CONTRIBUTIONS TO THE PHYSIOLOGY OF THE CALIFORNIA HAGFISH, POLISTOTREMA STOUTI. — II. THE ABSENCE OF REGULATIVE NERVES FOR THE SYSTEMIC HEART.

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The Cyclostomes, or hagfishes, have become classical in morphological literature. Their primitive vertebrate characters and their position in the vertebrate series make them in much demand for embryological and morphological research. Very little physiological work has been done with this group, yet their physiological interest promises to be great.

The hagfishes are relatively large and tenacious of life, two qualities very favorable to physiological research. The representative of the group on our western coast, especially abundant in Monterey Bay, reaches a length of forty to fifty centimetres. It is readily taken by means of the trawl or in traps, and can be kept alive in the aquarium with ease. Its tissues live under experimental conditions for hours or even days.

In this brief paper I shall present the results of a series of experiments made to establish the relation of the nervous system to the activity of the heart in Polistotrema stouti. The slight cartilaginous skeleton of the animal permits the preparation of organs with great facility. On the other hand, the slightest muscular movement of any part of the body is sufficient to displace the heart and to render the recording of its movements a matter of peculiar difficulty. The successful records finally made were secured by pinning the body muscles firmly to a holder and independently supporting the heart, and by curarizing the animal.

Stimulation of the vagus nerve. — Johannes Muller described the

¹ The experiments upon which this paper is based were performed at the Hopkins Seaside Laboratory, California. I take this opportunity to express my obligations to the Directors, Dr. C. H. Gilbert and Dr. O. P. Jenkins, for the facilities of the Laboratory.
vagus nerve of the hagfish as sending branches to the gill sacs, to the heart, and to the stomach-intestine. A small filament, doubtless Müller's cardiac branch, runs toward the heart in Polistotrema stouti but by macroscopic methods I have never been able to trace it nearer than the tissue just dorsal to the pericardial wall.

The motor fibres to the gill sacs furnish a ready means of determining whether or not a given stimulus is effective. The muscles of the gill sacs are always thrown into contraction by even a weak stimulus applied to the vagus, so also are the well developed constrictor cardiae at the entrance of the stomach. The strength of current necessary to produce contraction of these gill sacs is from twenty to thirty units by the proportionate scale of the Petzold inductorium used when fed by one Edison-Lalande cell.

The vagus was stimulated at different points along its course from the point of origin within the cranium to a point just anterior to the heart itself. It was stimulated with the nerve intact and with the nerve cut. Each nerve was stimulated by itself and also both together. The strength of the current was varied from one to one thousand units and the rate of interruption was also varied. The results of a series of such tests are presented in the table on page 320.

The heart rates given in this table are computed from counts made for equal periods of time immediately preceding, during, and following vagus stimulation. The error of measurement reaches a maximum of four to five tenths of a beat per minute. Slight variations due to causes which produce a general increase or decrease of rate may, of course, fall within the period under consideration. Of these outside factors among the most important are those influences affecting the return of blood to the heart. Taking into consideration these factors, it seems to me that in no case has a change of heart rate of sufficient magnitude occurred to justify the assumption of a direct
vagus influence. The accompanying figure gives one of a series of experiments in which the strength of current was varied from ten to one thousand units.

The failure to discover inhibitory nerves in the vagus of the hagfish was a great surprise to me, hence I immediately began experiments to determine by what other path the heart received its supply of regulative nerves. The spinal nerves are too delicate to isolate, hence experiments were necessarily confined to an exploration of the brain and spinal cord.

