

## REPRODUCTION VERSUS SOMATIC GROWTH: HORMONAL CONTROL IN *OCTOPUS VULGARIS*

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### SUMMARY

1. *Octopus vulgaris* can be forced into precocious maturity by removal of the subpedunculate lobe from the brain, an operation that releases the optic glands from inhibition, and allows them to secrete a gonadotropin.

2.  $^{14}\text{C}$ -leucine was injected into the bloodstream of immature animals and its subsequent incorporation into muscle protein followed by taking successive samples from the arms. The optic glands were then activated, and a further injection of  $^3\text{H}$ -leucine given and followed by means of further arm samples.

3. Optic gland secretion suppresses protein synthesis in the muscles. This is associated with an increase in the total amino acid pool in the muscles and with a considerable increase in the concentration of free amino acids circulating in the blood.

4. If an ovary is present these events are associated with a rapid growth of the ovary and its ducts, and a loss of weight elsewhere. In ovariectomized animals the ducts grow, but there is no yolk to absorb the large pool of free amino acids, and the animals gain weight by osmotic uptake of water into the muscles.

5. The developing ovary may produce a hormone that increases the release of amino acids from muscle, since the concentration circulating in the blood of intact animals remains at least as high as in ovariectomized octopuses, despite the demands of the developing ovary.

6. These matters are discussed in relation to other evidence for a gonadal hormone and in relation to the 'self-destruct' effect of the optic gland secretion in determining the post-reproductive death of octopuses.

### INTRODUCTION

Sexual maturation in *Octopus vulgaris* results from the increased secretion of a gonadotropin by the optic glands (Wells & Wells, 1959, 1972, 1975). Reproduction is the terminal event in the life cycle; the animals breed once and then die. In females egg laying is preceded by a period during which the ovary enlarges very considerably and followed by a period during which the female broods the eggs. The large ovary (up to 25 % of body weight) constricts the gut, the animals stop feeding as oviposition approaches and reject food while brooding. Digestive enzyme production is reduced (Sakaguchi, 1968). By the time the eggs hatch (4 to 6 weeks at 22 °C; Mangold &

Boletzky, 1973), the female appears notably emaciated and soon afterwards dies. The death of female octopuses is perhaps not very surprising; the animals starve and at the same time produce great quantities of yolk which contains protein accounting for 25 % of the wet weight – more than double the protein content of muscle (unpublished observations). But male octopuses also die, and at about the same time as their mates; they too cease to feed during the last few weeks of their lives, and, like females, they show degenerative changes that range from a failure to control skin colour and texture to the development of skin lesions that fail to heal (van Heukelem, 1973).

The situation in males suggests that the terminal condition of females is not simply due to starvation and the demands of a developing ovary. The optic gland hormone may have much more widespread effects upon metabolism.

An obvious experiment was to ovariectomise females, excise the optic glands by removing the source of their inhibitory nerve supply in the subpedunculate lobe (Wells & Wells, 1959) and to compare the condition in these precociously maturing ovariectomized animals with that in similar animals retaining their ovaries. To these we have added immature controls, starved immature controls, and males thrust into precocious maturity by the same operations as the experimental females. The experiments show that the optic gland hormone has effects upon the movements of amino acids and protein synthesis that cannot be attributed to the demands of a growing ovary.

#### METHODS

The experiments to be described below were carried out at the Stazione Zoologica Naples, during July and August 1975. A few additional observations were made at the Laboratoire Arago, Banyuls, during August 1977. The animals used throughout were *Octopus vulgaris* Cuvier, of between 250 and 400 g. Before any experiment was begun the octopuses, collected in trawls and traps, were examined for possible damage and kept in the laboratory for several days. Damaged individuals, and any that failed to feed regularly after the first 2 to 3 days, were discarded.

Because of limited facilities for keeping labelled animals in the partially recirculating seawater system at Naples, the number of animals used for the experiments at Naples was restricted to 17. For the principal experiment there were five groups of animals as follows:

1. Controls (C animals)  $n = 3$ .
2. Starved controls (SC)  $n = 3$ .
3. Precociously maturing females (MF)  $n = 4$ .
4. Precociously maturing females with ovaries removed (MO)  $n = 4$ .
5. Precociously maturing males (MM)  $n = 3$ .

Each group included animals ranging from 250 to 350 g in weight; the groups were matched so that each included a similar range of weights.

Each octopus, whether in Banyuls or Naples was treated as follows: after an initial 'settling down' period in the laboratory, the octopus was anaesthetized (3 % urethane at Naples, 2½ % ethanol in Banyuls), the central pallial adductor muscles were cut and the mantle folded inside out to examine the gonad. Females were always immature; males slide more gently into sexual maturity and all had spermatophores when examined, as is usual in octopuses of the size used (Wells & Wells, 1972). Some of the

females (MO group) had their ovaries removed at this time, the eggs being extruded through a slit in the ovisac and the ovarian blood vessels ligated. Three days later feeding ceased and two days after this the animals were injected with  $^{14}\text{C}$ -leucine, at  $0.0150 \mu\text{Ci g}^{-1}$ , passed into a branchial heart under light anaesthesia. Feeding began again 24 h after injection, and 2 days after this the animals were yet again anaesthetized. This time central brain operations were made with the subpedunculate lobes removed from the animals (MF, MO, and MM groups) that it was wished to drive into precocious maturity; controls had dummy operations.

The animals were then fed regularly until day 14 when a period of 2 days starvation preceded a second injection, this time of  $^3\text{H}$ -leucine given at  $0.15 \mu\text{Ci g}^{-1}$ . Again the animals were starved for 24 h after injection. By this time females from the MF and MO groups were already showing marked increases in the size of the ovaries (where present) and oviducts.

The animals were killed at between 28 and 35 days after the initial  $^{14}\text{C}$  injection. During the intervening period, blood and arm samples were taken at intervals, as specified in Fig. 1; one of the advantages of *Octopus* as an experimental animal is that successive muscle samples can be taken by cutting off the outer third of each arm in turn, an apparently brutal treatment that has surprisingly little effect upon the animals' behaviour. Our octopuses regularly fed within minutes of removing an arm sample (without anaesthesia). A proportion of any wild-caught sample of octopuses from the Mediterranean is found to have one or more regenerating arms, and the animals are evidently well adapted to survive such mishaps.

Because we were at first uncertain of the survival of animals subjected to multiple arm-croppings, only one (the 'master') of the animals in each group was sampled on all occasions; that is at 6 and 24 h and at 3 and 12 days after each injection. The rest of the octopuses had samples taken only at 3 days after each injection or only at 3 days following the  $^{14}\text{C}$  injections and at 6 and 24 h and at 3 and 12 days after the second,  $^3\text{H}$ , injection ('sub-masters'). In the event all of the animals survived, so that it would probably have been possible to take a complete set of samples from each octopus.

