sociable 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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7. All of the effects of surgery on the so-called peduncle complex (a group of structures including the optic glands, peduncles lobe, and olfactory lobe) reported by J. B. Messenger [*Proc. R. Soc. London Ser. B* **167**, 225 (1967)] were observed in one or another of the animals that were operated on. These effects include postural and locomotor disturbances (oscillation, spinning, and circling), loss of muscle tone, and changes in both the size and orientation of the pupil. In most cases the postural and locomotor disturbances disappeared during the long postoperative maintenance. The impairments often reappeared when, for example, the female was transferred to another tank for a sex behavior test.
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.
9. Noumura, cited in Wells (*10*).
10. M. J. Wells, *Bull. Natl. Inst. Sci. India* **27**, 61 (1964).
11. _____ and J. Wells, *J. Exp. Biol.* **222**, 293 (1969); J. Z. Young, *The Anatomy of the Nervous System of* Octopus vulgaris (Clarendon, Oxford, 1971).
12. Since secretion from the optic glands is not necessary, copulation in the octopus either may have a nonhormonal basis or other, as yet unidentified, sources of hormone (ovarian?) may be involved (*5*).
13. J. J. Barlow, A. V. Juorio, R. Martin, *J. Comp. Physiol.* **89**, 105 (1974).
14. H. Sakaguchi, *Bull. Jpn. Soc. Sci. Fish.* **34**, 716 (1968).
15. There have been numerous speculations concerning whether or not female octopuses must die after spawning, the most recent being K. Mangold and S. von Boletzky, *Mar. Biol.* **19**, 7 (1972); R. F. Thomas and L. Opresko, *Nautilus* **87**, 61 (1973); A. Guerra, *Invest. Pesq.* **39**, 397 (1975). The data of Sakaguchi (*14*), demonstrating the decline of proteolytic enzymes, and those of the present experiments would seem to answer these questions conclusively. All normal female octopuses lay eggs (fertile or infertile) or die egg-bound; all die after hatching of the young or while brooding. As in all biological systems, abnormal functioning of an endocrine gland may occur, resulting in individual deviations from the norm.
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.
17. C. Finch, *Q. Rev. Biol.* **51**, 49 (1976).
18. *Nautilus* is the only known cephalopod that does not have optic glands. This may mean that the optic glands are a recent evolutionary development. The absence of the optic glands in *Nautilus*, and the elaborate shell that it constructs, indicates that this animal may have a much longer life-span than other cephalopods. The mechanism described herein may cause the death of all other cephalopods. However, differences in the functioning of the optic gland secretion in other cephalopods must occur, since squids and cuttlefish typically die shortly after spawning without brooding the eggs as in octopus.
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

hypotension. Because collateral circulation in the cat is greater than in other species, carotid occlusion alone caused only transient alterations in the electroencephalogram (EEG) and had no effects on the concentrations of adenosine triphosphate (ATP), phosphocreatine, lactate, or NADH in the brain measured 20 minutes after occlusion. Blood withdrawal from a femoral arterial cannula to a mean arterial pressure of 30 torr resulted in a critical level of oligemia, as evidenced by the loss of EEG activity. After 20 minutes of oligemia, the brain was frozen in situ with liquid nitrogen, the blood pressure being maintained at 30 torr and ventilation being continued for at least 10 minutes of freezing. The brain was sectioned at −196°C with a cooled saw in the coronal plane into slices 1 cm thick. The NADH in the frozen slices was fluoresced with excitation light at 366 nm from a 200-watt mercury-arc lamp fitted with Corning filter 5840. The fluorescence of NADH (450 nm) was recorded photographically through Corning filters 3389 and 5562 on Polaroid high-contrast film (type 51). Tissue levels of ATP, phosphocreatine, lactate, and NADH were determined enzymatically (*3*) in 2-mg samples, dissected at −30°C from various regions of the brain slice.

