

ON THE SEXUAL BIOLOGY OF *PANDALUS BOREALIS* (CRUSTACEA DECAPODA)

III. THE INITIATION OF THE FEMALE PHASE

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(Text-figs. 1-2)

In the second paper of this series (Carlisle, 1959*b*) I have described the results of various experimental manipulations upon the termination of the male phase in the protandric hermaphrodite *Pandalus borealis* Krøyer. In this paper I shall be concerned with the effects of such interference upon the initiation of the female phase of the life history.

In a number of papers Panouse (1943-8) provided evidence, based upon extirpation experiments, that the sinus gland of the eyestalks of the common European prawn *Palaemon* (= *Leander*) *serratus* secretes a hormone which inhibits the development of the ovary and in particular vitellogenesis. His results were confirmed by Takewaki & Yamamoto (1950*a, b*), who worked upon the Japanese prawn *Paratya*. Since the above work was completed much evidence has accumulated that the sinus gland is not in itself an endocrine organ, but a neurohaemal organ (Carlisle & Knowles, 1953); that is to say it is the terminal organ of a neurosecretory system, whose main function is to release into the blood hormones produced elsewhere, within neurosecretory cells. It is generally (but not universally) accepted that the hormones stored in the sinus gland, and released into the blood there, are produced mainly within the neurosecretory cells of the ganglionic X organs (see discussion in Carlisle & Knowles, 1959). In agreement with this view I have found that, both in *Palaemon* (Carlisle, unpublished) and in the Mediterranean prawn *Lysmata seticaudata* (Carlisle, 1953*a, b*), it is possible to extract an ovary-inhibiting substance from the sinus gland and from the ganglionic X organ which lies in the medulla terminalis of the brain (see Carlisle, 1953*c*). This substance, after intravenous injection, produces involution of the developing ovary in the intact animal, or effectively counteracts the result of eyestalk ablation, so far as ovarian growth is concerned. Extirpation of the sinus glands alone leads to an increase in ovarian weight significantly less than does total eyestalk ablation—as indeed Panouse also found. Ablation of the ganglionic X organ of the medulla terminalis leads to a delayed onset of increase in ovarian size, but once this increase has begun it proceeds as rapidly as after total eyestalk ablation (Carlisle, unpublished). We may

suppose that the delay is a result of the hormone stored within the sinus gland: once this has been utilized, then ovarian increase can begin. The sinus gland seems nevertheless to be necessary for the efficient release of the hormone, for its removal does lead to some increase in ovarian size, until a new sinus gland is regenerated from the stump of the X organ-sinus gland neuro-secretory tract.

The evidence seems satisfactory that ovarian growth in these two species of prawns is inhibited by a hormone secreted by the X organ-sinus gland complex. This complex in *Pandalus* has been described in the first part of this series (Carlisle, 1959a). Unpublished data suggest that in *Palaemon*, during the juvenile phase, the ovary is inhibited by the action of this hormone; before the onset of maturity the secretion of the hormone ceases and the ovary begins to develop; ovulation succeeds and once more the ovary is inhibited by the hormone until the breeding season of the following year. There is thus an annual cycle in the secretion of the hormone once the animal is mature. Arvy, Echalié & Gabe (1954) have provided evidence that the immature gonad may be influenced by a secretion of the Y organ in the crab *Carcinus*, but this has not yet been confirmed in *Palaemon*, nor indeed in any natantian, nor do these authors suggest that this organ has any influence on the gonad after sexual maturity.

Whereas most of the Decapoda are normal bisexual creatures, three isolated genera have been found to show a functional sex reversal, at least in some of their species. The blind burrowing decapod *Calocaris macandreae* is known to show this phenomenon (Runnström, 1925; Balss, 1930); *Lysmata seticaudata* and *L. nilita* apparently show full obligatory protandric hermaphroditism, so that every male which survives long enough reverses sex to become a female, while there are no primary females in the population (Spitschakoff, 1912; Caroli, 1917; Dohrn, 1950; Dohrn & Holthuis, 1950; Carlisle & Dohrn, 1952, 1953; the terminology is that proposed by Carlisle, 1959c); certain species of the genus *Pandalus*, notably the deep-sea arctic prawn *P. borealis*, the subject of the present communication, *P. montagui* and *P. kessleri* are also protandric hermaphrodites (Berkeley, 1930; Jägersten, 1936; Rasmussen, 1953; Aoto, 1952) though according to Pike (1952) *P. bonnieri* is dioecious. In *P. montagui* and in *P. borealis* most populations show a variable percentage of primary females so that the hermaphroditism is partial but obligatory (Mistakidis, 1957; Allen, 1959). In some populations, however, including that upon which I have worked in the Gullmarfjord, Sweden, the hermaphroditism is full and there are no primary females in the population (Carlisle, 1959b). The analysis of the sex reversal is of course easier in such a population.