It was thus possible to follow the incorporation of labelled leucine into muscle in maturing and immature animals with and without ovaries. Control and experimental groups could be compared and it was also possible within each experimental group to compare samples taken from the same animals before and after activation of the optic glands.

#### RADIOMETRICS

The labelled materials used were L-(U- $^{14}\text{C}$ ) leucine,  $348 \text{ mCi/mmol}$ ,  $50 \mu\text{Ci/ml}$  and L-(4, 5- $^3\text{H}$ ) leucine,  $53 \text{ Ci/mmol}$ ,  $1.0 \text{ mCi/ml}$ . These were diluted 1:3 with sterile seawater prior to injection as indicated above. Weighed muscle samples of 1 to 2 g were prepared by carefully trimming the skin and gelatinous layer below the skin from the central core of muscle at the proximal end of the clipped arm tips. These were frozen immediately and later, after addition of 4 ml of distilled water, refrozen. After thawing, samples were homogenized to a uniform froth with a Super Dispax Tissumizer (Tekmar Co., Cincinnati, Ohio). One gram aliquots of froth produced clear, nearly uniform solutions when added to 10 ml of Instagel which, when counted on a Packard Tri-Carb scintillation spectrophotometer and corrected for quenching,

gave values for total radioactivity in disintegrations per minute (DPM) comparable to other solubilizing techniques. Trichloroacetic acid (TCA) soluble DPM were determined by adding 1.50 ml of 20% w/v aqueous TCA at 4 °C to 1.50 g of froth; one ml aliquots of the supernate were counted in Instagel as indicated above. The difference between total and TCA soluble radioactivity was used as an estimate of 'protein' DPM. When part of the TCA precipitates were processed by the method of Greengard *et al.* (1964) for the isolation of protein, 5 to 10% of the 'protein' DPM were extracted with hot TCA and an additional 5 to 10% with hot alcohol and solvents. The extractable proportion varied little in individual animals, however, and did not effect the conclusions drawn, so that only the simple difference figures for TCA insoluble DPM are reported. When samples contained both  $^{14}\text{C}$  and  $^3\text{H}$  the DPM due to each isotope were calculated according to the manufacturer's recommendation using standard window settings and an external standards ratio technique.

#### AMINO ACID ANALYSES

Aliquots of the TCA supernates from the final muscle samples were also taken for amino acid analysis. Samples of blood were taken at the same time (after a minimum of 36 h without food) and added to an equal volume of 20% TCA. The TCA supernates were lyophilized to dryness and redissolved in 0.2 M sodium citrate buffered to pH 2.2. Analyses were carried out in a three-buffer single-column system similar to that described by Benson (1972) using a 1.5 mm by 300 mm column of Durham DC-4A resin and a ninhydrin detection system.

#### RESULTS

##### *Wet and dry weights*

The animals were weighed when they arrived in the laboratory and subsequently when injected and at 3 and 12 days after each injection. Animals kept for longer than 12 days after the second injection were weighed again at 16 days and at 19 days when they were killed.

Figure 1 shows the average weight change for each group of female octopuses (males will be considered in a separate section, below) plotted relative to the weight of each animal at the time (day 0) of its first  $^{14}\text{C}$  injection.

Starved controls showed a steady weight loss throughout the experiment (and have been omitted from Fig. 1). The animals shrunk steadily, mainly by loss of flesh from the arms, so that by the end of the experiment, having lost up to 50% of their body weight, they appeared notably cadaverous, with disproportionately large eyes and heads. They remained, nevertheless, alert and active, capable, so far as we could observe, of the full range of colour and skin texture changes to be seen in animals kept in aquaria. Starvation, *per se*, does not lead to the apparently inevitable degenerative changes seen in mature octopuses. Fed controls gained little weight during the first week – they were anaesthetized twice during this period, had arm samples taken, and were in any case starving at the beginning of the experiment so perhaps it is not very surprising. After the first week, they showed a steady weight gain of about 3.25 g per day. The food ration of about 10 g per day (crabs alternating with fish, 1 day crab,

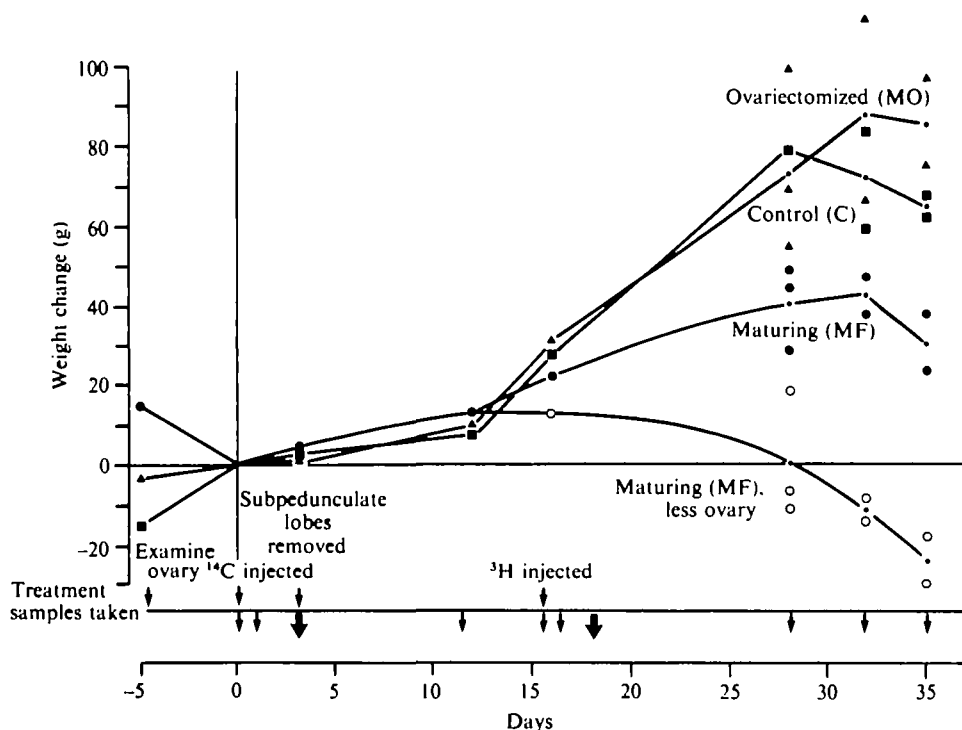


Fig. 1. Weight changes and injection and sampling schedule for female *Octopus* during the double label experiments. Timing of surgical procedures and injections described in the text are indicated above the lower scale. Times at which arm muscle samples were taken from all animals (heavy arrows) or from representatives from each group (light arrows) are indicated below the scale. Weight changes relative to day 0 (first injection) for immature females (C, squares,  $n=3$ ), maturing ovariectomized females (MO, triangles,  $n=4$ ) and maturing females (MF, filled circles,  $n=4$ ) are shown above. All the measurements made (and averages of these) are shown from day 28 onwards; before this points show averages only. The open circles show body weight of MF animals excluding measured or estimated ovary weights and indicate the rate of ovarian development. Estimated ovary weights (for the maturing, less ovary, plot) are calculated from weights at death and development rates in other animals (Wells, 1960; Wells and Wells, 1959). (All weight changes corrected for armtips removed).

