may be composite chromosomes (*31*) each consisting of a haploid set of genes or, alternatively, they may be smaller units that behave as a larger linkage unit at macronuclear division, perhaps through attachment to a common site on the nuclear membrane (*32*). During the formation of new linkage units some DNA sequences may be eliminated, and others may form heteroduplexes which lead to gene conversion. Since the behavior of H alleles implies that not all aspects of the process are random, we suggest that early-assorting loci are located near fragmentation or recombination sites which are particularly prone to gene conversion or gene conversion-like events (*33*). Tests of these ideas require further biochemical characterization of macronuclear development, particularly with respect to restriction-like endonucleases, further characterization of the mechanism of macronuclear division, and construction of detailed maps of both micronuclear and macronuclear genomes.

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21. F. P. Doerder and L. E. DeBault, in preparation.
22. The DNA content decreases from 132C to 90C between 50 and 100 fissions after conjugation (*21*). This would contribute to the delay in accumulation of stable subclones. However, since the matrix used to calculate the theoretical curves (*10*) cannot accommodate variable numbers of total subunits, points beyond 50 fissions are not shown in Fig. 3. Although they would be useful, they are not essential for the argument presented here.
23. J. W. McCoy (personal communication) has found evidence suggesting that the initial accumulation of subclones can vary with genetic background. Although the literature is unclear, apparently the same strains were used for the experiments in both (*14*) and (*16*). However, very few assortment experiments have been repeated, and the most consistent interpretation is that all assortment begins early after conjugation.
24. In assortment experiments clones are usually serially recloned only every 8 to 13 fissions, and often fission rates of individual clones are not monitored. Thus, it is possible that a stable, recloned cell could be the linear descendant of a stable cell that arose shortly after the previous transfer but, because of the length between transfers, such stability at an earlier fission would not be recorded. In computer simulations (Fig. 3) each fission is monitored.
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26. A careful reading of the literature shows that the delay in the onset of assortment is in fact overstated on the basis of 45 subunits. Specifically, the slower accumulation of stable subclones for 1 : 1 ratios (Fig. 2) has not been adequately considered. For example, Allen (*14*) found that the average delay between the expected and observed proportions of stable subclones for E-1 and E-2 heterozygotes was 40 fissions, and therefore concluded that for these loci each diploid subunit began to express only one allele at 40 fissions after conjugation. Yet in the same paper it is reported that at 40 fissions 1.9 and 3.9 percent of subclones heterozygous for E-1 and E-2, respectively, were stable. Since the probability of observing stable subclones soon after the onset of assortment is vanishingly small when the starting ratio of subunits is 1 : 1, the assortment process must have begun earlier. Similarly, for the P-1 locus assortment is said to begin at 50 fissions, although at 50 fissions 1 percent stable subclones were present (*16*). Overstatement also applies to loci identified by recessive mutations (not shown in Fig. 3). For example, Doerder (*6*) reported that assortment at two regulatory loci, r1 and r3, began between 20 to 30 and 30 to 40 fissions, respectively. However, close examination of the original data reveals patterns of gene expression that can only be accounted for by an ongoing process of assortment which began at least 15 to 25 fissions earlier.
27. Random distribution means that the replicated products have equal probability of moving to opposite poles (segregation) or the same pole (nondisjunction). Thus, in the case where a G_1 macronucleus posesses only one of a kind of subunit, there is a probability of 0.25 that one of the progeny cells will have no copies of it; that is, the probability of a stable subclone is 0.25. This behavior is independent of the total number of subunits.
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32. Independent behavior of subunits smaller than haploid genomes would, through random assortment and unequal macronuclear division, lead to aneuploidy unless there were compensatory differential subunit replication at each division. This appears unlikely since in *T. pyriformis* it appears that DNA molecules replicate only once each cell cycle [(*18*); H. A. Andersen and E. Zeuthen, *Exp. Cell Res.* **68**, 309 (1971)].
33. Since both alleles are almost always recovered from each new macronucleus, we suggest that the process leading to the formation of haploid subunits occurs after the 4C stage of macronuclear development (*13*). If gene conversion were to occur when two copies of each allele are present, a ratio of 3:1 could result; if conversion were also to occur at later stages, even further distortion could result. Alternatively, it is possible that following the assembly of new linkage groups, certain of these are preferentially over- or underreplicated. If such differential replication were a function of DNA sequences surrounding particular genes, deviation from 1 : 1 would result. This would be a gene-conversion-like event.
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Hormonal Inhibition of Feeding and Death in *Octopus*: Control by Optic Gland Secretion

Abstract. Female Octopus hummelincki *lays eggs, broods them, reduces its food intake, and dies after the young hatch. Removal of both optic glands after spawning results in cessation of broodiness, resumption of feeding, increased growth, and greatly extended life-span. Optic gland secretions may cause death of most cephalopods and may function to control population size.*

It is well known that the female octopus spawns once in its life, eats less while caring for the eggs, and invariably dies shortly after the eggs hatch. This report indicates that death is due to a secretion or secretions from the optic glands. When these glands are removed after eggs have been laid, the female ceases to brood the eggs, begins to eat again, gains weight, and lives for a prolonged period.