I have shown (Carlisle, 1953a, 1954) that the assumption of functional female form in *Lysmata seticaudata* is inhibited by a hormone of the X organ-sinus gland complex. I then suggested that it seemed probable that the

hormone responsible for this inhibition might well be the ovary-inhibiting hormone. The evidence that I am about to present suggests that in *Pandalus borealis* also, the ovary is inhibited by a hormone of the X organ-sinus gland complex, that the assumption of the female form is also inhibited by a hormone of this complex, and that there is a high degree of correlation between the two actions.

EXPERIMENTAL DATA

Change of the apparent sex of the external accessory and secondary sexual characters can only take place in a crustacean when the animal moults, shedding the old shell and growing a new one of different dimensions and with differing proportions. In a prawn possessing both functional ovarian and functional testicular tissue the functional sex is that corresponding to the external characters, for even if the testis is producing sperm, the prawn cannot mate as a male if it lacks the copulatory appendages and if the vasa deferentia terminate blindly with no opening to the outside; nor can it mate as a female, though possessing a functional ovary, if it lacks the female apertures and the ovigerous hairs. A prawn which has not moulted during the period of observation has had no chance to modify its sexual condition. In all experiments reported here, therefore, only individuals which had moulted during the experimental period are included in the data. All animals which did not moult before the end of the experiment were disregarded.

OVARIAN INHIBITION

Effects of the X organ-sinus gland complex

Eight groups of animals were selected, four of females and four of large males. Two groups of each sex were left intact and two groups had the eyestalks extirpated. One group from each division received a single injection of 4 sinus gland equivalents of an extract prepared from the sinus glands of females. Table 1 summarizes the treatments accorded to the different groups together with the mean ovarian weight in each group after a period of ten days had elapsed. Table 2 supplies a summary of the analysis of variance of these data. It will be seen that the operation of eyestalk removal is followed by a very significant increase in ovarian weight, while the injection of the extract is followed by the reverse. In the females, when both the operation and the injection are performed upon the same group of animals (group 4), it is the operation which has the greater effect—the injection is evidently inadequate to counteract the effect of the operation. In the smaller males, however, which received the same dose of the extract as the larger females and hence a larger dose per unit body weight, the effects of the injection and of the operation have effectively cancelled each other out, so that the mean ovarian weight in group 8 is the same as in group 5.

It is to be noted in the table of analysis of variance (Table 2) that there is a highly significant entry for 'interactions'. This is not unexpected for of course the males and females are in different physiological states, and it is not to be expected that either the injection or the operation will have exactly the same degree of action on the ovarian weight in the two sexes. Moreover, since all individuals, whatever their size, received the same dose of extract,

TABLE 1. OVARIAN INHIBITION

Group	No. in group	Sex	Operated	Injected	Mean ovarian weight (mg)	Standard deviation	Standard error of mean
1	15	♂	-	-	34.79	9.58	2.56
2	11	♂	-	+	23.63	8.22	2.60
3	14	♂	+	-	123.71	30.33	8.41
4	10	♂	+	+	42.51	4.41	1.47
5	24	♂	-	-	19.58	6.95	1.45
6	25	♂	-	+	16.45	7.94	1.62
7	19	♂	+	-	27.86	8.49	2.00
8	27	♂	+	+	19.44	6.99	1.37

TABLE 2. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 1 (OVARIAN INHIBITION)