2 days fish) was evidently sufficient to sustain a modest growth in normal animals. On day 28 the 'master' animals were killed; the two remaining controls actually lost weight after this, perhaps because the temperature was abnormally high ( $27^{\circ}\text{C}$  against  $24 \pm 1.0^{\circ}\text{C}$  during most of the experiment) at the time.

The experimental groups showed the same basic pattern of initial loss followed by a steady weight gain during the second or third weeks of the experiment. The maturing animals with intact ovaries (MF group) gained weight more slowly than the controls. Almost all of their limited weight gain was in the ovary. When the final actual weight (or, for the intermediate points, the estimated ovary weight, see Fig. 1) is deducted, the MF animals can be seen to have lost weight from about 20 days after removal of the subpedunculate lobes.

Ovariectomized animals with activated optic glands showed a surprising gain in weight. Their total weight rose by an average of  $3.7\text{ g}$  per day, a little faster than that

Table 1

Animal	Wt at operation (g)	Wt of ovary removed (g)	Body wt at death (g)	Wt of ducts (g)	Duct wt + body wt ( $\times 10^3$ )	Days since operation
1. MF animals (mature females)						
212	250	29.7*	230	3.32	12.2†	33
213	350	50.8	375	4.18	11.1	25
214	250	8.8†	210	2.80	9.1†	32
525	270	27.9	260	2.23	8.5	25
2. MO animals (ovariectomized when immature)						
454	350	0.53	420	2.64	6.2	20
457	220	0.30	300	2.30	7.6	33
461	300	0.36	430	3.02	7.0	34
509	220	0.40	260	1.95	7.5	32

\* 212 laid eggs; weight of eggs in aquarium when killed = 40.2 g.

† 214 laid eggs; weight of eggs in aquarium when killed = 97.7 g.

‡ Ratios with weight of eggs from aquarium added to body weights; without these eggs 212 = 14.4, 214 = 13.3.

of controls. In these ovariectomized octopuses the oviduct and oviducal glands increased very greatly in size, at a rate comparable with that found in the MF group (Table 1). The ovariectomized animals were apparently maturing normally, in so far as it is possible to deduce this from the condition of their sex ducts. Yet they gained weight, unlike octopuses with intact ovaries.

The apparent anomaly was at least partially resolved when the *dry* rather than the *wet* weights of the successive muscle samples taken from the master animals were examined. In the fed control the percentage of protein (dry weight protein to total wet weight of sample) remained steady at  $10.7 \pm 1.2\%$ . In the maturing female it was  $8.8 \pm 0.8\%$ . In the ovariectomized female it dropped progressively from 10.3% on day 12 to 6.8% on day 28. Much of the weight gain in the ovariectomized animal was thus water; in terms of dry weight, the animal behaved as expected, like an MF octopus. As we shall see below, the low protein content of ovariectomized animals is associated with the retention of large quantities of amino acid in the muscles, apparently leading to an osmotic uptake of water that more than compensates for the weight of protein lost.

#### *Incorporation and loss of $^{14}\text{C}$ - and $^3\text{H}$ -leucine into arm protein*

Figure 2 summarizes the data arising from successive arm sampling of the 'master' and 'submaster' animals from groups C, MF and MO. Preliminary examination of the  $^{14}\text{C}$ -leucine data showed no significant differences between intact immature and ovariectomized immature animals (as was to be expected since the immature ovary forms only about 1/500th of the body weight) and both types are considered together as controls at this stage.

The incorporation of  $^3\text{H}$ -leucine did, in contrast, show considerable differences between controls and the two groups of experimental animals. Injections of  $^3\text{H}$ -leucine were made 12 days after removal of the subpedunculate lobes from the MF and MO