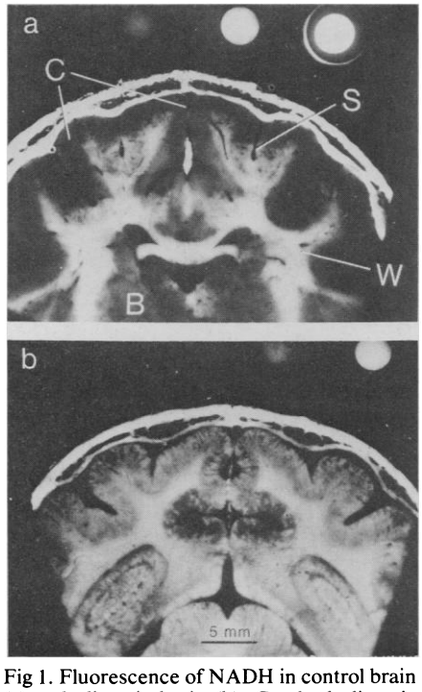

Fig 1. Fluorescence of NADH in control brain (a) and oligemic brain (b). Cerebral oligemia was produced with bilateral carotid occlusion followed by systemic arterial hypotension. After 20 minutes of cerebral oligemia, the brain was frozen in situ and frozen sections were fluoresced with illumination at 366 nm. In the control brain (a) low fluorescence was present in cerebral cortex (*C*) and basal ganglia (*B*). High fluorescence was present in several cortical sulci (*S*) and in white matter (*W*). In the oligemic brain (b) the increase in NADH fluorescence exhibited microheterogeneity throughout the cortex. The frozen NADH standards located above both brain slices were 20, 50, and 100 μM, from left to right. The 20-μM standard in (b) was not visible.

Since the freezing process itself might induce ischemic change, regional NADH fluorescence and metabolite levels were determined in control brain. Figure 1a shows the NADH fluorescence in a slice from control brain frozen in situ. The cerebral cortex and basal ganglia exhibited low NADH fluorescence and contained low levels of NADH (0.012 mmole/kg) and lactate (1.7 mmole/kg), and high levels of ATP (2.5 mmole/kg) and phosphocreatine (5.2 mmole/kg). Thus in these regions with low NADH fluorescence, freezing in situ did not cause significant ischemic alterations. High fluorescence intensity was present in white matter and in the depths of several of the cortical sulci. In those sulci with increased fluorescence, tissue levels of NADH were threefold higher, lactate was twofold higher, ATP was 10 percent lower, and phosphocreatine was 50 percent lower than in the surface cortex. The ischemic change in these sulci presumably occurs because their blood supply, which originates at the cortical surface, is occluded by the freezing front prior to the time of tissue freezing. The lack of ischemic alteration in the basal ganglia indicates that deep brain regions, which are supplied from the ventral surface of the brain, continue to be perfused until the arrival of the freezing front. White matter, despite high fluorescence intensity, had low levels of NADH (0.008 mmole/kg) and lactate (1.5 mmole/kg), and high levels of ATP (2.2 mmole/kg) and phosphocreatine (3.2 mmole/kg). Thus in white matter, high fluorescence is not due to an ischemic elevation of NADH, but probably is caused by lower quenching of fluorescence or by nonspecific fluorescence.

Figure 1b shows the NADH fluorescence in a slice from an oligemic brain. Within the gray matter there was a striking pattern of heterogeneous fluorescence. The cortex contained numerous patches of increased fluorescence, ranging in size from 0.1 to 1 mm, and frequently exhibiting a columnar orientation. In Fig. 2 the columnar pattern of fluorescence can be more easily distinguished. In regions with a high density of fluorescent patches (A) there were alternating bands of high and low concentrations of NADH. Regions with fewer patches (B) contained isolated columns of fluorescence which were also oriented perpendicular to the surface of the cortex. Metabolite levels were greatly altered from control values in areas with a patchy increase of fluorescence. In region A (Fig. 2) of cerebral cortex, ATP was reduced to 1.5 mmole/kg, phosphocreatine was 0.7 mmole/kg, lactate was 25 mmole/kg, and NADH was 0.034 mmole/kg. Since the sample size (2 by 3 mm) encompassed several fluorescent patches, the metabolite levels represent average values for a composite of microregions with high and low fluorescence.

