Table 1. Results of 720 observations of the number of deflections of the trigger hair of *Dionaea muscipula* in order to cause closure, dependent upon locations and prior conditioning. Results are averages ± standard error.

Location of stimulus	Number of stimuli causing closure of trap		P
	Conditioned	Unconditioned	
Marginal hair	1.4 ± 0.14	2.0 ± 0.10	< .001
Outside trap	1.6 ± .11	2.1 ± .10	< .001
Stem	1.8 ± .08	2.2 ± .10	< .05
Inside trap	1.9 ± .15	1.8 ± .10	> .7

Twenty-five pods of adult *D. muscipula* were maintained in a greenhouse near Philadelphia from May to September. The plants were grown in sphagnum moss, in about 50 percent shade and with a relative humidity of at least 50 percent. The temperature in the greenhouse was the same as that outside. Fertilizer or other feeding was not given. Plants maintained in this way grew rapidly; they were vigorous and very sensitive. Daily, in the morning, each leaf trap was stroked either on the marginal hair, the outside surface of the trap, the stem, or the inside surface of the trap with a moderately stiff-bristle brush 4 mm in diameter. An attempt was made to apply approximately the same pressure and motion in each instance. Traps that were treated by this method were termed conditioned. A similar number of traps in which no stroking was applied served as unconditioned controls. Within 1 minute after stroking with the brush, the sensitive trigger hair of the inner leaf was deflected as many times as was necessary to cause trap closure. On the average, it required two deflections of the sensitive trigger hair to cause closure (*2*). Results shown in Table 1 indicate that conditioning causes the trigger hair to become more sensitive because of the fewer deflections required to effect closure. This was especially true for conditioning of the marginal hair and the outer leaf trap, where numerous receptors exist. A questionable effect was obtained for the stem, where there are few receptors, while stroking the inner trap, where none are present, had no effect. Because the stellate trichomes protrude prominently from the surface of the leaf, they would naturally be the structures most disturbed by the brush stroke. No other specialized structures are present on the leaf except the stomata cells, and these are flush with the surface (*5*). It is possible that the brush stroke actually stimulated the surface epidermal cells, but this is unlikely because the inner surface of the trap, which does not contain the stellate trichomes, is not sensitive to this stimulus. Although the function of these structures is definitely secretory in some plants, their actual purpose in others has been a matter of conjecture (*5*). Our results suggest that they may serve as touch sensors and that in some manner they are capable of causing a change in the internal environment of the leaf trap, which renders it more susceptible to closure by deflection of the trigger hair. Pathways of nervous conduction have not been described in leaves, but it has been suggested that the veins and tracheids might subserve this purpose (*5*). Proof of the stellate trichome's function might be secured by individual stimulation with a microelectrode. This has been attempted but thus far has been inconclusive because of technical difficulties. A mechanism of touch interpretation would have obvious utility for the plant. Certainly it would increase the probability of insect capture and might also serve as a protective device against the mechanical trauma of other predators.

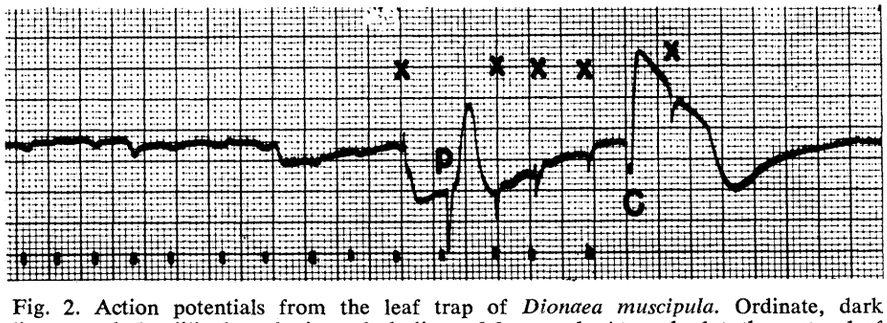

Fig. 2. Action potentials from the leaf trap of *Dionaea muscipula*. Ordinate, dark lines equal 5 millivolts; abscissa, dark lines, 0.2 second. At each dot the outer leaf blade was stroked with a fine wire; at the crosses, a small potential was presumed to arise from the touch receptors. *P* represents a propagated potential, and at *C* the leaf closed. Oscillations prior to the first small potential are movement artifacts.

