THE EFFECTS ON THE FAT AND STARCH METABOLISM OF *GEBIA* BY THE PARASITE *GYGE BRANCHIALIS*

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INTRODUCTORY

An account of the effects of *Gyge branchialis* on its Decapod host has been published by Tucker (1930). He points out that as in the case of *Inachus* parasitized by *Sacculina*, there is a feminization of infected males but no corresponding masculinization of infected females. Unlike *Inachus*, *Gebia* continue to moult after infection, which occurs in the majority of cases when the carapace is between 3 mm. and 9 mm. long. The infected animals attain normal adult size. In this respect they would appear to occupy a position intermediate between *Inachus* and *Eupagurus* parasitized by *Peltogaster* (investigated by Potts, 1906); the infected *Eupagurus* moult apparently rather more frequently than the uninfected.

The effects of parasitization on the general metabolism of the host have been investigated in the case of *Inachus* and other brachyuran crabs by Smith (1911, 1913) and Robson (1911). They found that sexually mature female crabs differ from males in having an excess of fatty material in the liver, which in the case of *Inachus* also floods the blood. In parasitized specimens of *Carcinus* (Smith) and *Inachus* (Robson) an excessive production of fat in the liver was found to occur.

Since the effects of the simple blood-sucking *Gyge* upon the morphology of its host are at least as marked as the influence of *Sacculina* on *Inachus*, the changes in the metabolic processes should be equally well marked. The following piece of work was undertaken in order to try to discover whether such changes did in fact occur.

MATERIAL

The specimens used were obtained from Naples, those for a study of the fats and fatty substances being preserved in formol in sea water, the specimens investigated for starch in 70% alcohol.

It may be objected that preserved material might well give unreliable results, but the fixation might be reasonably expected to affect parasitized and unparasitized animals alike, having no very marked effect on their relative fat or starch contents.

METHOD

The method of grouping the animals used for a quantitative estimation of the total fat content was this:

(1) Normal males.
(2) Parasitized males.
(3) Normal females.
(4) Parasitized females.

The normal females were then subdivided into two groups:

(*a*) Those carrying ova attached to the abdominal appendages or with ovaries showing orange with well-developed ova through the body wall.
(*b*) Those showing no visible sign of ova.

METHOD OF FAT ESTIMATION

This was done gravimetrically, the fats and allied substances being estimated *en masse* as an ether soluble extract.

A known weight of *Gebia* was saponified by boiling with excess of strong KOH solution under a reflux condenser. The fatty acids were then liberated by the action of concentrated H_2SO_4 on the solution of soaps when it had cooled. An extract was then made by shaking the mixture with a known volume of petroleum ether for 2 hr. On standing, the solution of fatty substances in ether formed an orange-yellow layer on top of the contents of the flask. This layer was raised into the neck of the flask by the addition of distilled water and a known volume of the ether solution was pipetted off into a dry weighed beaker. The ether having been evaporated off over a water-bath, the beaker and residual fat were weighed. From this the quantity of fat in the sample of *Gebia* was calculated. About fifteen *Gebia* were taken in each sample, weighing 10–20 g.

METHOD OF GLYCOGEN ESTIMATION

The glycogen was estimated as glucose volumetrically by titration against Benedict's solution. A known weight of *Gebia* was boiled, with strong KOH solution to break up the tissues, for 4 hr. under a reflux. The glycogen was then precipitated by alcohol and separated by filtration. After washing with alcohol and ether the precipitate was dissolved in boiling water and hydrolysed to glucose by refluxing with HCl for 2 hr. The solution of sugar thus obtained was then made up to a known volume and titrated against Benedict's solution. From the amount of sugar in the sample the quantity of starch was then calculated.