Using the NADH fluorescence technique, we have found regional microheterogeneity to be a common result of cerebral oligemia in the cat. Although the precise location and size of fluorescent patches were variable, the cortical orientation was consistently columnar. Furthermore, midline cortex (region B, Fig. 2) characteristically contained fewer patches of fluorescence and had smaller ischemic alteration of metabolite levels than did other gray-matter regions.

There are two fundamentally distinct explanations for the observed regional heterogeneity during oligemia: (i) an uneven reduction of cerebral blood flow or (ii) a homogeneous reduction of flow, with regions of higher metabolic rate acquiring greater O_2 debt, higher concentrations of NADH, and earlier energy shortage. The metabolic rate is known to vary widely in different brain regions (*4*). Moreover, within the striate cortex, columnar variations in the rate of glucose consumption have been demonstrated (*5*). However, the columnar orientation of cortical NADH fluorescence in the present study does not closely resemble the regularly repeating pattern shown for glucose uptake (*5*). In general, the distribution of brain regions with increased NADH fails to correspond to any recog-

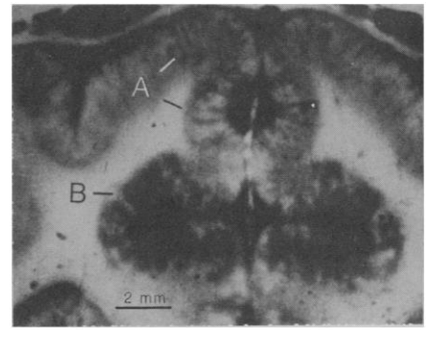

Fig 2. Columnar orientation of NADH fluorescence in oligemic brain. The midline regions of the brain slice pictured in Fig 1b contained microbands of fluorescence that were oriented perpendicular to the cortical surface. In highly fluorescent regions (*A*), there were alternating columns of high and low NADH fluorescence. In regions with fewer micropatches (*B*), isolated stripes of fluorescence often had a columnar orientation.

nizable pattern of metabolic or functional activity. Thus it is unlikely that the heterogeneity of NADH fluorescence can be attributed solely to variations in metabolic rate.

The alternative explanation for regional heterogeneity of fluorescence is patchy perfusion, which has been suspected in other models of diffuse cerebral oligemia (*6*). The vertically oriented columns of increased NADH separated by a column of normal NADH parallels the orientation of penetrating arteries that descend through the gray matter perpendicular to the surface of the cortex. The pattern of microheterogeneity in cortex suggests either (i) that flow ceases in individual penetrating arteries, while neighboring vessels continue to be perfused or (ii) that there exists between penetrating arteries a microwatershed zone analogous to the macroboundary zone between major cerebral arteries. These findings indicate that the location of the initial alterations in cerebral ischemia is determined largely by vascular factors. However, the location of ultimate cellular damage is likely to depend on additional factors, such as intrinsic vulnerability, which is poorly understood. By using NADH fluorescence as a sampling guide, it should be easier to study the pathological changes in the precise brain regions where ischemic damage is occurring.