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28 February 1966

Arterial Constrictor Response in a Diving Mammal

Abstract. *Angiograms were obtained in the harbor seal,* Phoca vitulina, *in air and during diving. During diving there is arterial constriction of the vascular beds of muscle, skin, kidney, liver, spleen, and presumably of all vascular beds except those perfusing the brain and heart. There is sudden constriction and narrowing of muscular arteries close to their origin from the aorta. Constriction of small arterial branches is so intense that blood flow is essentially lost in all involved organs.*

The ability of certain aquatic mammals to dive for extended periods in the absence of external oxygen supply is of great interest to biologists. A number of adaptations have been described which presumably permit prolonged diving. Of these, diving bradycardia is historically the first to be described (*1*) and has received the greatest attention. This emphasis on bradycardia may in large measure be based on the relative ease of its demonstration in the laboratory or field. A priori, it is difficult to visualize the functional basis by which bradycardia operates to permit prolonged diving. Recent work has shown that diving bradycardia is accompanied by a fall in cardiac output (*2*). However, the precise role that this decreased cardiac output plays in permitting prolonged diving is obscure.

Another adaptation which has been described is the development of arterial

constriction during diving (*2, 3*). Arterial constriction limits the blood supply to peripheral tissues and thus reduces oxygen consumption in these organs. Thereby, available oxygen is conserved for use by tissues having an obligatory oxygen requirement (central nervous system). The role of arterial constriction in permitting prolonged diving seems to be critical. However, the evidence for the existence of arterial constriction, as well as evidence concerning its precise role as an adaptive mechanism, has been largely indirect.

In recent years, angiographic techniques have been developed which permit accurate assessment of regional arterial circulation. These techniques have been used to determine the location and extent of vascular disease in human patients. It seemed that these methods would be suitable for giving detailed information concerning the operation and extent of the arterial constrictor response. This report describes the results of such studies in a diving mammal, the harbor seal, *Phoca vitulina*, and defines the organs involved in the arterial constrictor response, the precise site of vascular constriction, and the degree of constriction involved.

Four female harbor seals were flown from Maine to Pittsburgh. The animals were then maintained in a fresh-water tank for approximately 2 weeks in the Children's Zoo of Pittsburgh. During this 2-week period the animals adapted well to their new environment, regained their appetite, and generally thrived. Following this initial period, angiographic studies were performed in ambient air and during diving, as follows.

At the time of an experiment the animal was comfortably restrained in a canvas sling so that gross movements were prevented, but there was no interference with breathing. After the administration of local anesthesia (procaine, 1 percent) an indwelling Teflon catheter (outside diameter 2.3 mm) was inserted in a flipper artery and advanced upwards into the aorta under fluoroscopic control. For visualization of the renal, lumbar, and lower extremity circulations, the catheter was positioned approximately at the level of the renal arteries. For visualization of the hepatic, splanchnic, and splenic circulations, the catheter was positioned approximately at the level of the celiac artery. The cerebral circulation was studied with the catheter tip positioned in the proximal aortic arch. Renografin, 76 percent, was used as the contrast medium in doses of 30 and 20 ml for visualization of the visceral and cerebral circulations respectively. The contrast material was automatically injected with a Gidlund pressure injector set at 6 kg/cm^2. Serial angiograms were obtained with a Schonander film changer, programmed to yield more films during the early injection phase and covering a time span of 16 seconds.

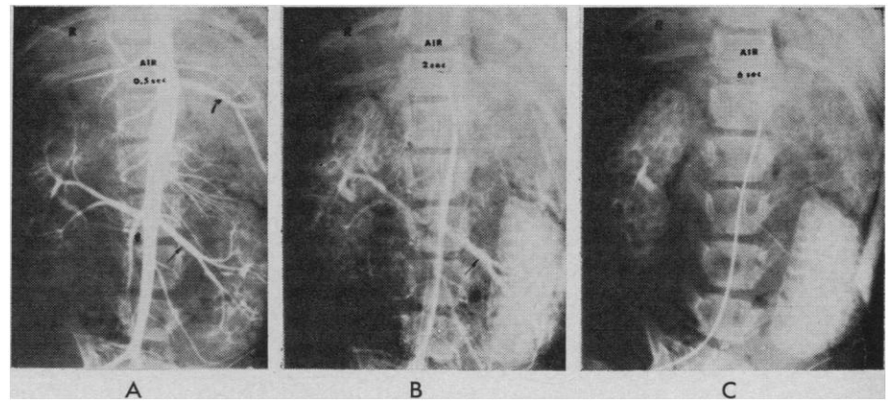

Fig. 1. Aortographic series of a nondiving (breathing) seal. The early arterial phase (*A*) shows the aorta and its visceral branches: the celiac (curved arrow), the superior mesenteric (arrow head), and the renal (straight arrow) filled with contrast material. The late arterial phase (*B*) at 2 seconds demonstrates washout of the aorta by non-contrast-laden fresh blood and beginning of clearance of the branch vessels. The nephrogram phase (*C*) at 6 seconds outlines the striking cystic appearance of the renal parenchyma (renculi); note the complete clearance of contrast material from the aorta and its branches.