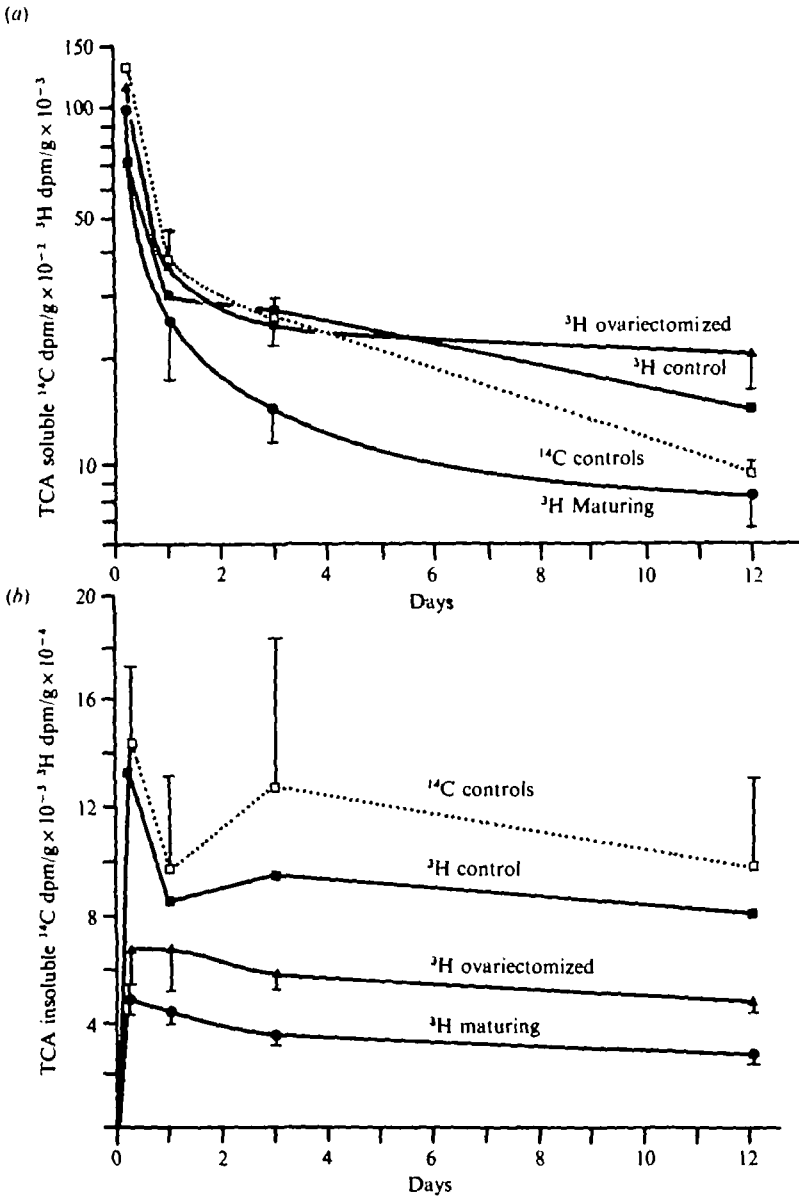


Fig. 2. Movement of leucine tracer through the free (TCA soluble, (a)) and 'protein' (TCA insoluble, (b)) compartments in the muscle of immature, maturing and maturing-ovariectomized female *Octopus*. Bars indicate 1 S.D. See text for details.

groups. This is long enough to have induced considerable secretion by the optic glands and a notable growth of the ovary, which would by now be laying down considerable quantities of proteinaceous yolk (O'Dor & Wells, 1973). Growth of the ovary is reflected in the somewhat reduced uptake and more rapid subsequent loss of leucine from the MF animals (Fig. 2a); MO animals more closely resemble controls and,

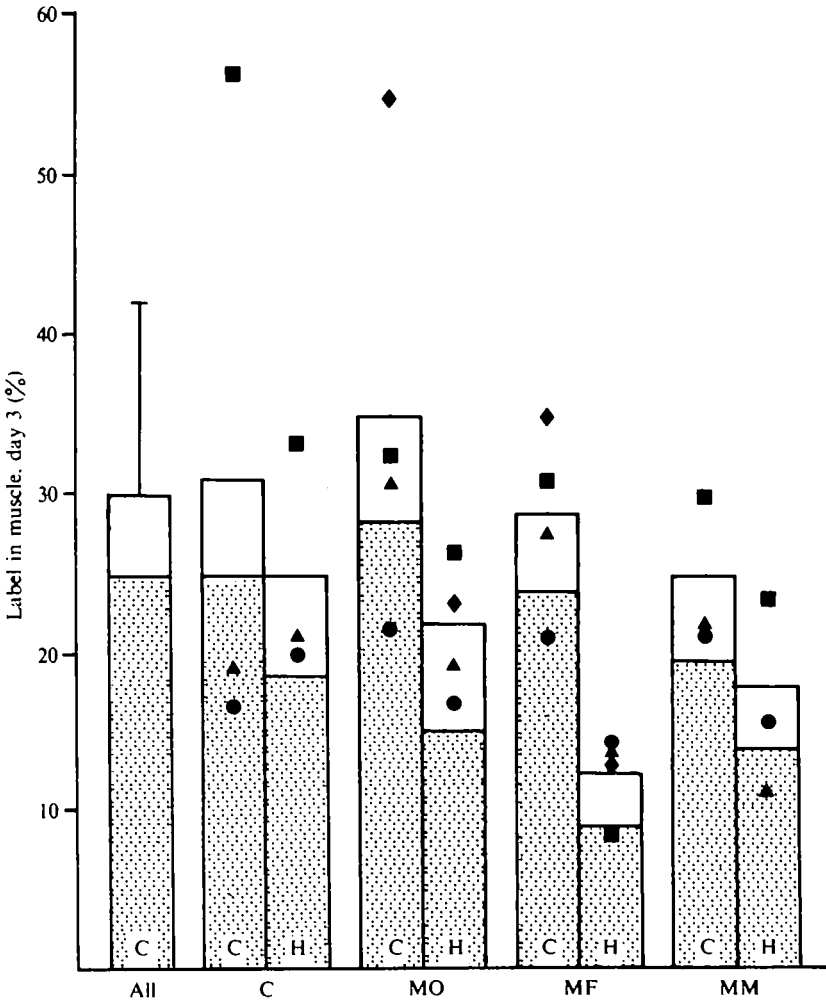


Fig. 3. Comparison of <sup>14</sup>C-leucine uptake by muscle in immature octopuses to <sup>3</sup>H-leucine uptake in the same animals after induction of precocious maturation. Bars indicate average total label in muscle after 3 days as a percentage of the injected dose. Shaded areas are the TCA insoluble portion ('protein'). The first bar also shows mean and standard deviation in <sup>14</sup>C content for all 14 animals while immature. Paired bars show average <sup>14</sup>C (C) and <sup>3</sup>H (H) content in groups receiving the same treatment (C, no treatment; MO, maturing-ovariectomized; MF, maturing females; MM, maturing males). Symbols show measured values for each animal in each group.

indeed, eventually show more TCA-soluble counts per milligram of muscle than these, for reasons that have already been discussed above.

Incorporation of <sup>3</sup>H-leucine into arm muscle protein is reduced in both the MF and the MO animals; the effect is greatest in the octopuses with intact ovaries (Fig. 2*b*). A second indication of reduced protein synthesis in the muscle can be seen when the figures for label present at 24 h are compared with those for label present at 3 days (Fig. 2*a*). Controls (all groups for <sup>14</sup>C, and the control animal for <sup>3</sup>H as well) all show an increase in protein label between the 24 h and 3 day samples. This presumably