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Melatonin Induction of Gonadal Quiescence in Pinealectomized Syrian Hamsters

Abstract. *Pinealectomized Syrian hamsters were injected thrice daily with 25 micrograms of melatonin per injection. The injections were administered at 3-hour intervals either during the day or during the night of a photoperiodic cycle of 14 hours of light and 10 hours of darkness. After 6 weeks of treatment with melatonin during the night, both pinealectomized and intact hamsters had reduced testis weight, and pinealectomized hamsters showed decreased levels of serum gonadotropins. Injection of melatonin during the day for 7 weeks either once (75 micrograms) a day or thrice (25 micrograms per injection) daily caused a reduction in testis weight in pinealectomized hamsters. Both pinealectomized and intact females injected with melatonin thrice daily during the day became anovulatory by week 7 of treatment. These results are similar to those observed when hamsters are exposed to a short photoperiod, suggesting that melatonin may be acting as a hormone in mediating the effects of photoperiod on the reproductive system of the Syrian hamster.*

The pineal gland participates in the regulation of seasonal reproductive cycles in the Syrian hamster (*1*). When hamsters are maintained in photoperiods of less than 12.5 hours of illumination daily, testicular regression in males or a state of anovulation in females ensues after 6 to 8 weeks (*2, 3*). These responses to short photoperiods can be prevented by pinealectomy. Therefore, it has been suggested that in the wild the decreasing day-length of autumn induces the pineal to secrete an "antigonadal" hormone which leads to a state of gonadal quiescence (*4*).

A physiological response similar to that observed in a short photoperiod has been achieved in hamsters kept on a long photoperiod by the implantation of melatonin-filled Silastic capsules (*5*). This observation might be interpreted to suggest that melatonin is the pineal "antigonadal" hormone. However, it should be noted that implantation of melatonin in Silastic capsules or in beeswax pellets prevented gonadal atrophy in hamsters maintained in short photoperiods (*5, 6*). Also, the autoimmunization of hamsters to melatonin induced testicular atrophy (*7*). Thus, the precise role of melatonin is not clear. The animals used in the melatonin implant studies (*5, 6*) were not pinealectomized, so it is possible that (i) exogenous melatonin is additive to or interferes with the action of endogenous melatonin, (ii) exogenous melatonin alters the secretion pattern of endogenous melatonin, or (iii) exogenous melatonin stimulates or inhibits the release of another pineal product, with this second product serving the role of an "antigonadal" hormone.

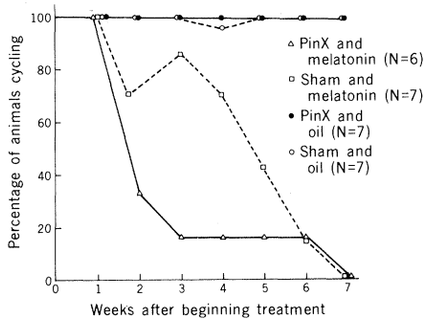

Fig. 1. Effect on estrous cyclicity of daily injections of melatonin (25 μg of melatonin per injection) or 0.05 ml of oil sesame (vehicle) at 1000, 1300, and 1600 hours in pinealectomized (*PinX*) and sham-pinealectomized (*Sham*) female hamsters maintained in a 14L:10D photoperiod. The percentage of females cycling was determined at the end of each week of treatment and injections were administered for 7 weeks.

We have reported that daily injections of melatonin cause gonadal quiescence when administered to hamsters during the afternoon or evening but have no effect when given in the morning (*8*). However, this daily injection regimen failed to inhibit the reproductive systems of pinealectomized hamsters. Thus, for the reasons outlined above it became important to determine whether exogenous melatonin could cause gonadal quiescence in pinealectomized animals. It seemed possible that injections of melatonin given once daily were effective when administered in the afternoon or evening because at those times the exogenous melatonin might be temporally additive to endogenous melatonin (which is produced in greatest quantities during the night in other species). We reasoned that if this were the case, multiple daily injections might be effective in inducing gonadal regression in pinealectomized hamsters.

Hamsters born and reared in our colony were housed in groups and maintained on a photoperiod of 14 hours of light and 10 hours of darkness (14L:10D). Adult animals were pinealectomized by a modification of the technique of Hoffman and Reiter (*9*) and allowed at least 2 weeks of recovery time