For diving, an animal was strapped to a tester board and its head was immersed in water contained in a plexiglass tank. Nondiving films were obtained with the animal in precisely the same position as during diving but with the water removed from the tank. Heart rate was monitored by means of an electrocardiogram, and the occurrence of a diving reflex was verified by the development of bradycardia. Injection of contrast material was performed at variable times, but at least 1 minute after submersion. Four animals were studied. For two animals sequential angiographic studies were obtained while they were diving and while they were in air. One animal died approximately 2 minutes after diving was initiated and approximately 1 minute after the injection of contrast material. Only nondiving films could be obtained with one of the animals because of technical difficulties.

The typical visceral arterial pattern of the seal in the nondiving state is seen in Fig. 1. The relative aortic origin and distribution of the celiac, superior mesenteric, and renal vessels are remarkably similar to those found in man. Though the arterial circulation and kidney contour approximate those observed in man, the nephrogram pattern of the seal is quite distinctive (Fig. 1*C*). The kidney of the seal is composed of multiple individual units (renculi) and has a striking appearance. The peripheral circulation to the flanks and hind flippers has a rich arterial pattern (Fig. 1*A*). An important feature to note in the nondiving state of the animal is the rapid disappearance or washout of contrast material from the aorta and its branches during each successive systolic period by fresh blood not containing contrast material. Thus, at 2 seconds, hardly any contrast material remains in the aorta (Fig. 1*B*), though some remains in the aortic branch vessels.

The arterial circulation of the seal during diving is demonstrated in Fig. 2. The striking feature observed is the virtual lack of peripheral organ filling. The contrast material flows into the proximal portions of the visceral aortic branches and then abruptly stops. The medium and small arterial vessels appear most affected by vasoconstriction, and the majority are not visualized by the opaque medium (Fig. 2*B*). In sharp contrast to the arterial pattern in air, even at 14 seconds (Fig. 2*C*) there is no noticeable nephrogram pattern, and the aorta, even at this time, still retains contrast material.

The vasoconstriction evident in the

visceral circulation during diving also occurs in the peripheral extremities, as seen in Fig. 3*B*. This is the same animal as in Fig. 3*A*. At 4 seconds a meager amount of contrast material is observed in the vessels to the hind flippers, which represents the maximal degree of filling found while a seal was diving.

The uneven distribution of the opaque material in the aorta is not due to aortic constriction but rather to layering of the contrast material. This is a function of the specific gravity of the contrast material in comparison to that of blood. This phenomenon is more evident during diving than in air, because of the prolonged retention of contrast material in the aorta in the former situation.

There was no variation in degree of constriction regardless of the interval between diving and performance of angiograms, which suggests that the constriction persists throughout the dive and is not pulsatile.

The seal that succumbed shortly after the injection of contrast material during diving, developed convulsions prior to death. These were presumably due to a local overdose of contrast material to the brain, resulting from retrograde flow in the aorta as a consequence of the pronounced peripheral vasoconstriction.

No alteration was noted in the cerebral angiograms during the diving and nondiving states. These findings were clearcut, but the technical quality of the films was the least satisfactory of any of the angiograms, and they were not suitable for reproduction.

Direct confirmation of the occurrence of arterial constriction during diving is provided by these studies. In addition, a semiquantitative estimate of the degree of constriction may be deduced. Arterial constriction involves the vascular beds of muscle, skin, kidney, liver, spleen, and presumably all the vascular beds except those perfusing the brain and heart. There is sudden constriction and narrowing of muscular arteries beginning close to their origin from the aorta. This finding eliminates the possibility that the lack of filling is related primarily to a decrease in cardiac output. Constriction of small arterial branches is so intense that blood flow is essentially lost in all involved organs. At the same time the angiograms indicate that perfusion of the brain is maintained.

In terms of overall oxygen availability, diving produces cessation of external ventilation and hence of external oxygen supply. The animal is now required to live on the oxygen stores present in lung air and circulating blood. Arterial constriction prevents oxygen utilization by peripheral tissues and the available oxygen stores are utilized for oxygen-dependent metabolism in the central nervous system.