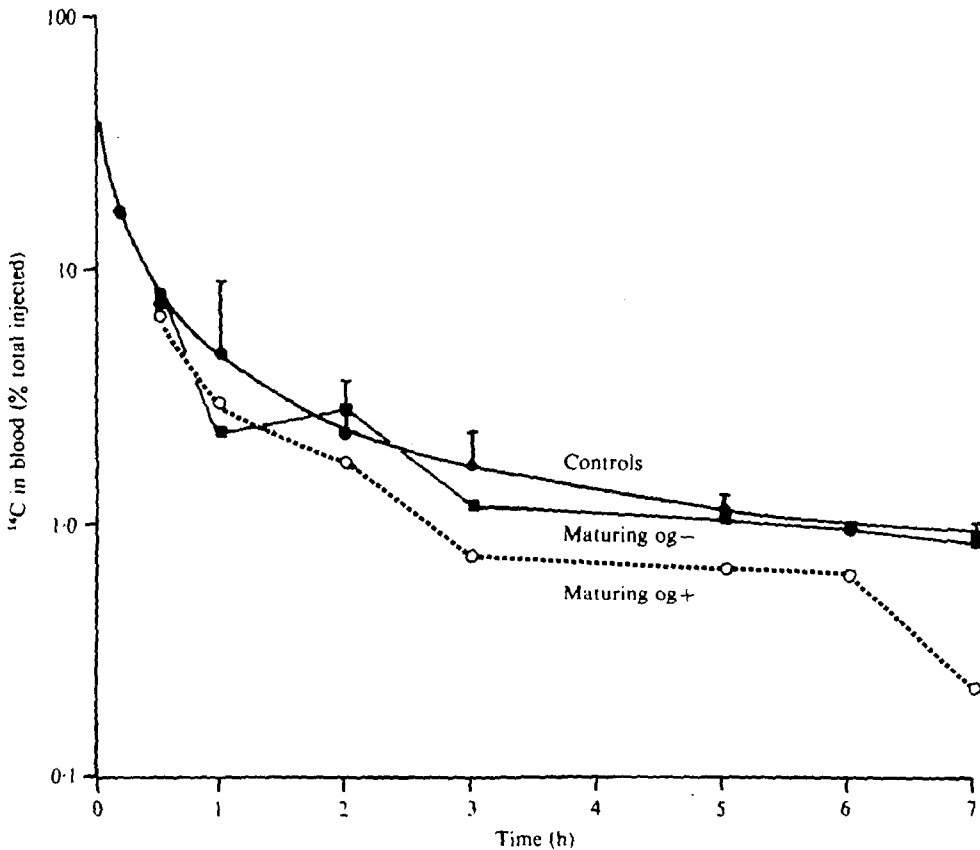


Fig. 4. Disappearance of  $^{14}\text{C}$ -leucine from the blood of immature (filled circles) and maturing (open circles) octopuses and maturing octopuses with optic glands removed (filled squares).

results from the 'washing-out' of labelled leucine in other tissues when the animals return to feeding after the 24 h sample. The effect is not seen in MF or MO animals where protein synthesis by the muscles is reduced and (as will be shown below) more free leucine is retained in the blood.

Further data confirming these results were obtained from animals not subjected to the full sequence of arm samples. These are included in Fig. 3; incorporation of  $^3\text{H}$ -leucine into the muscle protein of animals with active optic glands was always considerably lower than the incorporation of  $^{14}\text{C}$ -leucine into the muscles of the same animals prior to gland activation. Only one control animal showed a reduction in incorporation and on average the differences were not significant in this group. Comparison of  $^{14}\text{C}$  and  $^3\text{H}$  incorporation data from Figs. 2 and 3 by paired-data  $t$ -test indicates significant reductions (MO,  $P < 0.05$ ; MF,  $P < 0.02$ ; MM,  $P < 0.01$ ) in  $^3\text{H}$  in muscle protein; in group MO this occurs despite the absence of the yolk-forming tissue. Protein yolk is formed in the follicle cells within the ovary itself (Buckley, 1977; O'Dor & Wells, 1973).

*Amino acid levels in the blood*

In a previous report (O'Dor & Wells, 1973), we have described the rapid removal of injected leucine tracer from the blood of normal octopuses; 95 % of the radioactivity is removed within the first hour. Fig. 4 records the disappearance of label over 7 h from the blood of OG+ animals (equivalent to MF octopuses in Figs. 1 and 2) and from octopuses with developing ovaries which had had their optic glands removed five days prior to the injection (OG-octopuses). These data are from the same series of experiments reported in 1973 and were obtained by killing animals at the stated intervals after injection.

The label remaining in the blood of OG+ animals at any time was consistently less than in controls, but removal of the optic glands returned the levels to those of controls. One explanation of this would be that rapid leucine uptake by the ovary removes free leucine from the blood and lowers circulating leucine levels. This would remove tracer directly and also increase its removal indirectly by increasing specific activity in the blood. With reduced ovarian uptake after optic gland removal (O'Dor & Wells, 1973), conditions should return to normal. The situation is not this simple, however, since analysis of the free amino acids in the blood of control, MF and MO animals from the double label experiment (Table 2) indicates that blood free amino acid levels in MF animals average more than three times, and in MO animals more than double those in controls.

In a further experiment carried out in Banyuls in 1977 blood samples were taken after two days starvation from two females which had had their ovaries removed in an advanced stage of development 2-4 weeks earlier (one was naturally maturing and the other had had its subpedunculate lobe removed 23 days before ovariectomy). The optic glands were then removed and 5 days later a second set of samples was taken; as before the animals were starved for two days before sampling. In each case the total concentration of the common amino acids dropped sharply after optic gland removal (from 1616 to 1173 nmol/ml in the naturally maturing animal and from 1958 to 1111 in the surgically activated one) and the same effect was seen on free leucine (105 to 31 and 106 to 55 nmol/ml respectively). All of this evidence points to effects of the optic glands acting outside the ovary to increase amino acid levels in the blood.

*Amino acid levels in muscle*

Muscle amino acid levels (Table 2) were reduced in MF animals, but not apparently in the MOs. It should be borne in mind, however, that the MOs had considerably less protein (per wet weight) in their muscle than either the C or MF animals. If, as seems likely, solid muscle protein is replaced by fluid, the total pool of free amino acids in the MO animals is likely to have been much greater than in either of the others. This would be consistent with the findings from the experiments summarized in Figs. 2 and 3, which show reduced leucine uptake and protein synthesis in both the MF and the MO animals. The optic gland hormone would appear to inhibit muscle protein synthesis: in maturing animals with an intact ovary much of the amino acid thus rendered available is taken up by the growing ovary; in ovariectomized octopuses with active optic glands there is no place for amino acids taken up with food to go and it