Source of variation	Degrees of freedom	Sum of squares	Probability
Sex	1	48,313	< 0.001
Operation	1	20,346	< 0.001
Injection	1	19,136	< 0.001
Interactions	4	45,918	< 0.001
Error	137	20,626	—
Total	144	154,339	—

$$s^2 = 150.59. \quad s = 12.25.$$

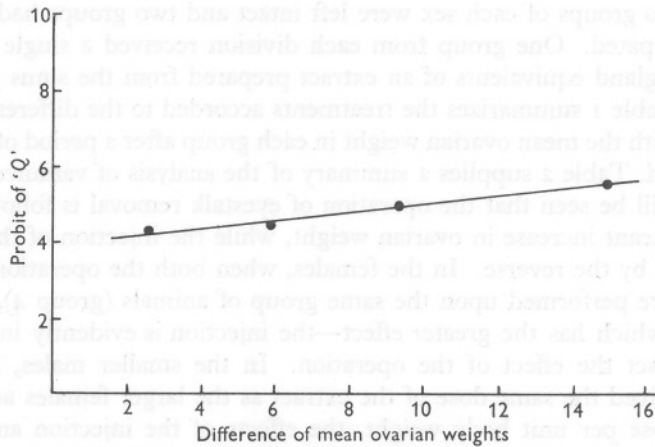


Fig. 1. The correlation in response to various treatments of the ovarian weight, with proportion becoming female.

whereas the operation would have, presumably, a uniform effect per individual, it might be expected that these two factors would interact. In fact, if the analysis of variance is carried further to separate the various possible interactions orthogonally, each of them is found to be significant. This, however, is irrelevant to the main argument. There is no doubt that eyestalk ablation leads to a rapid increase in ovarian weight and that injection of sinus gland extract prevents this wholly or in part, while in the intact animal such an injection is followed by involution of the ovaries. The case is clear for the hormonal inhibition of ovarian development by the X organ-sinus gland complex.

Effects of other organs and tissues

Complete selective extirpation of other tissues has not been attempted in this series of experiments. Removal of the eyestalk necessarily involves the ablation of other organs besides the sinus glands and the ganglionic X organs. Attempts at replacement therapy with tissues of the eyestalk other than these had no effect. In particular injections of extracts of the sensory pore X organ (*SPX*), of retroretinal tissue (*RT*) or of the medulla externa (*ME*) had no effect on ovarian weight in either males or females, intact or lacking eyestalks. Injections of extracts of the medulla terminalis ganglionic X organ (*MTGX*), however, acted like sinus gland extract injections. The data are summarized in Tables 3 and 4. The experiment was carried out in like manner with like dosages and times as in the first experiment reported above. In each group only the first twelve animals caught at the end of experiment were dissected and find inclusion in these tables. The remainder were discarded. It is abundantly clear that injection of extracts of the *SPX*, *ME* and *RT* are without any effect on ovarian weight in any of the conditions of the experiment.

The vas deferens gland

This gland, which has been shown to be necessary for maintaining the male phase (Carlisle, 1959*b*), is absent from the later non-sexual stages and from the female. Injection of extracts of the gland in dosages which had a profound effect upon the male (Carlisle, 1959*b*) had no effect upon oocytes that were undergoing vitellogenesis, or upon ovulation. Ovaries which were sectioned ten days after a single injection of vas deferens gland extract, however, showed the presence of cells which appeared to have the character of spermatogonia, while these were more clearly defined in animals which had had two such injections. Much more work is required upon this point, especially in view of the results of Charniaux-Cotton (1954) with *Orchestia*, and I will refrain from further comment until I have had the opportunity to experiment once again upon the vas deferens gland of *Pandalus*.