A knowledge of the available oxygen stores would permit quantitative evaluation of the role of the arterial constrictor response. No such data are available. However, prevention of the diving response by atropine in trained seals during voluntary diving results in drowning in approximately 4 minutes after submersion (*4*). The product of a 4-minute period and the resting oxygen consumption of the seal of 250 ml/min (*5*) indicates that the magnitude of available oxygen stores is approximately 1000 ml. It may be estimated that approximately 20 percent of resting oxygen consumption is utilized by the central nervous system (40 to 50 ml/min). Given total stores

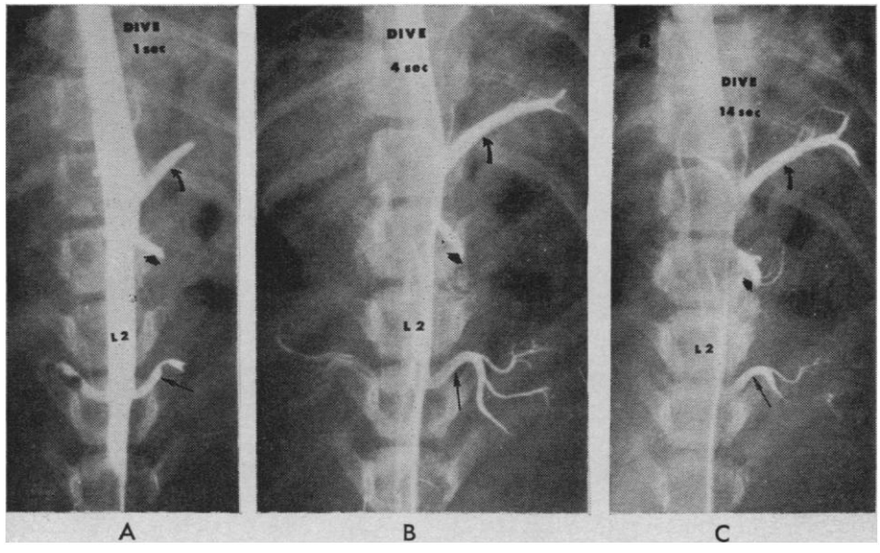

Fig. 2. Aortographic series of a diving seal. The early arterial phase (*A*) demonstrates the abrupt cutoff of contrast material in the visceral branches (arrows) due to vasoconstriction. (Compare this with the findings on nondiving seals in Fig. 1*A*.) In the late arterial phase (*B*) at 4 seconds, there is still only minimal peripheral vessel filling, as noted in the left renal artery (thin arrow). No nephrogram is evident even at 14 seconds (*C*), and contrast material remains in the aorta and its branches. Note the constricted appearance of the medium-sized arteries in the celiac (curved arrow), superior mesenteric (arrow head), and left renal (thin arrow) branches.

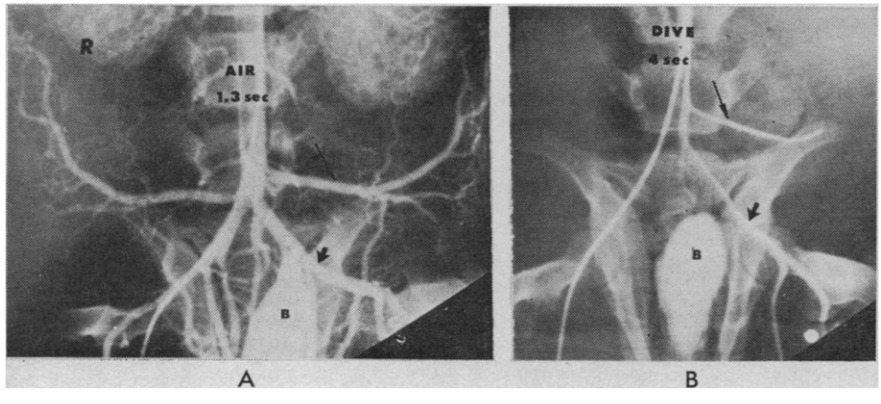

Fig. 3. The peripheral arteries of a seal in air (nondiving) and diving. The arterial phase in air (*A*) shows the rich vasculature of the flanks (thin arrow) and hind flippers (arrow head). The arterial phase during diving (*B*), in the same animal, demonstrates the marked vasoconstriction and the consequent lack of vessel filling even at 4 seconds. Note the "drop-shaped" bladder (marked *B*) filled with contrast material from the previous test injection.

of 1000 ml used at the rate of 50 ml/min, the central nervous system could be supplied from internal stores for 20 minutes without external oxygen source. And, indeed, the upper limit of diving time in this animal is approximately 20 minutes (*6*).