Table 2. Free amino acid levels in *Octopus vulgaris*

Number of animals	Blood (nm/ml) $\pm$ s.d.				Muscle ( $\mu\text{m/g}$ ) $\pm$ s.d.			
	C (3)	MF (4)	MO (4)	MM (3)	C (3)	MF (4)	MO (4)	MM (3)
Alanine	53 $\pm$ 28	345 $\pm$ 65	135 $\pm$ 83	233 $\pm$ 290	9.4 $\pm$ 3.4	8.8 $\pm$ 4.8	9.5 $\pm$ 6.3	9.6 $\pm$ 1.8
Arginine	8 $\pm$ 14	31 $\pm$ 4	13 $\pm$ 17	23 $\pm$ 4	13.6 $\pm$ 6.7	9.4 $\pm$ 6.4	11.6 $\pm$ 5.9	11.7 $\pm$ 3.2
Aspartic acid	20 $\pm$ 5	32 $\pm$ 12	60 $\pm$ 29	85 $\pm$ 120	4.9 $\pm$ 1.6	3.9 $\pm$ 1.5	4.6 $\pm$ 2.4	5.1 $\pm$ 0.0
Cystine	33 $\pm$ 6	29 $\pm$ 9	86 $\pm$ 35	122 $\pm$ 151	0	0	0	0.8 $\pm$ 1.0
Glutamic acid	11 $\pm$ 9	257 $\pm$ 292	21 $\pm$ 16	123 $\pm$ 180	8.7 $\pm$ 1.7	5.8 $\pm$ 1.8	11.1 $\pm$ 8.6	7.5 $\pm$ 0.2
Glycine	27 $\pm$ 6	60 $\pm$ 18	29 $\pm$ 9	76 $\pm$ 85	2.0 $\pm$ 0.2	1.1 $\pm$ 0.5	1.7 $\pm$ 0.8	2.4 $\pm$ 1.4
Histidine	22 $\pm$ 20	64 $\pm$ 45	42 $\pm$ 49	46 $\pm$ 24	1.1 $\pm$ 0.7	1.4 $\pm$ 1.1	2.6 $\pm$ 1.6	1.4 $\pm$ 0.8
Isoleucine	15 $\pm$ 5	62 $\pm$ 4	43 $\pm$ 25	54 $\pm$ 42	1.1 $\pm$ 0.3	0.8 $\pm$ 0.5	1.2 $\pm$ 0.6	1.9 $\pm$ 1.3
Leucine	25 $\pm$ 0	84 $\pm$ 15	53 $\pm$ 31	62 $\pm$ 51	3.1 $\pm$ 1.6	2.3 $\pm$ 1.5	3.3 $\pm$ 1.8	4.3 $\pm$ 2.0
Lysine	30 $\pm$ 9	97 $\pm$ 68	70 $\pm$ 33	73 $\pm$ 61	3.5 $\pm$ 1.6	2.7 $\pm$ 2.3	3.3 $\pm$ 1.4	4.0 $\pm$ 0.9
Methionine	3 $\pm$ 6	6 $\pm$ 11	6 $\pm$ 6	7 $\pm$ 6	0.8 $\pm$ 0.8	0.2 $\pm$ 0.3	1.0 $\pm$ 0.6	1.6 $\pm$ 0.9
Phenylalanine	5 $\pm$ 9	18 $\pm$ 16	0	12 $\pm$ 11	1.0 $\pm$ 0.1	0.9 $\pm$ 0.4	0.9 $\pm$ 0.2	1.9 $\pm$ 1.4
Proline	42 $\pm$ 23	117 $\pm$ 31	86 $\pm$ 27	85 $\pm$ 90	12.0 $\pm$ 4.6	6.1 $\pm$ 2.7	7.5 $\pm$ 4.6	5.8 $\pm$ 1.6
Serine	18 $\pm$ 8	59 $\pm$ 44	66 $\pm$ 27	128 $\pm$ 170	4.5 $\pm$ 2.2	3.4 $\pm$ 2.1	5.1 $\pm$ 3.1	4.8 $\pm$ 0.1
Threonine	15 $\pm$ 5	25 $\pm$ 35	85 $\pm$ 65	119 $\pm$ 160	2.0 $\pm$ 0.0	1.4 $\pm$ 1.5	3.0 $\pm$ 2.8	3.4 $\pm$ 0.3
Tryptophan	32 $\pm$ 16	68 $\pm$ 48	60 $\pm$ 37	46 $\pm$ 5	0.8 $\pm$ 0.1	0.8 $\pm$ 1.0	0.9 $\pm$ 0.4	1.7 $\pm$ 1.1
Tyrosine	20 $\pm$ 9	44 $\pm$ 11	99 $\pm$ 56	74 $\pm$ 82	1.0 $\pm$ 0.1	0.8 $\pm$ 0.7	1.6 $\pm$ 0.9	2.0 $\pm$ 1.6
Valine	35 $\pm$ 37	108 $\pm$ 25	66 $\pm$ 38	83 $\pm$ 104	1.5 $\pm$ 0.4	1.0 $\pm$ 0.8	1.8 $\pm$ 1.1	2.0 $\pm$ 1.1
Total (common)	414 $\pm$ 121	1,506 $\pm$ 225	1,020 $\pm$ 428	1,451 $\pm$ 1571	71.0 $\pm$ 8.4	50.8 $\pm$ 21.4	70.7 $\pm$ 35.5	71.9 $\pm$ 10.0

accumulates, causing osmotic uptake of water and a considerable increase in wet weight despite the reduced synthesis of new protein.

### *Quantitative changes in leucine utilization*

Because of the complex relationship between muscle, blood and ovary the movement of tracer into the various free amino acid and protein pools is influenced by changes in free leucine concentration, leucine uptake and protein synthesis in any of these compartments. Tracer leucine movements and metabolism in feeding, fasting (unfed for 1–3 days) and long-term starving octopuses are predicatable from a mathematical model of leucine utilization with a dietary input of  $26\ \mu\text{mol}$  leucine/h, and a consumption of  $16\ \mu\text{mol}$ /h for energy in animals of the size used in the present experiments (O'Dor, Durward & Wells, in preparation). The difference allows for the observed formation of  $3.25\ \text{g}$  of muscle tissue per day (muscle contains about  $70\ \mu\text{mol}$ /g of total leucine). The formation of egg yolk in MF animals increases the leucine demand by about  $14\ \mu\text{mol}$ /h (about  $50\ \text{g}$  of eggs containing  $135\ \mu\text{mol}$  leucine/g formed in 20 days) to  $30\ \mu\text{mol}$ /h,  $4\ \mu\text{mol}$ /h more than the diet supplies. The loss of  $25\ \text{g}$  of muscle tissue from MF animals during this period would make up this deficit. When the model of a Control (C) animal (O'Dor, Durward & Wells, in prep.) is adjusted to account for this additional demand and for the changes in free leucine concentration in blood and muscle, various hypotheses about the cause of decreased incorporation of label into muscle protein can be tested.