TABLE 3. OVARIAN INHIBITION

Group	No. in group	Sex	Operated	Injected MTGX	Injected SPX	Injected ME	Injected RT	Mean ovarian weight (mg)	Standard deviation
9	12	♀	-	-	-	-	-	35.01	8.4
10	12	♀	-	-	-	-	+	35.27	8.93
11	12	♀	-	-	-	+	-	34.20	9.01
12	12	♀	-	-	-	+	+	34.35	8.70
13	12	♀	-	-	+	-	-	36.07	8.25
14	12	♀	-	-	+	-	+	35.05	8.26
15	12	♀	-	-	+	+	-	34.76	8.78
16	12	♀	-	-	+	+	+	34.60	8.99
17	12	♀	-	+	-	-	-	23.14	8.21
18	12	♀	-	+	-	-	+	23.77	8.20
19	12	♀	-	+	-	+	-	23.09	7.95
20	12	♀	-	+	-	+	+	22.91	8.12
21	12	♀	-	+	+	+	-	25.05	8.88
22	12	♀	-	+	+	-	+	24.73	8.70
23	12	♀	-	+	+	+	-	23.68	8.76
24	12	♀	-	+	+	+	+	23.50	8.90
25	12	♀	+	-	-	-	-	120.01	30.24
26	12	♀	+	-	-	-	+	110.37	33.76
27	12	♀	+	-	-	+	-	130.25	31.09
28	12	♀	+	-	-	+	+	104.38	33.02
29	12	♀	+	-	+	-	-	114.46	27.89
30	12	♀	+	-	+	+	+	137.50	26.66
31	12	♀	+	-	+	+	-	90.92	27.84
32	12	♀	+	+	+	+	+	127.76	35.21
33	12	♀	+	+	-	-	-	44.26	7.22
34	12	♀	+	+	-	-	+	42.57	7.20
35	12	♀	+	+	-	+	-	39.20	7.91
36	12	♀	+	+	-	+	+	40.11	6.93
37	12	♀	+	+	+	-	-	39.34	7.29
38	12	♀	+	+	+	-	+	40.31	7.28
39	12	♀	+	+	+	+	-	42.41	6.91
40	12	♀	+	+	+	+	+	42.00	7.35
41	12	♀	-	-	-	-	-	18.75	7.04
42	12	♀	-	-	-	-	+	18.85	6.82
43	12	♀	-	-	-	+	-	19.34	6.64
44	12	♀	-	-	-	+	+	19.39	7.32
45	12	♀	-	-	+	-	-	19.42	7.49
46	12	♀	-	-	+	-	+	19.04	7.34
47	12	♀	-	-	+	+	-	19.58	7.14
48	12	♀	-	-	+	+	+	18.63	7.06
49	12	♀	-	+	-	-	-	15.82	7.03
50	12	♀	-	+	-	-	+	16.13	7.09
51	12	♀	-	+	-	+	-	16.60	6.88
52	12	♀	-	+	-	+	+	16.55	7.41
53	12	♀	-	+	+	-	-	15.90	6.97
54	12	♀	-	+	+	+	+	16.03	7.32
55	12	♀	-	+	+	+	-	16.07	7.58
56	12	♀	-	+	+	+	+	16.51	6.99
57	12	♀	+	-	-	-	-	27.83	7.95
58	12	♀	+	-	-	-	+	25.97	8.47
59	12	♀	+	-	-	+	-	25.96	8.99
60	12	♀	+	-	-	+	+	26.34	7.63
61	12	♀	+	-	+	-	-	26.51	8.60
62	12	♀	+	-	+	-	+	26.70	7.64
63	12	♀	+	-	+	+	-	26.03	7.70
64	12	♀	+	-	+	+	+	27.58	8.94
65	12	♀	+	+	-	-	-	19.91	7.00
66	12	♀	+	+	-	-	+	18.87	7.32
67	12	♀	+	+	-	+	-	18.92	7.56
68	12	♀	+	+	-	+	+	19.73	8.39
69	12	♀	+	+	+	-	-	19.21	7.34
70	12	♀	+	+	+	-	+	19.87	7.43
71	12	♀	+	+	+	+	-	20.03	8.09
72	12	♀	+	+	+	+	+	20.11	8.81

TABLE 4. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 3

Source of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Sex	1	219,882.8	219,883	≤ 0.001
Operation	1	146,440.1	146,440	≤ 0.001
MTGX	1	112,447.7	112,448	≤ 0.001
Interactions of Sex, op., MTGX	4	265,671.1	66,418	≤ 0.001
Other injections	3	372.8	124.3	N.S.
Other interactions	53	17,798.2	335.8	c. 0.01
Error	704	121,591.9	172.7	
Total	767	885,701.8		

$s = 13.1$. N.S., not significant.

ATTAINMENT OF THE FUNCTIONAL FEMALE STATE

Effects of the X organ-sinus gland complex

To determine the effects of the X organ-sinus gland complex upon the attainment of the functional female state animals in the intersexual condition were chosen. The individuals so chosen were allocated to groups at random. The animals were treated like those of groups 5-8 in the first experiment reported above, but three types of extract were used:

- (1) Extract of the sinus gland of females, which at this season of the year (April) might be presumed to be moderately active in secreting ovary-inhibiting hormones;
- (2) Extract of the sinus glands of young males, which might be presumed to be fully active in the secretion of the ovary-inhibiting hormone;
- (3) Extract of the sinus gland of old males and non-sexual individuals, which might be presumed to be relatively inactive in the ovary-inhibiting hormone.