This finding suggests that the primary adaptive mechanism permitting prolonged diving is selective arterial constriction, that it operates to conserve available oxygen stores for cerebral metabolism, and that once oxygen stores are depleted, diving must cease or death will occur.

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Courtship in Spiders without Prior Sperm Induction

Abstract. *Experiments with a lycosid spider demonstrated that courtship behavior is displayed by males independently of whether sperm induction had occurred following the final molt. Contrary to earlier suggestions, proprioceptive feedback from sperm-filled palps is not essential for the onset of reproductive display.*

Studies of the ontogenesis of courtship display in the wolf spider *Lycosa rabida* Walckenaer showed that such behavior appeared 5 to 6 days after the final molt in normal males and in males that were not allowed to fill their palps with sperm.

Copulation in all spiders (class Arachnida, order Araneae) involves insertion by the male of sperm-filled palps into the genital opening of the female. A number of days after the final molt, a small sheet-like web is constructed by the male, and seminal fluid is deposited upon the upper surface. Male *L. rabida*, standing above the web, bend the palps in alternation beneath the sternum and press them against the lower surface of the web to absorb the seminal fluid through the web fibers (*1*). This act of filling the palps with sperm, which Montgomery (*2*) called "sperm induction," obviously must precede copulation if fertilization of the female is to result.

Petrunkevitch (*3*), referring to *Dugesiella hentzi*, said "My observations leave no room for doubt that a male with empty palpi does not court and avoids contact with the female." After observing reproductive behavior in numerous species, Gerhardt (*4*) concluded that this statement was true for all spiders. Both authors apparently based their assumptions on the fact that the male does not court when presented with a female during a variable period of time following the male's final molt. A tendency to display first appears at about the same age that sperm induction is observed to occur. On the basis of this correlation, Gerhardt (*4*) suggested that the readiness of a male to court depends on a sense of fullness in the palps; that is, that proprioceptive feedback by way of palpal nerves is a prerequisite for courtship behavior in spiders.

Later authors (*5*, *6*), when discussing the relation between sperm induction and courtship, have relied on the statements of Petrunkevitch and Gerhardt. Gering (*6*) suggested that the stimulus for sperm induction "seems to be instrumental in initiating the chain-reflex sequence that apparently constitutes the sexual biology of spiders." Heretofore, experimental approach to this problem has been lacking.

The relation of sperm induction to the onset of courtship was examined in *L. rabida*, a wolf spider abundant in grassy fields of the eastern half of the United States. The 55 individuals used in this study were collected in Maryland as immature instars during June and early July of 1964 and 1965. All animals were maintained in isolation and did not see conspecifics until they were tested. Penultimate males could be identified by their swollen palpal tarsi. A few days prior to molt, the body and leg coloration became darker, and such animals were checked frequently thereafter to find out when the final molt occurred. In this way it was possible to insure that sperm induction was not performed by males prior to any experimental treatment. When the spider drank molting fluid from the cast skin, an indication that molt was completed (*7*), the animal was treated. Two groups of males were treated during the penultimate instar stage to preclude the possibility of sperm induction.

Autotomy of the palps was induced by tying the tibiotarsal joint of both palps to one side of the cage while the animal was under carbon dioxide anesthesia. After recovery, the spider pulled away, removing the distal four segments (Fig. 1*a*). Melted paraffin was used to close the male genital pore, to seal and immobilize the spinnerets, or to fix the palps in a position dorsal to the cephalothorax (Fig. 1*b*). All animals were inspected before and after testing to insure that paraffin seals were intact. The temporal patterning of courtship in this species is not affected by palp autotomy (*8*).

Experimental males were divided into five groups of five animals each and one group (group F) of 10. Individuals of group A autotomized both palps during the penultimate instar; members of group B, also palpless as penultimate instars, in addition had their spinnerets sealed and immobilized with paraffin immediately after the final molt; and group C males autotomized their palps after the final molt. In group D the palps were fixed with paraffin dorsal to the cephalothorax; after the final molt, the spinnerets were sealed and immobilized in group E; and a seal was placed over the genital pore of males in group F as soon as molt was completed.

As a result of these treatments, members of groups A, B, and C lacked palps which could be filled with sperm; males in B and E were unable to construct sperm webs; and those in D could not place the palps in the proper position for sperm induction. Members of group F had empty palps because they could not release sperm from the genital pore; however, they were not prevented from performing the movements associated with sperm-web construction and sperm induction.

Two groups of ten males each served as controls. One group underwent no treatment; the other was subjected to carbon dioxide anesthesia immediately