Dissociation of Bradycardia and Arterial Constriction during Diving in the Seal Phoca vitulina

Abstract. *Bradycardia associated with diving in the harbor seal has been dissociated from the arterial constrictor response by intracardiac pacing. Development of arterial constriction does not depend upon the development of bradycardia. During pacing, arterial constriction continues in the absence of bradycardia. Increases in heart rate to values greater than 120 beats per minute during a dive produce a progressive decrease in mean aortic pressure, which suggests that one major function of bradycardia is to reduce cardiac output, thus matching left ventricular output to the restricted vascular bed and decreased venous return associated with diving.*

In the dive response of the seal, the principal circulatory adaptive changes are bradycardia with decrease in cardiac output (*1, 2*) and arterial constriction which conserves oxygen by restricting perfusion of peripheral tissues (*3, 4*). The role of each of these responses and the relation between them have not been clearly defined. For this purpose it would be necessary to dissociate bradycardia from arterial constriction during diving, in order to study one response without the other. In the past, attempts to individually block one or the other response in the seal by means of drugs have not been entirely successful (*5*).

In this study the technique of electrical cardiac pacing was used to dissociate bradycardia from the arterial constrictor response. It was found that arterial constriction occurred without bradycardia in response to diving and also that the seal could remain submerged safely for long periods without bradycardia.

Four young female harbor seals, *Phoca vitulina,* were trained to dive under controlled laboratory conditions. A teeter board was used to support the seal during the study and to regulate submersion time, as previously described (*2*).

A polyethylene catheter (PE 90) was inserted into a femoral artery and advanced to the abdominal aorta. This catheter was used to record aortic pressure with a Statham strain gauge and a Polygraph recorder. Small subcutaneous needle electrodes were used to record the electrocardiogram by means of the same Polygraph recorder. An intracardiac pacing electrode (Elecath pacing stylet No. 550; diameter, 0.1 cm; length, 39 cm) was inserted through the chest wall, via a thin-walled 18-gauge needle, and positioned in a cardiac chamber. The intracardiac pacing electrode was connected with insulated leads to a battery-operated pacemaker (Medtronics, model 5800) with rate and amperage adjustable from 50 to 180 per minute and 1.1 to 22.0 ma, respectively. Procaine was used for local anesthesia during placement of the aortic catheter, intracardiac pacing electrode, and electrocardiogram electrodes.

