I must leave the converse of this open for the present—namely, whether fat is absent when lipochrome is likewise absent.

This pigment is not a constant characteristic of the blood of Inachus. The following table gives in percentages the number of normal and sacculinised animals of both sexes

which exhibited lipochrome in either condition in the blood, during the months December–April, 1910–1911:

TABLE I.

	Normal ♂ per cent.	Number of observations.	Normal ♀ per cent.	Number of observations.
December	22	58	20	50
January	24	158	25	127
February	26	116	68	122
March	17	189	68	186
April	5	97	62	181
	Infected ♂.		Infected ♀.	
December	30	10	66	9
January	46	30	69	33
February	50	24	70	37
March	47	17	61	31
April	23	17	42	19

We observe that the percentage increases in the normal female from a figure about the same as that of the normal male to one about twelve times as large, month for month; and we observe that in the infected crabs the figures are considerably higher for male and female alike than in the case of the normal animals, e.g. in January those for the infected males are about twice as great as those of the normal male, and those for the infected females are over two and a half times as great as those of the normal females. Finally, the figures for the normal male decrease from their maximum (in February) to the low amount of 5 per cent.

Now the winter months in the Gulf of Naples constitute the season for the active moulting of the young crabs. In those months we find, in crabs about to moult, not only the blood, but also the skin of the carapace and joints deeply coloured by the lipochrome. On the other hand, crabs that have moulted and show the soft fresh carapace of the stage succeeding a moult, invariably have colourless blood and skin. Again, the months February–April fall in the very height of the breeding season of Inachus, in the Gulf, and at that time we find along with the appearance of the bright orange-coloured

ovary of the mature female a marked increase in the blood-lipochrome—in this case yellow.

I think it possible from the above data, taken in conjunction with our knowledge of the habits and development of these animals, to formulate the following explanation.

Fat is indicated in the blood of the male and female both in the normal and infected conditions, but it is signally increased at the period of rapid moulting, in the breeding season, and in the sacculinised condition in both sexes. These three phenomena are, then, comparable in their effects on the fat-metabolism of the crab. But of these three, as far as numerical proportion goes, the state of the breeding female and that of the infected animals of both sexes seem to be more closely akin, and to represent a development of fat more constant or more intense than in the moulting animals. I think it is a legitimate deduction to suggest that the action of the Sacculina closely resembles the function of the ovary, namely, to collect a nutritive substance circulating in the blood. I do not propose to attribute to this increase of fatty material any mysterious and ill-defined power of "conditioning the formation" of the secondary sexual characters, though I admit the propriety of such speculation. All I propose to say at present is, that the constancy of a fatty substance as a property of the blood of sacculinised animals is yet another expression of the profound physiological disturbance set up in the infected crab, a disturbance which always seems to end by metamorphosing the male and the immature female into a condition corresponding to that of female sexual maturity.

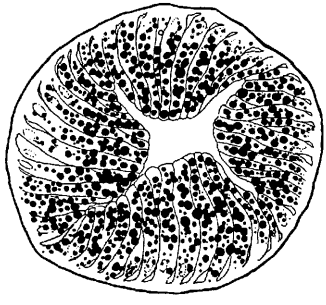
Thus far we have dealt with the blood alone and have based certain conclusions thereupon. It will now be convenient to show that the effects of moulting, the period of female sexual maturity and sacculinisation are similarly registered upon the liver,[1] regarded in the light of its function as a fat-storing organ.

[1] I use the term "liver" merely for the sake of convenience, though readers may substitute "hepato-pancreas" or "gastric gland" according to their views.

The deposition and development of fat in the form of globules within the "fat-cells" of the liver was followed by micro-chemical methods. Owing to the smallness of Inachus (the thoracic width averaging only 1·7 cm., in the total number observed) quantitative measurements of fat contents were deemed impracticable, and recourse was had to sections stained with osmic or Sudan mixture.

It is open to critics to urge that a comparative study upon these terms may be deceptive, but by admitting only such

TEXT-FIG. 1.



Transverse section through liver-lobe of Inachus mauritanicus infected by Sacculina, showing large quantity of fat in the liver-cells. (Semi-diagrammatic.)