The animals in the first experiment received an injection of type 1 and the data from groups 5-8 of this experiment will be considered here also. The treatments accorded to the various groups are summarized in Table 5, together with the numbers which did and which did not attain a functional female condition at the succeeding moult. Table 6 summarizes the statistical analysis of these data. It will be seen that eyestalk removal has led to a very significantly higher proportion of attainment than in the unoperated animals, while injection of sinus gland extracts has had the opposite effect. The most effective type of extract was type 2 and the least effective type 3 which had an insignificant effect. Perusal of Table 5 will show that an injection of type 1 or type 2 effectively counteracted the effects of the operation, affording adequate replacement therapy in this respect; statistical analysis confirms this impression. It is evident that the attainment of a functional female state from the non-sexual condition is inhibited by some hormonal emanation from the sinus gland.

TABLE 5. ATTAINMENT OF FEMALE STATE: TREATMENTS AND RESULTS IN THE VARIOUS GROUPS

Group	No. in group	Operated	Injected (type 1)	Injected (type 2)	Injected (type 3)	Number becoming female	Number remaining non-sexual
73	24	-	-	-	-	12	12
74	25	-	+	-	-	9	16
75	19	+	-	-	-	16	3
76	27	+	+	-	-	14	13
77	28	-	-	-	-	15	13
78	26	-	+	-	-	9	17
79	23	+	-	-	-	18	5
80	28	+	+	-	-	13	15
81	30	-	-	-	-	14	16
82	23	-	-	+	-	5	18
83	22	+	-	-	-	17	5
84	31	+	-	+	-	12	19
85	29	-	-	-	-	15	14
86	31	-	-	-	+	15	16
87	31	+	-	-	-	24	7
88	30	+	-	-	+	23	7

Effects of other organs and tissues

Once more no attempt has been made at complete selective extirpation of other organs. An experiment similar to that summarized in Tables 3 and 4, but utilizing non-sexual animals, made it plain that injection of extracts of the *MTGX* acted in precisely the same way as extracts of sinus glands upon the attainment of the functional female condition, while injection of extracts of the *SPX*, *ME* and *RT* had no significant effect. The results of this experiment will not be reported in full.

I could find no evidence that injection of vas deferens gland extract affected the attainment of the functional female state, but this requires further investigation.

THE CORRELATION BETWEEN OVARIAN GROWTH AND ATTAINMENT OF THE FUNCTIONAL FEMALE STATE

As a measure of the effectiveness of the various treatments in interfering with the normal rate of attainment of the female state the coefficient of association (Q) was calculated (Yule, 1900; Yule & Kendall, 1945). The values of Q are listed in Table 6 and repeated in Table 7. The differences in mean ovarian weight brought about in the same individuals by the same treatments were calculated, as a measure of the effectiveness of these treatments in interfering with ovarian weight; these values are also listed in Table 7. If the phenomena of hormonal inhibition of attainment of the female status and of hormonal inhibition of ovarian growth are not interconnected these values should show no significant regression one upon another, whereas if they are connected there should be a significant regression.

Now Q is a coefficient which has the limits $+1$ and -1 , and the graph of a function which is constrained to lie between limits is of necessity sigmoid, hence some linearizing transformation is needed if we are to calculate a linear regression of Q upon the difference in mean ovarian weights. According to Finney (1952) there is little to choose between the various linearizing transformations of sigmoid curves especially over the middle of the range, when we do not know on *a priori* grounds which sigmoid to expect. It is possible that the logit transformation might give a closer fit, but for ease of computation the probit transformation was chosen. The expected probits corresponding

TABLE 6. ATTAINMENT OF FEMALE STATE: χ^2 AND COEFFICIENTS OF ASSOCIATION (Q) BETWEEN ATTAINMENT AND DIFFERENT TREATMENTS

Treatment	$\chi^2_{(1)}$	Probability	' Q '	Standard error of ' Q '
Operation	19.112	< 0.001	+0.412	0.083
Injection (type 1)	10.718	= 0.001	-0.430	0.120
Injection (type 2)	8.462	< 0.01	-0.525	0.155
Injection (type 3)	0.96	—	-0.058	0.377

Second order comparisons:

Injection (type 1):Injection (type 2) 1.808 = 0.15.
 Injection (type 1):Injection (type 3) 6.098 = 0.02.
 Injection (type 2):Injection (type 3) 9.672 < 0.01.