In the model, even the combined effects of leucine uptake by the growing ovary and the decrease in specific activity caused by the increase in unlabelled leucine in the blood cannot account for the decrease in muscle protein label. Qualitatively, a decrease in free leucine uptake by muscle is consistent with both the increase in blood leucine and the decreased label in muscle protein, but quantitatively the reduction in uptake needed to account completely for the decreased label in muscle is too great to allow the observed levels of label in the free amino acid pool. The change in the model which best predicts the experimental observations is an 80% reduction in protein synthesis in muscle. This same reduction in synthesis in a model adjusted to the leucine demands and concentrations of MO animals predicts both the intermediate value for incorporation of label into protein in this group seen in Fig. 2*b* and the prolonged retention of label in the muscle free leucine pool seen in Fig. 2*a*. The only change in either pool size or label movements in MF and MO animals which is not a predictable consequence of a reduction in muscle protein synthesis is the much higher blood to muscle ratios in MF animals compared with MO animals, a matter which is discussed below.

### *The situation in males*

Three male animals were used in the double-label experiments. All had the sub-pedunculate lobes removed and all had enlarged optic glands at the end of the experiment. In males, the testis enlarges considerably and the number of spermatophores made increases greatly after the optic glands are activated (Wells & Wells, 1972). The metabolic demand of the male system hardly seems likely, however, to equal that of an ovary in vitellogenesis. The optic gland hormone, so far as we know, is the same in males and females (Wells & Wells, 1975; Wells *et al.*, 1975), and if one assumes that it

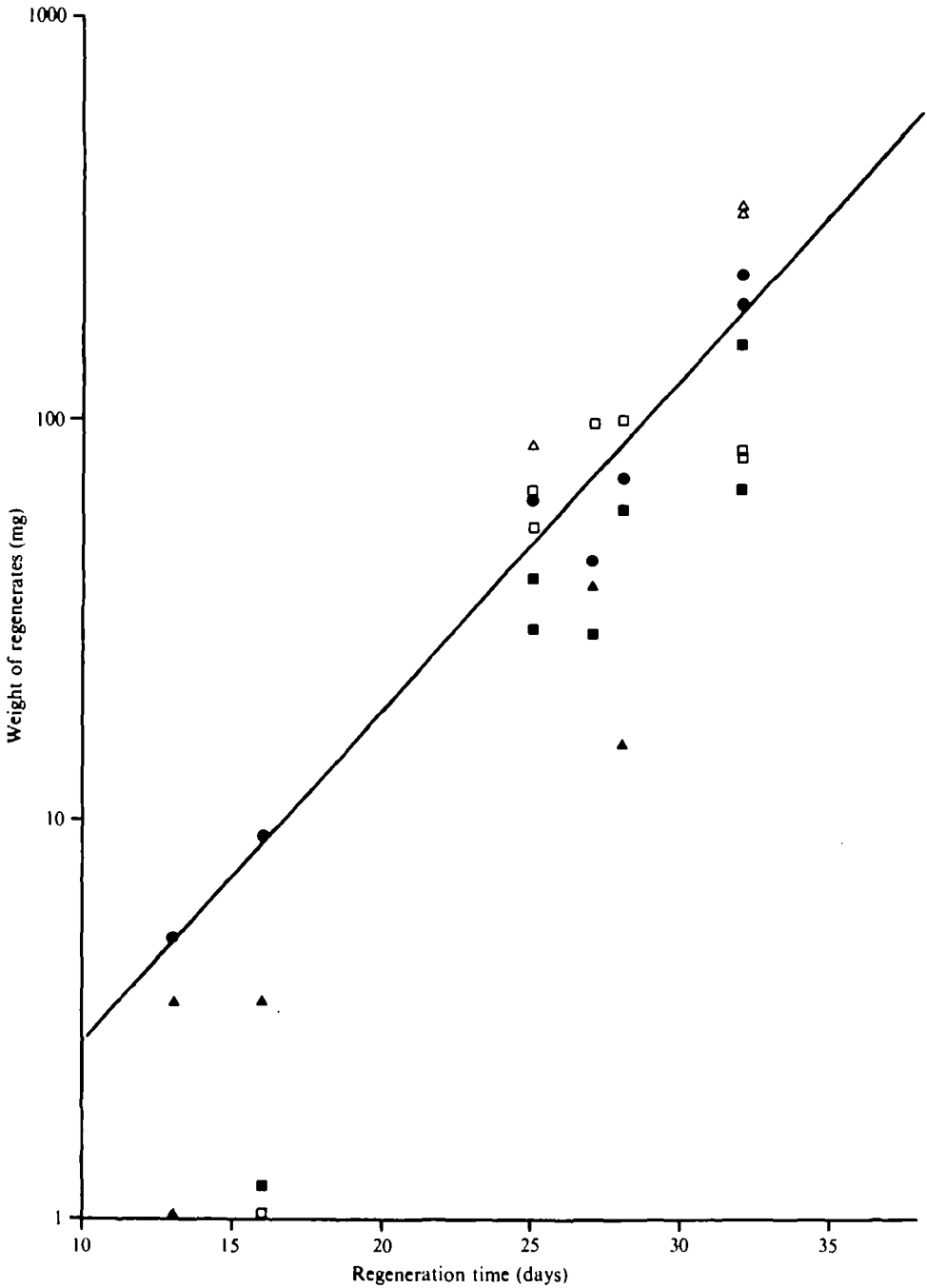


Fig. 5. Growth of regenerating armtips. Immature females (filled circles), maturing females (filled squares), ovariectomized maturing females (open squares) and maturing males (filled triangles). Hectocotyli of maturing males are shown as open triangles. Line is the least-squares regression for the control data.

will have the same effects upon amino acid uptake and protein storage in the two sexes, one might expect to find levels of amino acids in blood and muscle somewhere between the levels found in MF and MO animals. This expectation was, broadly, confirmed. As Table 2 shows, males with activated optic glands show the same increase in blood amino acid levels as their female counterparts. But the results obtained were very variable – one of the animals grew more rapidly than any of the other animals in the experiment and another became notably oedematous and had the highest blood amino acid levels of any animal measured. It would be unwise to conclude much from these data alone; insofar as it goes, it implies that the effect of optic gland activity is similar in males and females.

### *Regeneration of lost armtips*

In nature, octopuses are often found to have arms that are regenerating; loss of one or more armtips is a hazard that the animals evidently face in the course of their everyday life. It has, moreover, been noted that wound repair is difficult in fully mature animals, which develop skin lesions that fail to heal (van Heukelem, 1973).

Arm samples were taken at various stages during the present experiment, and the armtips had in most cases begun to regenerate by the time the experiments were complete. The data are sparse but quoted here (Fig. 5) because they provide yet further evidence that sexual maturation is associated with a reduction in somatic growth. The regenerating armtips of MF and MM animals seem to grow less rapidly than those of controls, despite the high amino acid levels in their blood, which one might otherwise have expected to aid the growth of new tissues (figures for MO animals are also included in Fig. 5; but it should be remembered that these tips may have a lower protein content than the rest, see p. 20). An interesting apparent exception was in the case of the hectocotylized arms of males, which appeared to regenerate faster than the other arms of the same animals; the reduction in somatic growth may allow an exception in the case of this sexually important organ.