RESULTS

Gebia. *Fat expressed as a percentage of body weight*

	Normal male	Parasitized male	Ovigerous female	Non-ovigerous female	Parasitized female
	0·9 1·02 1·00 0·93 1·21 1·16 0·97 1·06 1·09	1·55 1·66 1·42 1·49 1·74 1·81	1·80 1·795 2·510 2·210 2·733 2·755 1·930 1·930	1·193 1·217 1·184 1·223	1·065 1·075 1·087 1·023 1·610 1·380 1·190 1·360
Mean	1·04	1·61	2·21	1·204	1·22
Variance δ^2/N	0·0094	0·0188	0·1485	0·000262	0·0371
S.D. $\pm\sqrt{V}=\sigma$	0·09694	0·1371	0·3854	0·016	0·1926
S.E. $\pm\sigma/N$	0·03231	0·05599	0·1362	0·004	0·06813

Percentage of fat found in 130 specimens of *Gyge* which weighed 7·2 g. was 4·4 %.

Gebia. *Starch expressed as a percentage of body weight*

	Normal female	Parasitized female	Normal male	Parasitized male
	0·1831 0·1802 0·1816 0·1898 0·1889 0·1879 0·1906 0·1890	0·1819 0·1921 0·2260 0·2165	0·1148 0·1148 0·1389 0·1412	0·1418 0·1658 0·1540
Mean	0·1864	0·2041	0·1276	0·1538
Variance δ^2/N	0·000014	0·000317	0·000164	0·000096
S.D. $\pm\sqrt{V}=\sigma$	0·003741	0·01799	0·01266	0·009797
S.E. $\pm\sigma/N$	0·000467	0·00449	0·00316	0·003265

STATISTICAL ANALYSIS OF RESULTS

Fats

Comparison of the normal and parasitized ♂:

$t=8{\cdot}45$.

$n=13$.

P is less than 0·01, ∴ the difference in the means is significant.

Comparison of the non-ovigerous ♀ and normal ♂:

$t = 11{\cdot}93$.
$n = 13$.
P is less than 0·01, ∴ the difference in the means is significant.

Comparison of the non-ovigerous and parasitized ♀:

$t = 0{\cdot}1894$.
$n = 10$.
P is greater than 0·8, ∴ the difference in the means is not significant.

Starches

Comparison of normal ♀ and parasitized ♀:

$t = 7{\cdot}240$.
$n = 10$.
P is less than 0·01, ∴ the difference in the means is significant.

Comparison of normal ♂ and normal ♀:

$t = 10{\cdot}91$.
$n = 10$.
P is less than 0·01, ∴ the difference in means is significant.

Comparison of normal ♂ and parasitized ♂:

$t = 6{\cdot}067$.
$n = 5$.
P is less than 0·01, ∴ the difference in means is significant.

DISCUSSION OF THE RESULTS

Tucker has reviewed the literature on parasitic castration up to 1930 and has reached the conclusion that Smith's theory of metabolic stimuli, notwithstanding the criticisms that have been made of it, is still the only one which affords a satisfactory explanation of the facts. The present results appear to be in entire agreement with and to afford additional support to Smith's theory.

Considering first the fat analyses, it is evident that there is a significant difference in fat content between normal male and non-ovigerous females. There is a definite increase in fat in parasitized males associated also with the presence of typically feminine secondary sexual features. There is no increase in fat in parasitized females, ovigerous females have a fat content considerably in excess of non-ovigerous ones. Parasitism of the female is associated with a degeneration and disappearance of the ovary but no change in secondary sexual features. The gonad in infected males shows an increased number of the oocytes often found in normal testes and the testicular tissue may degenerate and disappear (Tucker, 1930).

The effect of the parasitic *Gyge* on the male *Gebia* seems to consist of creating an insistent demand for fats which it withdraws from the blood of its host, thereby stimulating the anabolic processes and causing a rise in fat content from 1·04 to

1·6%. That *Gyge* does remove fat from its host is shown by the high percentage (4·4) which it contains, and which must originally have come from the host.

In the case of the parasitized females, the fat production of the host appears to be sufficient for host and parasite, but insufficient for host, parasite and ovary. The natural result of this is degeneration of the ovary due to malnutrition. The parasite in the Cryptoniscid stage fixes on to a *Gebia* of a carapace length of less than 6·5 mm., consequently it seems legitimate to assume that the growing *Gyge* brings a metabolic drain to bear on the host of the same nature and at least the same magnitude as a functional ovary. The growing female parasite is itself developing relatively large ovarian structures and it seems feasible to assume that it needs similar substances in this respect to its host.