TABLE 7. CORRELATION BETWEEN EFFECT ON OVARIAN WEIGHT AND EFFECT ON ATTAINMENT OF FEMALE STATE

Source of variation	No. of animals	Difference of mean ovarian weights (x)	' Q ' (from Table 6)	$p = \frac{Q+1}{2}$	Expected probit (y)
Injection (type 1)	200	4.9367	-0.430	0.2853	4.432
Injection (type 2)	106	2.5979	-0.525	0.2375	4.287
Injection (type 3)	121	9.3511	-0.058	0.4708	4.927
Operation	427	14.9577	+0.412	0.7060	5.542

After appropriate weighting of the data the regression equation is $y = 3.95 + 0.106x$ with variance of gradient 0.000 084 67, so that gradient = 0.106 ± 0.0092 ; hence $t_{[400]} = 11.538$ and $P < 0.001$. Columns 3 and 6 and the regression equation are illustrated graphically in Fig. 1.

to each value of Q are listed in Table 7. A regression equation of these expected probits (y), suitably weighted, upon the difference in mean ovarian weights (x), was then computed by the usual probit method (Finney, 1952). After appropriate weighting the equation obtained was

$$y = 3.95 + 0.106x.$$

The regression coefficient was 0.106 ± 0.0092 , whence $t_{[400]} = 11.583$ and $P < 0.001$. The equation and the four points upon which it is based are illustrated graphically in Fig. 1. It will be seen that the regression of the probit of Q upon difference in mean ovarian weight is highly significant; that

is to say there is an extremely close correlation between the effects of the various treatments upon sex reversal and the effects of these same treatments upon ovarian growth.

OVARIAN SIZE AT THE MOMENT OF ATTAINMENT OF A FUNCTIONAL FEMALE CONDITION

In all groups it was found that those animals, regardless of body weight, which had the largest ovaries had become females, while those with the smallest ovaries had remained in the non-sexual condition; there was a very narrow zone of overlap, i.e. the animal with the smallest ovary of those which had become females, had, in some groups, an ovary just a little smaller than the largest of those which remained non-sexual. Fig. 2 illustrates the range

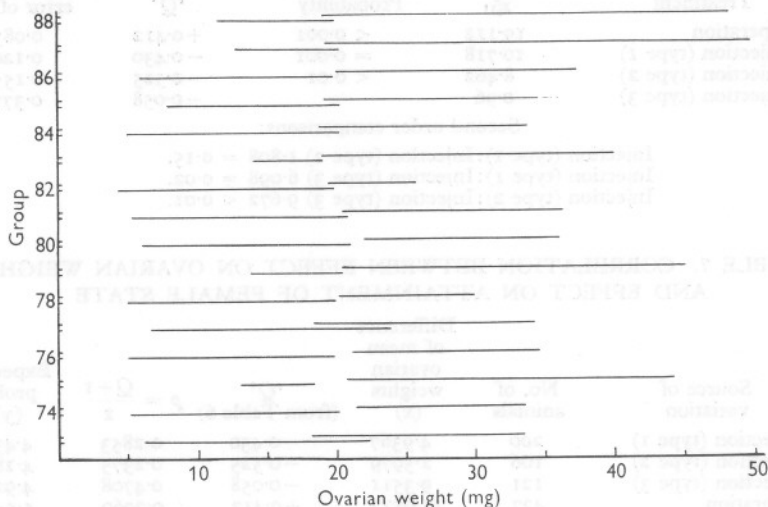


Fig. 2. The range of ovarian weights in animals of groups 73-88 (see Table 5). The lower line of each pair represents the range of weights found in those animals which remained non-sexual after the moult following experimental interference; the upper line the range in those which became functional females.

of ovarian weights in the various groups of animals referred to in the previous sections. Statistical analysis confirms visual impressions that there is a very strong correlation between a large ovary and attainment of the female condition. The correlation coefficient is 0.99. Inspection of the graph also suggests that there is little variation in the ovarian weight at the boundary between those which had and those which had not become females. In all groups the largest ovary of a prawn which had remained non-sexual and the smallest ovary of a prawn which had become female were approximately 20 mg. Once more statistical analysis confirms this impression. Curve-fitting techniques applied to this whole assembly of ovarian weights and to the individual

groups reveal insignificant departures from expectation with a $\chi^2_{[15]}$ of 11.85, whence $P = 0.7$. In other words the ovarian weight at the moment of attainment of a functional female state was not modified by any of the treatments which I had applied.