The optic glands are the only definitely

Table 1. Mean, standard deviation (S.D.), and range for body weights and longevity for the four groups of animals: group 1, normal; group 2, both optic glands removed; group 3, one optic gland removed; and group 4, sham-operated control.

Group	*N*	(A) Maximum body weight prior to egg laying (g)			(B) Time from egg laying to death (days)			(C) Weight at death (g)			Ratio of (C) to (A) (%)		
		Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
1	12	203.5	65.34	104 to 300	42.7	6.80	31 to 51	188.0	70.70	74 to 277	81*	7.35	71 to 95
2	14	210.6	62.71	130 to 326	175.1	60.02	75 to 277	223.5	85.31	82 to 383	109	40.69	61 to 200
3	8	196.0	71.65	116 to 347	77.4	9.68	64 to 91	104.5	63.88	70 to 254	51	12.05	39 to 73
4	3	191.7	87.03	91 to 247	45.3	11.24	33 to 55	145.4	81.09	52 to 200	72	12.90	57 to 81

**N* = 8 for group 1 only.

identified endocrine glands in octopus. They are small, paired, spherical structures located in the orbital sinus, sitting on the optic tract in the hilum of the optic lobes. They are composed of nonnervous secretory (chief) cells and supporting cells and are neurally connected to the subpedunculate lobe of the brain (*1, 2*). Previous studies suggested that these glands are involved solely in the control of gonadal maturation (follicle cell proliferation and vitellogenesis) (*3–5*). However, that conclusion was based either on short-term experiments or on long-term experiments conducted during periods of relative glandular inactivity. In the present experiments, the optic glands were removed from females after they had spawned, and effects upon feeding, growth, sex behavior, and time of death were studied.

The reproductive behavior of *Octopus hummelincki*, Adam, 1936, is similar, if not identical, to what is currently known of other octopods. It copulates when immature (before egg maturation) as well as during egg manufacture, and stores sperm in its oviducal glands up to a maximum of 4 months. About 2 weeks prior to spawning, the female rejects all efforts of the male to copulate and there is a large decrease in feeding. Eggs are laid in clusters, attached to a substratum, and are constantly protected and cleaned by the female. During the brooding period all females eat and may leave the eggs to catch crabs or snails, but the amount ingested is much less than before spawning. In addition to the quantitative decline in food intake, there is a qualitative change in the specialized method of feeding upon shelled molluscs (*6*). Before spawning, *O. hummelincki* bores holes in gastropod shells, into which it injects a secretion from the posterior salivary glands, and then pulls the weakened snail out and eats it. After spawning, the female ceases to bore and secrete; instead, it forcefully pulls the body of the snail from its shell but may then not eat it. Brooding continues until hatching of the eggs which is temperature-dependent and occurs in about 32 days under the conditions of the present experiment (23°C). The female dies about 10 days after the eggs hatch. By the time of its death, the female has lost much of its maximum body weight prior to egg laying (Table 1 and Fig. 1).

Male *O. hummelincki*, on the other hand, become sexually mature (copulate and pass spermatophores containing sperm) at a very small size (at least 20 g) and live for no more than 7 to 9 months thereafter. They grow to a maximum body weight that is typical for the species, that is, about 50 to 60 percent of the female's maximum weight. Aging males begin to eat less with increasing intervals between meals, gradually lose weight, copulate erratically, and die.

In the present experiments, four groups of female *O. hummelincki* were observed both before and after each female laid eggs. Group 1 was composed of 12 normal females. Group 2 included 14 females whose optic glands were removed bilaterally in a single-stage operation between 4 and 17 days after eggs were laid. Group 3 was composed of eight females from which only one optic gland was removed between 1 and 22 days after eggs were laid. Group 4 included three surgical controls (sham-operated) from which neither gland was removed although all surgical procedures were otherwise identical to those for groups 2 and 3. A fifth group was composed of 13 males from which the optic glands were removed bilaterally.