The parasitized females do not ever show the great increase in fat content found in ovigerous females; this may be due to one of two causes:

(1) There is no accumulation of fat in the body as in the case of an ovary crammed with yolk-filled eggs.

(2) Whatever stimulus a mature ovary, developing eggs, has on fat production is effectively removed.

Of these two alternatives, I consider the former the more probable. We know that in the parasitized male there is considerable stimulus to fat production and it seems likely that this is the general effect of the parasite, but that in the female the anabolic processes are unable to supply fat for host, parasite and ovary, the latter degenerates and consequently there is no ovarian accumulation of fats as in an ovigerous female. That *Gyge* creates a demand for fat at least equal to a resting ovary is shown by the fact that parasitized females contain as much fat as non-ovigerous females, i.e. females with ovaries in an inactive state.

There is a certain amount of evidence to show that the female formative substance (hormone of Goldschmidt) is involved in some way with fat production. Whether in parasitized males or in unparasitized females there is an accentuation of feminine features and there is an increase in fat content which is due presumably to an increase in hormone production. The maturation of the ovary causes an increase in fat production, and at the same time the setae appear on the first abdominal appendage (Tucker, 1930). In parasitized males, fat production is stimulated and at the same time feminine characters make their appearance. The evidence supports the view that the production of female hormone or sexual formative substance is involved with the production of fatty substances and that changes brought about in this aspect of anabolism are responsible for the sexual inversions found in parasitized males. It is, of course, possible to regard the fact as a secondary factor and to relate the hormonic changes to some more subtle and hitherto undiscovered change in metabolism. At the time of writing there is no direct evidence in support of this view. On the evidence Smith's assumption was in the main correct. The parasite by bringing a demand of a similar nature to that of an ovary to bear on the animal produces such changes in metabolism as lead to a feminization of infected males, or an accentuation of feminine features in infected females where such metabolic changes occur.

As regards the starch content of *Upogebia*, a definite difference exists between normal males and females. Parasitization is followed by a slight increase in both sexes. Smith correlated the inability of *Inachus* and *Carcinus* parasitized by *Sacculina* to moult, with the inability to store glycogen owing to demands on its metabolism, by the parasite. This view is further supported by the fact that inability to moult and approach of maturity of the parasite coincide. *Gebia* goes on moulting after infection and its glycogen content is maintained as high, if not higher, than in uninfected animals. These facts are in support of Smith's views but a further analysis of animals at various stages of ecdysis would be needed to establish a connexion between glycogen anabolism and chitin production. There is evidence that in Insecta glycogen and chitin production are linked (Paillot, 1938). The slight increase of glycogen in parasitized animals makes it conceivable that moulting might occur more frequently in these than in normal animals. There is no evidence that this is so. Potts (1906) was of opinion that *Eupagurus* parasitized by *Peltogaster* moulted more frequently than normal. The effect of *Peltogaster* on the metabolism of the host has not yet been investigated, but the conditions in *Gebia* suggest that increased frequency of moulting is not *a priori* impossible. It is, of course, possible that the increase in glycogen is merely due to regression of the gonad lessening glycogen katabolism, rather than an active stimulus to anabolism of glycogen by the parasite's presence.

Owing to the methods employed larger numbers of animals are needed for glycogen estimation than I had at my disposal, and I therefore hesitate to give any definite opinion on the relative increase of glycogen due to parasitization. I think it fairly certain that a slight increase does occur.

SUMMARY

1. Males of *Upogebia littoralis* parasitized by *Gyge branchialis* show a marked increase in fat content from 1·04 to 1·6 %.

2. Parasitized females have a fat content practically the same as that of ordinary non-ovigerous females, 1·2 %.

3. Ovigerous females have a fat content considerably higher than that of parasitized males or non-ovigerous females, namely 2·2 %.

4. The parasite *Gyge* has a relatively high fat content, 4·4 %.

5. There is a significant difference in glycogen content between normal males and females.

6. There may be a slight increase in glycogen following parasitization.

7. The results are essentially in agreement with the theory of metabolic stimuli put forward by Geoffrey Smith.

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