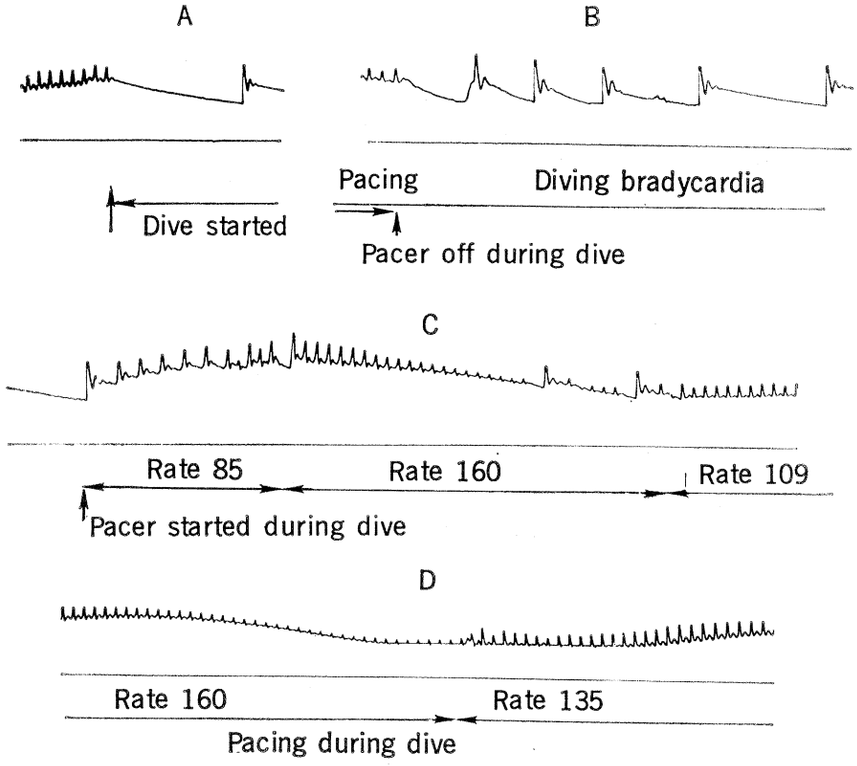

Fig. 1. Aortic blood pressure in a seal at onset of dive (A) and during changes in electrical pacing (B–D). The aortic pressure in this animal before the dive was 135 mm-Hg (systolic) and 80 mm-Hg (diastolic).

Three types of studies were made. (i) Control dives without cardiac pacing were conducted to ascertain that bradycardia occurred normally in the animal in response to diving at the time of study. (ii) The seal was submerged and pacing was instituted after the onset of bradycardia to determine whether bradycardia could be stopped and the heart rate electronically controlled during diving. (iii) Pacing was instituted prior to the dive, and bradycardia was prevented. In one seal arteriograms were obtained, by use of the methods described previously (*4*), during normal control diving and during diving when bradycardia was prevented by cardiac pacing.

The heart rate was easily controlled by use of the intracardiac pacer, the rate of which was set slightly higher than the electrocardiographically determined heart rate of the seal. The pacer was set at 1.1 ma, and the amperage was increased progressively until the impulse from the pacer produced a ventricular response. If no ventricular response occurred, the position of the pacer electrode was adjusted and additional attempts to capture control of ventricular rate were undertaken. With proper electrode placement, 10 to 17 ma were adequate to insure repeated ventricular response to the pacemaker.

Bradycardia occurred promptly in a normal fashion at the onset of diving (Fig. 1A). After bradycardia was established the rate of ventricular contraction could be controlled by the pacer. When pacing was started before the dive, bradycardia could be prevented despite diving. Turning the pacer

off during a dive resulted in a return of bradycardia (Fig. 1B). When the pacer was turned on during diving, the ventricular response could be captured at about 85 impulses per minute (Fig. 1C) and then regulated at any desired rate above that required for ventricular capture. Such controlled pacing during diving was sustained without distress to the animals for periods in excess of 6 minutes, a period of diving not compatible with survival if arterial constriction is prevented (*6*).

Heart rates comparable to the nondiving rate (120 to 150 beats per minute) were compatible with prolonged diving but generally resulted in a gradual decrease in systolic and diastolic aortic pressure and in pulse pressure. Rates greater than 150 beats per minute produced a sharp progressive decrease in aortic pressure (Fig. 1, C and D). If the rate was then decreased, aortic pressure and pulse pressure increased progressively (Fig. 1D). At rates of about 100 to 120 beats per minute, aortic pressure remained fairly constant (Fig. 1C). During diving the aortic blood pressure could be manipulated simply by altering the heart rate by means of the intracardiac pacer. Occasionally, during diving with pacing, ventricular escape from the pacer occurred. The next ventricular response was accompanied by an increase in aortic pressure (Fig. 1C).

Arteriograms performed during diving with pacing demonstrated the same degree of profound arterial constriction that occurred during a normal dive (*4*). The role of the arterial constrictor response in oxygen conservation seems clear. Profound constriction of arteries perfusing all peripheral tissues that were studied (except brain and possibly heart) (*4*) prevents further delivery of oxygen to these sites, thus conserving available stores of oxygen for the oxygen-dependent central nervous system. Peripheral tissues affected by the arterial constrictor response convert to the process of anaerobic glycolysis when the oxygen already present in these tissues is consumed (*7*).

Heretofore the precise role of bradycardia during diving has not been clearly demonstrated. Failure of the normal dive response in the harbor seal generally produces death from anoxia within 4 minutes of diving. The present finding that the seal can remain submerged, with an intact arterial constrictor response but without bradycardia, for more than 4 minutes demonstrates that the arterial constrictor response is the more important circulatory adaptation during diving. The finding that the arterial constrictor response occurs without initial bradycardia suggests that arterial constriction is not mediated in response to bradycardia. It appears that bradycardia is not essential for the conservation of oxygen or for the institution of the dive response.