The octopus was anesthetized for 9 minutes in 2.5 percent ethyl alcohol. A single incision was made along the midline between the eyes; muscles and membranes were separated, the right (or left) orbital sinus was opened, the white body was retracted, and the optic tract was hooked and raised and the optic gland was picked off. The left (or right) gland was similarly removed. The orbital sinuses were entered bilaterally for all animals in groups 2, 3 and 4 even when the optic gland was not removed (*7*).

Table 1 and Fig. 1 show weight changes and time to death for groups 1 to 4. Females subjected to sham operative procedures (group 4) did not differ from normal females that received no surgery (group 1). Females with one optic gland removed (group 3) behaved similarly to those in group 1 but they survived significantly longer (77 days compared to 43 days) (*8*). However, their increased longevity was not due to an increase in food intake, since these females did not eat any more than those in group 1, and during the last month or so they refused all food whatsoever. The reduced food intake extending over a longer survival period resulted in a significantly greater weight loss by the time of death. Only one octopus lost as little as 27 percent of its maximum weight prior to egg laying, the other seven animals losing between 41 and 61 percent.

A strikingly different pattern of events occurred in females with bilateral removal of the optic glands (group 2). Typically, but not invariably, the female ceased to incubate the eggs upon recovery from the anesthetic (3 to 4 minutes) (*9*). Within 2 weeks after surgery all females in group 2 began to eat more regularly and take larger amounts of food than those in group 1. Moreover, they fed in the same way as they did before egg laying, boring holes in the shells of snails. Despite the resumption of eating, females in group 2 continued to lose weight for about a month (30 ± 16.7 days) after the eggs were laid (Fig. 1). The continuing body weight loss was a specific effect of egg laying, and not an effect of surgery, because the males ate and gained weight immediately after their optic glands were removed. Food intake and body weight gradually increased in group 2 females; several of them attained weights significantly above the maximum typical for the species. Females with optic glands usually do not permit copulation by 60 days after spawning but typically will resume copulation by about 90 days, permitting the male to transfer spermatophores (without further egg production).

Males without optic glands continued eating and surpassed the species-typical maximum body weight of normal males. They also lived longer than normal males but, since there is neither a reliable method for estimating the age of octopods nor an event comparable to egg laying, there is no fixed reference point against which to evaluate when they would have died under normal conditions.

The major effect of optic gland removal in the female octopus was increased longevity. While the longest-lived female died 51 days after laying eggs and the longest lived one-gland female died 91 days after spawning, octopuses with both glands removed lived for as long as 9 months after spawning (mean 175.1 days; Table 1). One octopus died suddenly on the 75th day after laying eggs. The remaining 13 lived 4 months or longer. The five longest-lived animals survived for 221, 233, 242, 243, and 277 days. We do not know what caused death in these animals. There may have been several contributing factors; for example, some of the animals developed infections and cysts of head and ovary, which are rarely seen in normal animals. Some animals died suddenly and unexpectedly while eating normally and at peak body weights, whereas others had stopped eating and were losing weight at the time of death. Deterioration of water quality in the recirculating seawater system may have contributed to some deaths.

The optic gland of octopus has been compared to the pituitary gland of vertebrates (*1, 3, 4, 10*). However, while the vertebrate pituitary has been implicated in a variety of neuroendocrine functions, optic gland function in cephalopods has been related solely to gonadal maturation (*11*). The present results suggest that optic gland secretion (or secretions) subserves several different functions, including control of copulation, broodiness, food intake, and longevity.