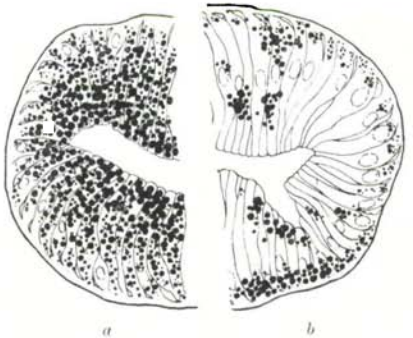
evidence as was based upon a large or small amount of fat in the liver, I venture to think solid results are attained.

If sections of a liver of a sacculinised crab be examined, they will invariably be found to contain a good quantity of fat, provided that the crab has acquired some of the characteristic adult female modifications (vide Text-fig. 1). The roots of the Sacculina will also be found to be heavily charged with fat, as Smith has already observed. Turning to the normal males and females, we find no such constant localisation of fat in any one type; with an exception, to be cited later on,

a considerable variety in the quantity is noticed—at one time it is great (vide Text-fig. 2A), at another, small (2B), in one and the same type. This is indeed what one would expect, if one regards the fat supply as dependent upon the animals' casual opportunities for obtaining food.

These observations upon the liver of infected crabs are based upon a considerable number of individuals. Unfortunately, I did not take a sufficient number of animals among the sexually mature females to enable me to state readily

TEXT-FIG. 2.



a. Transverse section through liver-lobe of Inachus mauritanicus (normal), showing large quantity of fat in the liver-cells. *b*. Another lobe, showing loss of fat from the liver-cells. (Semi-diagrammatic.)

that the phenomenon of increased fat in the "liver-cells" is, or is not to be seen in their case. But the following observations may be adduced as bearing upon the point.

The liver of Inachus exhibits a considerable amount of variety in its superficial coloration, due, as several authors have observed, to the presence or absence of colouring matter in the "ferment cells"[1] of the lining epithelium. At

[1] It is open to doubt whether the term "ferment cells" expresses the function of these correctly: I merely adopt the name faute de mieux.

one time it is quite pale, at another of an intense black-brown, with every possible intermediate stage between the two. Now, I have observed in crabs about to moult and showing lipochrome in the blood, that the liver tends to become pale, as the following table shows:

TABLE II.

Inachus, Normal (January–April, 1911), showing Lipochrome in the Blood.

	Liver condition.		
Total.	Pale.	Intermediate.	Dark.
94 . .	75 . .	13 . .	6

Again the infected crabs have the same tendency, though in a less degree:

TABLE III.

Inachus, Infected (January–April, 1911), showing Lipochrome in the Blood.

	Liver.		
Total.	Pale.	Intermediate.	Dark.
69 . .	39 . .	15 . .	15

These infected crabs show a constant amount of fatty material in the liver, and the same is true of those young crabs just dealt with (Table II) and described as having colourless livers. It may be only a chance coincidence that the latter condition is found correlated with a constant supply of fat in the liver, or it may be an invariable and necessary correlation. If the latter is true, then we may state that the mature female in the breeding season may be similarly presumed to have an increased or more constant supply of fat in the liver-cells, as I found that towards April the liver of such animals was invariably pale. I admit that, in default of an extensive investigation of the liver of breeding females, we are compelled to resort to this pale

condition of the liver as a basis of comparison between the breeding females and infected animals of both sexes, and I make such a comparison with due regard to this qualification. Indeed, if we are not prepared to allow such a comparison, at all events we are left with yet another characteristic in common between the adult female and the infected crabs, viz. the loss of colour in the liver.

It is a curious point to observe that in the normal male the blood, as the summer approaches, loses its lipochrome, while the liver tends to become pale. A possible interpretation of this, though as yet quite unverified in fact, is, that the liver, though containing fat, has not yet received the appropriate stimulus (e.g. the moult of infection) to liberate its contents into the blood. Finally, I may say that the insufficiency of our knowledge of the function of the "ferment cells" relegates much of this reasoning to the region of tentative hypothesis.

III. The Nature and Function of the Lipochrome of Inachus.

We have hitherto regarded the two lipochromes, pink and yellow, as one, and have indicated their relation to the development of fat. It now remains to state the little that seems to be justified from my observations as to their nature and function.