### DISCUSSION

The first objective of the present series of experiments was to investigate the hypothesis that activation of the optic glands causes changes to the physiology of *Octopus* that cannot be attributed to changes in the state of the gonads.

One effect of activating the glands is to put the ovary into vitellogenesis. There is a massive demand for materials to form yolk proteins that is apparently met by breaking down the arm muscles; the animals cease feeding and become notably emaciated as egg-laying approaches. These visible changes are reflected in the movement of labelled leucine injected into the bloodstream; what happens to the label presumably reflects what must happen to food entering the bloodstream by the more normal digestive route. The changes observed in the two series of experiments outlined above were:

(1) Label is cleared from the blood more rapidly in maturing female animals than from their immature controls. This effect is apparently attributable to the demands of the vitellogenic ovary and it disappears when the optic glands are removed (Fig. 4),

a finding in keeping with the results of *in vitro* experiments on amino acid uptake and protein synthesis by eggs (O'Dor & Wells, 1975; Wells *et al.* 1975).

(2) In experiments of longer duration, protein synthesis in the muscles is reduced (Fig. 2).

(3) The level of free amino acid in the blood is considerably elevated in animals with active optic glands (Table 2).

Since changes of types (2) and (3) are found in ovariectomized animals (and (3) at least is found in males) with active glands, they are not attributable to the demands of the developing ovary. It is, in any case, difficult to see how the demand of an ovary could actually increase rather than reduce blood amino acid levels. It is concluded that the optic glands have effects upon amino acid levels and protein synthesis in muscles as well as effects on the growth of the gonads.

From the muscle tissue amino acid levels in maturing ovariectomized (and male) animals, it seems probable that the optic gland effect is to inhibit protein synthesis rather than to reduce amino acid uptake. Increased amino acid levels in the blood would result (see p. 26) from the reduction in synthesis so that there is no need to postulate an effect upon release from the larger amino acid pool ( $100\times$  blood concentrations) in the muscles.

We do not know whether this effect of the optic glands on somatic growth is direct or indirect; in the absence of *in vitro* experiments on muscle protein synthesis (which are envisaged as a subsequent stage in this analysis) there is no way to be certain that the effect is direct, at present it is simply the most economical hypothesis. It is also just possible that the effect in somatic growth is not due to a product of the optic glands at all, but to activation of some further system also regulated by the subpedunculate lobe; Froesch (1974) has pointed out that this lobe also contains neurosecretory cells that appear to have no connection with the optic glands and has suggested that some of the effects attributed to the optic glands could be elicited by destroying these. Three lines of evidence argue against this possibility. One is that removal of the subpedunculate lobe does not alter growth rates if the optic glands are removed as well (Buckley, 1977; Wells & Wells, 1959). A second is that secretion by the optic glands drives the gonads to increase relative to body weight at very much the same rate however the glands are excited; implanted glands (Wells & Wells, 1975), and glands excited by cutting the optic tract close to the glands (Wells & Wells, 1959; an operation that would cut the nerve supply to the glands without interfering with the subpedunculate lobe or the neurosecretory axons running from there to the preorbital vein) have the same quantitative effect as removal of the subpedunculate lobe. Finally, there are the data from maturing animals (see p. 24) sampled before and after optic gland removal; the effect of the operation is to produce a sharp drop in circulating amino acid levels.

One further result of the present experiments deserves attention because it raises the possibility of a gonadal hormone. The ratio of blood to muscle amino acid levels was markedly different in animals maturing with and without ovaries; the animals with ovaries intact showed a high blood to muscle ratio that could not possibly be accounted for if the only action of the ovary was to withdraw amino acids from the blood (Table 2). It is possible that the ovary produces a hormone that increases the release of muscle amino acid into the blood from the pool in the muscles.

This is interesting because there has previously been little evidence for a gonadal hormone in *Octopus*. Taki (1944) claimed that castration led to swelling and eventual collapse of the male ducts and Richard & Lemaire (1975) have more recently shown that the weight of the mature female ducts is increased by ovariectomy in *Sepia*. Taki attributed his results to dependence upon a testicular hormone, Richard & Lemaire to the elimination of an inhibitory feedback acting upon the optic glands. Wells & Wells (1972) repeated Taki's experiments and showed that castration was only sometimes followed by swelling of the male ducts, an effect they attributed to surgical interference with the blood supply. Despite a single instance (in a male) where castration was followed (or, more probably, by chance, accompanied) by an increase in the size of the optic glands (Wells & Wells, 1969) they concluded from the effects of castration in a further 31 males that no gonad to optic gland feedback was occurring (Wells & Wells, 1972). In immature females, ovariectomy is not followed by enlargement of the ducts (Callan, 1940; Taki, 1944) as one might expect if optic gland secretion were being held back by products of the ovary. If Richard & Lemaire's (1975) interpretation is correct, the situation must be different in mature and immature females, and in mature males and females, or in *Octopus* and *Sepia*.

One possible explanation of the apparent anomaly has arisen from the results summarized in the present paper. We now know that maturing octopuses (whether ovariectomized or not) have a higher level of blood amino acids than their immature controls, or mature animals with their optic glands removed. We have seen that the absence of the ovary accompanied by a suppression of protein synthesis elsewhere almost certainly causes an increase in the muscle amino acid pool and a consequent osmotic uptake of water. Whether an increase in water content is the explanation of the increase in duct weights observed by Richard & Lemaire (but not, incidentally, confirmed in the smaller series of our own experiments summarized in Table 1) cannot be known, since they only measured wet weights. But it must, for the moment, remain a possibility, and it is the most economical hypothesis.

Wodinsky (1977) has recently published the results of experiments in which the optic glands were removed from mature *Octopus hummelincki*. The animals included females that had laid and were brooding eggs. He found that the animals generally abandoned their eggs, resumed feeding and growth and lived for considerably longer than mature animals retaining the glands. A resumption of growth is, of course, exactly what one would expect from the findings of the present series of experiments, and it is nice to have this corroborative evidence, although from a different species. All the evidence points to activation of the optic glands being an irreversible process, so that the post-reproductive animals continue to waste away until they die. Removal of the optic glands has been tried several times in immature *O. vulgaris* (see, for examples, Buckley, 1977, Wells & Wells, 1972) and the animals survive well though they have never been kept long enough to see whether they will eventually exceed the 'normal' size at death.

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