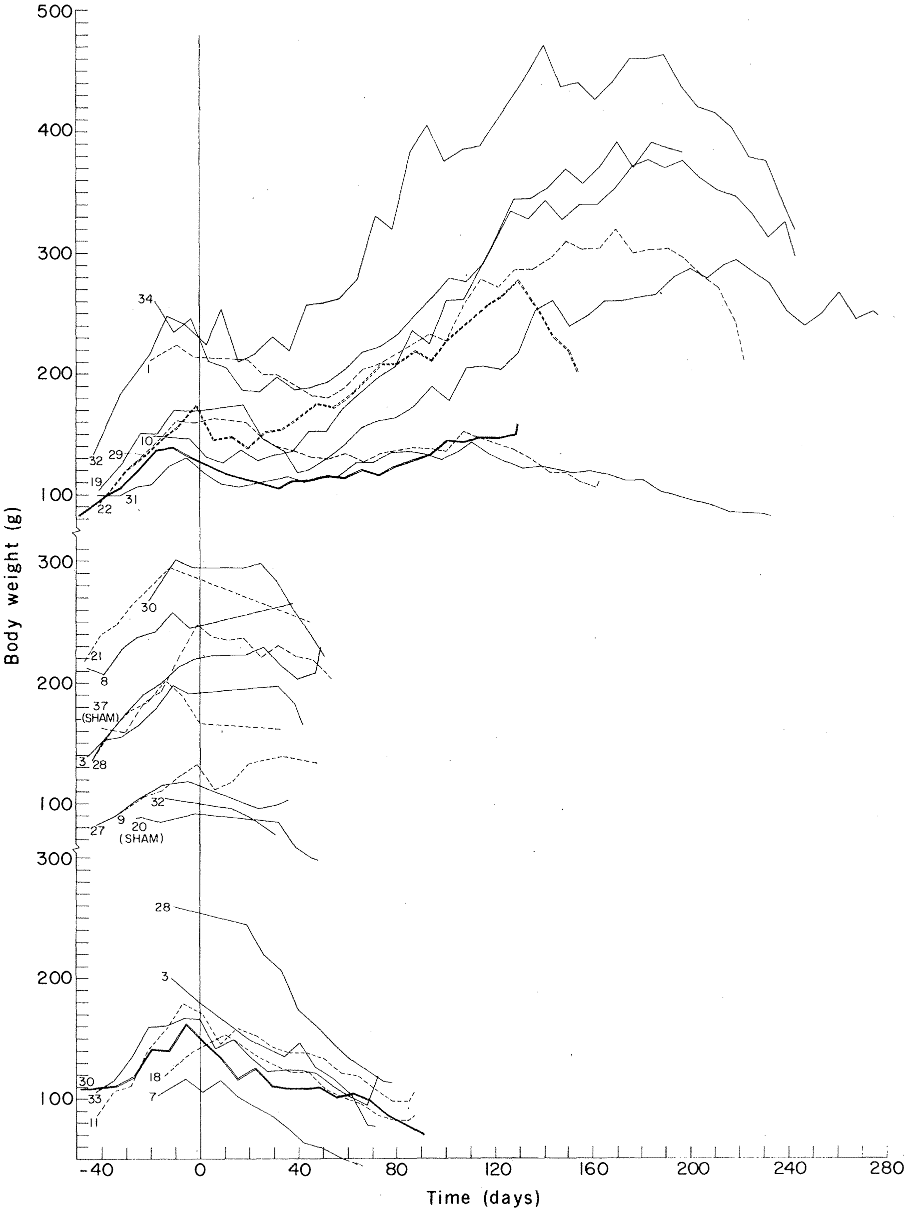

Fig. 1. Body weight in grams of individual octopuses in the four groups as a function of longevity in days. Group 1 (normal, $N = 9$) and group 4 (sham-operated controls, $N = 2$) are in the middle section of the graph; group 2 (both optic glands removed, $N = 9$) in the upper section; and group 3 (one optic gland removed, $N = 7$) in the lower section. Day 0 is the first day on which eggs were laid. The number on each curve is the number of the individual animal.

That optic gland secretions contribute to the control of sexual and parental behavior is suggested by the following observations. (i) Prior to gonadal maturation, when optic gland secretions are presumably at their lowest level, females will copulate readily. (ii) Two weeks prior to spawning when optic gland secretions are presumably at their highest levels, sexual behavior ceases permanently. (iii) Optic gland removal results in an immediate and permanent cessation of parental behavior (broodiness). (iv) Females without optic glands eventually resume copulation. These data suggest that a maximum secretion of the optic gland principle or principles has an anticopulatory function, perhaps analogous to that of the vertebrate hormone prolactin (*12*).

Although the resumption of feeding after removal of the optic glands suggests that optic gland secretions have an inhibitory effect upon eating, the relation cannot be a simple one because at one stage of the reproductive cycle high levels of secretion are not incompatible with voracious feeding. For example, 2 to 5 weeks prior to spawning, when the ovary is rapidly enlarging, the female eats between 5 and 10 percent of its body weight per day. It is only during the 2 weeks immediately preceding spawning, and thereafter, that the females' quantitative intake declines precipitously. These data indicate that the secretions of the optic glands must reach a quantitative threshold before feeding is inhibited (or that additional releasing factors are involved).