Previous studies in the seal indicate a reduction in heart rate with a relatively proportional decrease in cardiac output during diving as the perfused vascular bed is decreased. Although there are no precise data concerning the acute changes in venous return upon diving, a number of factors might be anticipated to reduce venous return. These include (i) the reduced cardiac output per se, (ii) pooling of blood in the unusually large hepatic sinuses and numerous abdominal veins of this animal, (iii) reduction in pressure gradient between extrathoracic and intrathoracic compartments produced by immersion of the whole animal in water, (iv) loss of respiratory pumping of blood into the thorax during the apnea of diving, and (v) possible changes in venomotor tone in the extrathoracic venous system. Thus, when the heart rate is increased above a critical level there is a decrease in aortic pressure, presumably reflecting impairment of diastolic filling caused by the decrease in venous return. The sudden increase in aortic pressure, when there is ventricular escape from the pacer during diving, is consistent with this hypothesis.

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References and Notes

1. P. F. Scholander, *Hvalraadets Skrifter Norske Videnskaps-Akad. Oslo*, No. 22 (1940), pp. 1–131.
2. H. V. Murdaugh, E. D. Robin, J. E. Millen, W. F. Drewry, E. Weiss, *Amer. J. Physiol.* **210**, 176 (1966).
3. S. W. Grinnell, L. Irving, P. F. Scholander, *J. Cell. Comp. Physiol.* **19**, 341 (1942).
4. K. M. Bron, H. V. Murdaugh, J. E. Millen, R. Lenthall, P. Raskin, E. D. Robin, *Science* **152**, 540 (1966).
5. H. V. Murdaugh, W. W. Pyron, J. K. Brennan, W. L. Mitchell, *Physiologist* **5**, 186 (1962).
6. H. V. Murdaugh, J. C. Seabury, W. L. Mitchell, *Circ. Res.* **9**, 358 (1961).
7. P. F. Scholander, L. Irving, S. W. Grinnell, *J. Biol. Chem.* **142**, 431 (1942).
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Conformation of Blood-Group and Virus Receptor Glycoproteins from Red Cells and Secretions

Abstract. *Optical rotatory dispersion of human blood-group and virus receptor glycoproteins from erythrocytes and secretions was studied in the far-ultraviolet. Erythrocyte membrane blood-group glycoproteins, with potent virus receptor activities, contained significant alpha-helical and extended beta conformations, whereas the glycoproteins of secretions were largely disordered. These conclusions were supported by determination of the Moffitt constants* (b_0) *and by measurements of circular dichroism.*

Blood-group substances seem to have been studied by optical rotatory dispersion by only Beychok and Kabat (*1*). They limited investigation to the main representatives of the first human blood-group system, the A,B, and H(O) antigens isolated from ovarian cyst fluid. These glycoproteins contain 75 to 80 percent carbohydrate, including approximately 30 percent hexosamine, and 20 to 25 percent peptide; their molecular weights are around 300,000 (*2*). The substances showed weak optical rotatory dispersion, and the curves had a low negative extremum near 220 mμ. Beychok and Kabat attribute the negative extremum at least in part to the *N*-acetyl(2-acetamido) group of hexosamines, especially β-linked *N*-acetyl-D-glucosamine; they found no evidence of α-helical conformation in these ABH(O) substances from epithelial secretions (*1*).

The main antigens of the second human blood-group system to be discovered (*3*), the M and N substances, have been isolated from human erythrocyte membranes and characterized as glycoproteins (see *4*). They are among the most powerful inhibitors of influenza virus hemagglutination yet described (*5*). These glycoproteins contain between 50 and 55 percent carbohydrate, including 12 to 25 percent *N*-acetylneuraminic acid and 10 to 13 percent *N*-acetylated hexosamines; they have been obtained in homogeneous form in various stages of aggregation from molecular sizes of 12×10^6 down to approximately 3×10^4. Blood-group and antiviral activities increase in parallel with increase in molecular size. Harsh treatment readily disaggregates the larger molecules into smaller ones consisting of units that are multiples of 30,000 (*5*).