DISCUSSION

These experiments make it evident that ovarian growth and maturation in *Pandalus borealis*, as in *Palaemon*, *Lysmata*, *Carcinus* and other species of decapods, is regulated, at least in part, by an ovary-inhibiting hormone produced by the X organ-sinus gland complex. This hormone is found in the medulla terminalis ganglionic X organ and in the sinus gland, but has not been found in the sensory pore X organ. It seems probable, therefore, that it is produced in the *MTGX*, stored in the sinus gland and released into the blood from there. No other tissue in the eyestalk which has been investigated had any effect upon ovarian growth or maturation. This is to be contrasted with the conclusions of Aoto & Nishida (1956) who, working with *Pandalus kessleri*, likewise found that eyestalk removal led to premature ovarian growth (at least during early summer). On histological grounds, however, they concluded that an ovary-inhibiting hormone was produced by the X organ (by which term they appear to mean the sensory pore X organ), while an antagonistic hormone was produced by the 'circum-orbital gland'. In *P. borealis* I have been able to find no trace of any hormone affecting gonadal or sexual development in the sensory pore X organ. Certainly there is a seasonal cycle in the histological appearance of this organ (Carlisle, 1959a), and since breeding is also seasonal, these two cycles must perforce be correlated. This is not, however, to be taken as a causal relationship without any other supporting evidence, and a diligent search has failed to produce any such evidence. We must conclude then that the seasonal changes in the *SPX* do not correspond to seasonal secretion of an ovary-inhibiting hormone, but rather are an expression of some other seasonal secretion, or annual cycle of metabolism. The same arguments apply also to the retinotectal tissue, which likewise undergoes a seasonal cycle in histological appearance. Here too I have failed to find any evidence of the secretion of hormones regulating any aspect of the sexual cycle. I believe that this tissue is probably homologous with that described as the circum-orbital gland by Aoto & Nishida. I have been able to find no evidence for any eyestalk hormone controlling any aspect of the sexual cycle of *P. borealis*, except the ovary-inhibiting hormone secreted by the neurosecretory cells of the *MTGX* and released into the blood by the neurohaemal endings of the sinus gland.

Similarly I could find no evidence that any hormone except one emanating from the *MTGX*-sinus gland complex, played any part in governing the change from the non-sexual to the female phase. Extracts of the *SPX*, of the

retroretinal tissue or of the medulla externa were without effect, while eyestalk removal increased the proportion which became females at the next moult, an effect which was countered by the injection of extracts of sinus gland or of *MTGX*. We must conclude that some hormone produced in the *MTGX* and released into the blood at the sinus gland is responsible for restraining the attainment of the functional female condition. It is possible, but unlikely, that the vas deferens gland plays some part in this; the point requires further investigation. I have no evidence as to any part played by the Y organ.

The high degree of correlation found between the effects of the various treatments upon the attainment of the female form and upon ovarian inhibition makes it seem probable that but one hormone is concerned in inhibiting premature ovarian growth and in restraining the change from the non-sexual to the female condition. At the moment of this change the ovary seems always to have reached a certain minimum size. This seems unlikely to be a causal relationship but rather an expression of the same agent acting upon both the change of status and upon ovarian development.

I should like to acknowledge the friendly assistance afforded to me by the former director, Dr G. Gustafson, of the K. Svenska Vetenskapsakademien Kristinebergs Zoologiska Station, and to thank him for all he has done to make three visits to the laboratory pleasurable as well as profitable.

SUMMARY

Ovarian growth and vitellogenesis are inhibited by the ovary-inhibiting hormone secreted by the medulla terminalis ganglionic X organ and the sinus gland. No other tissue within the eyestalk has been found to have any action upon ovarian growth. Similarly the change from the non-sexual condition to the functional female state is inhibited by a hormone emanating from the same centres, while no other tissue within the eyestalk has been found to have any effect. The two inhibitions show a high degree of correlation which makes it probable that they represent two responses to the same hormone.

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