The causal mechanisms underlying the shift from a "boring" to a "pulling" mode of feeding are not known, but two facts implicate neurosecretory processes. Barlow *et al.* (*13*) ligated the posterior salivary duct, in the walls of which run secretory and efferent nerves from the superior buccal lobe of the brain to the posterior salivary glands, and found an accumulation of neurosecretory products (noradenaline and 5-hydroxytryptamine) on the brain side of the ligature. I found that ligation or cutting of the duct in male and pre-egg-laying female *O. vulgaris* caused permanent cessation of boring. Nevertheless, the animals lived and gained weight for up to 5 months after surgery. Thus the specialized method of predation is independent of, and dissociable from, the quantitative intake of food. These data indicate that brain neurosecretions may be necessary for boring to occur and are inhibited in the normal spawning female. The present results implicate the optic glands in the control of these neurosecretory processes, since their removal in the spawning female is followed by a resumption of boring.

A relation between gonadal function, cessation of feeding, and death was suggested by Sakaguchi (*14*) who found a 92 and 96 percent reduction in the level of proteolytic activity in the posterior salivary glands and digestive gland (hepatopancreas), respectively, of postspawning *O. vulgaris* (*14, 15*). It was assumed that in the absence of digestive enzymes starvation, body weight loss, and death ensued. However, some normal males and, in the present study, a significant proportion of the females with optic glands removed live for periods of 2 to 4 months after the cessation of feeding, a period far exceeding the postspawning longevity of normal females. These observations indicate that death in the postspawning normal female octopus cannot be due solely to starvation. It appears that optic gland secretions are responsible both for the cessation of feeding and the reduced longevity but that different mechanisms may mediate the two effects.

The octopus apparently possesses a specific "self-destruct" system. When the secretions of the optic gland reach a quantitative threshold, feeding is inhibited and death ensues (*16*). The finding that removal of an endocrine gland leads to an increased life-span supports the hypothesis that aging is a function of extrinsic effects upon cellular aging phenomena (*17*). In many species (fishes, insects, arachnids, and molluscs) the female ceases to feed, spawns, and dies. The hormonal control of such processes in the octopus may provide an excellent model system for analysis of the mechanisms involved (*18*).

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8. The finding that one optic gland alone is sufficient to maintain the normal postspawning events is similar to the report (*3*) that experimental enlargement of only one optic gland is sufficient to induce precocial egg production. However, Wells and Wells (*3*) did not compare the number of days from surgery to spawning to determine whether females with both glands lay eggs sooner than females with only one actively secreting gland.
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16. The function that this mechanism serves in the female may be to ensure survival of the eggs by preventing the predatory female from eating them (although this occurs occasionally). The probability that the female will leave the eggs while she forages and expose them to parasites and predators (although this occurs in the laboratory and may also occur in the field), is reduced, with the female becoming strongly attached to the egg site, this enabling her to clean and to aerate the eggs and mechanically to assist the eggs in the hatching process. In the male, the linkage of inhibition of feeding and death to reproductive activities seems to be looser than in the female, since the male lives many months after gonadal maturation. However, in both sexes, this mechanism guarantees the elimination of old, large predatory individuals and constitutes a very effective means of population control.
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19. I thank L. Silverstein for his surgical instruction. The first two successful operations were performed by him. I also thank L. Garibaldi, curator, and the New England Aquarium, for their generous supply of seawater. This work was supported by N.I.H. Biomedical Research Support Grants at Brandeis University.

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Regions of Cerebral Ischemia Located by Pyridine Nucleotide Fluorescence

Abstract. *The fluorescence of the reduced form of the endogenous pyridine nucleotide nicotinamide adenine dinucleotide was used to map regions of ischemia in cat brain. A remarkably microheterogeneous pattern of increased fluorescence resulted from a critical level of incomplete cerebral ischemia. The fluorescence pattern suggests that ischemia occurs initially in microwatershed zones between penetrating cerebral arteries.*

Brain function is altered by hypoxia or ischemia often before significant metabolic changes can be detected (*1*). It has been argued, however, that altered metabolism in a few vulnerable regions could be responsible for cerebral dysfunction. Thus a method which locates ischemic regions within the brain would greatly facilitate studies of the biochemical substrates of cerebral ischemia. Recently, we have shown that concentrations of the reduced form of nicotinamide adenine dinucleotide (NADH), which increase rapidly in the brain during ischemia, can be detected fluorometrically in frozen brain slices (*2*). Consequently, regions with early ischemic change can be located by scanning frozen brain sections for areas of increased NADH. In the experiments reported here we used this fluorescence technique to map regions of metabolic alteration following incomplete cerebral ischemia (oligemia). In addition, the concentrations of metabolites in various regions were measured to confirm the presence of ischemic change.

Cats were anesthetized with ketamine (Ketalar, Parke-Davis), paralyzed with gallamine (Flaxedil, Davis & Geck), and ventilated with 75 percent N_2O and 25 percent O_2. Cerebral oligemia was produced by occlusion of both common carotid arteries followed by arterial