It has been observed above that, though clearly distinguishable as pink and yellow in their more pronounced stages, they tend to merge insensibly into each other. But so much is clear, that a rich orange yellow characterises the blood-pigment of the female in the breeding season, and a pink shade the male and female at the moult and under the influence of Sacculina. It may possibly be of interest from the point of view of "heterozygosis" to remark that the male occasionally exhibits a pure yellow lipochrome in the blood.

With regard to the moulting animals, the lipochrome may be traced from the blood into the skin of the joints and

carapace, after which the blood becomes pale. The animal in this condition has a bloated appearance, markedly different from the fresh pale condition of the stage succeeding a moult. I think we may conclude that some sort of fatty substance is lodged in the tissues in question, and assists in the formation of the new epidermis. The fate of the lipochrome can only be vaguely suggested; probably it is got rid of at the moult, as the colourless condition of the new skin would suggest; but I have never examined the freshly-cast carapaces to satisfy myself upon the point.

The rôle of the yellow pigment is but generally indicated by the fact that the fat it accompanies is stored up in the ovary. It is certainly specific for this function, but its actual rôle, if it has any, is not necessarily indicated by the association. One is tempted, of course, to assign to each of these two lipochromes a differentiated physiological function and identity. Maly (**4**) found that they could be separated by appropriate chemical reactions from each other in the yolk of the ovary of Maia squinado. But it is permissible to observe that while the blood of a mature female Inachus is more often yellow, the ripe ovary of the same animal is orange (pink + yellow). Finally, when we take into consideration the passage from pink to yellow in the blood lipochrome (p. 275), it will be seen that, on the other hand, there is an equal possibility that these two colouring bodies are not rigidly determined each from each.

IV. A Note on the Origin of the Fat Supply of Inachus in Relation to Sacculinisation.

There seems to be little reason to doubt that the fat contained in the liver-cells of Decapoda is directly supplied by the food-stuffs of the animal. In addition to the experience of other authors I may quote my own results on this point.

After a fortnight's or eighteen days' starvation fat begins to disappear from the liver. If a number of crabs which have been thus starved are taken and fed on a fat-con-

taining diet for some hours, the liver-cells of these, upon examination after some time, will be found to contain a considerable quantity of fat. Crabs which have not been thus fed exhibit none or very little. If one deduces from this the immediate dependence of the animal for its fat supply on its food-stuffs, then we are naturally led to ask how the infected animals exhibit such a constant supply. Moulting male and female and breeding females might also be made subject to such a question, but we must remember that the periods are but temporary with them, and they return again to their normal condition, while the infected animal has to continue playing the host to the parasite's Gargantuan appetite, though no doubt what is true of the infected animal is, to a certain though limited degree, true of the two others.

Is, then, the increased fat in the blood of the infected animals the result of an increased activating of fat that would otherwise have been stored up? Or is it due to an increased initial supply?

I do not think we can resolve this interesting question yet, but I am inclined to think that the infected animal obtains an abnormal supply of fat. Unfortunately I have not made any comparative observations upon the appetite and feeding of Inachus, any more than to observe that infected animals are as active in obtaining food as are the normal ones. Mr. Smith also informs me that he has noticed that the former eat very greedily and die much more rapidly than uninfected individuals, when starved. Finally, I have reason to doubt whether infected crabs live over the summer in the Gulf of Naples.

When observations are carried out to satisfy this question it will possibly be discovered that a great mortality of the infected ultimately sets in, due to the failure of the crab to obtain enough nutrition and reserve material for itself and for the parasite.

V. Summary.

The following appear to be the most salient points that can be deduced from the evidence cited above:

(1) that infection by Sacculina induces the maintenance of an abnormal quantity of fat in its host's liver and blood;

(2) that this condition resembles that found in normal females and males preparing for the moult and in sexually mature females, the ultimate destination of the fat being functionally similar in the case of the mature females and the infected crabs;

(3) that in all probability the ultimate fate of the infected crab is death from starvation, arising from its inability to obtain enough fatty material for itself and its parasite;

(4) that a pink-coloured lipochrome is found in the blood of moulting and infected animals of both sexes, while a rich yellow characterises that of the sexually mature female.

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