### TABLE SHOWING HEART RATE WITH VAGUS STIMULATION.

<table>
<thead>
<tr>
<th>Date 1899.</th>
<th>Nerve stimulated</th>
<th>Strength of stimulus</th>
<th>Duration of stimulation</th>
<th>Rate before stimulation</th>
<th>Rate during stimulation</th>
<th>Rate after stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec. 28</td>
<td>Vagus</td>
<td>200</td>
<td>18 sec.</td>
<td>26.6</td>
<td>26.4</td>
<td>26.5</td>
</tr>
<tr>
<td>“ ”</td>
<td>Vagus</td>
<td>400</td>
<td>18.4 sec.</td>
<td>25.2</td>
<td>25.2</td>
<td>25.2</td>
</tr>
<tr>
<td>“ ”</td>
<td>Right vagus</td>
<td>500</td>
<td>10 sec.</td>
<td>25.2</td>
<td>24.8</td>
<td>24.8</td>
</tr>
<tr>
<td>“ ”</td>
<td>Right and left vagus</td>
<td>500</td>
<td>30 sec.</td>
<td>24.5</td>
<td>24.6</td>
<td>24.5</td>
</tr>
<tr>
<td>“ ”</td>
<td>Right and left vagus</td>
<td>10</td>
<td>13 sec.</td>
<td>24.0</td>
<td>23.8</td>
<td>24.0</td>
</tr>
<tr>
<td>“ ”</td>
<td>Right and left vagus</td>
<td>100</td>
<td>17.6 sec.</td>
<td>24.0</td>
<td>24.1</td>
<td>24.0</td>
</tr>
<tr>
<td>“ ”</td>
<td>Right and left vagus</td>
<td>1000</td>
<td>18 sec.</td>
<td>23.3</td>
<td>23.3</td>
<td>23.3</td>
</tr>
</tbody>
</table>

**Cranial stimulation.** — The brain was stimulated with platinum electrodes, the bipolar and unipolar methods both being used. The stimulation of different parts of the brain, especially of the medulla and of the roots of the cranial nerves, gave entirely negative results in so far as any influence affecting the heart contractions is concerned.

**Stimulation of the spinal cord.** — It was hoped that by stimulating different sections of the spinal cord any regulative nerves that might reach the heart by this path would be discovered. My results here also were wholly negative, although numerous experiments were performed. It is true that in this series of experiments the heart rate was sometimes slightly increased. The stimulation of the cord was always followed by vigorous contractions of the great lateral muscles, and any one who has ever handled a live hagfish will realize what a pronounced effect the muscular action will have on the tension of
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The heart. These effects cannot be absolutely eliminated except in the curarized animal and the changes in tension and pressure are great enough to produce a change of one or two contractions per minute. I have reached the conclusion, therefore, that regulative nerves for the heart do not pass out through the spinal nerves in the hagfish.

At the suggestion of Dr. Howell, to whom I am greatly indebted for inspiration and advice, I have repeated the experiments along two lines, namely, stimulation of the vagus with the heart not exposed to the air, as it was in the experiments quoted above, and stimulation by applying the electrode directly to the venous sinus. These experiments were kindly performed for me by Mr. W. F. Allen during the summer of 1900, and I have since repeated the experiments myself. Stimulation of the vagus in the neck had no effect on the undisturbed heart. Upon stimulating the venous sinus there was an increase of two or three beats per minute following the first stimulation, an increase that remained permanent for that series. In the experiment quoted the rates were counted in the order given.

**Hagfish No. X.**

| Natural heart rate per minute | 28, 27, 28, 28, 28 |
| Vagus stimulated, heart not exposed to air | 30, 30, 31, 30, 30 |
| Sinus venosus stimulated | 30, 31, 30, 31, 31 |

If the experiments occurred in the order: 1 normal rate, 2 vagus stimulation, 3 sinus stimulation, 4 normal, 5 vagus stimulation, etc., it was found that an increase in rate of one or two beats per minute followed the first stimulation but that the rate did not return afterward to the former normal. My interpretation of this slight change is that it is a direct effect of the general muscular contractions of the animal. When the heart is left in the pericardium it is covered by a sheet of ventral muscle and the pericardial epithelium. Any body movements occurring greatly facilitate the flow of the blood and at the same time produce a decided change in the pressure on the heart as suggested above. These two factors act to increase the heart rate.

Curara acts in the usual way when injected into the hagfish. However, it takes a much larger dose to produce paralysis, and it acts more slowly than on a frog or a mammal. Curara eliminates the influence of the motor nerves of the cord on the body muscles, and also the vagus influence on the muscles of the gill sacs. Stimulation
of the vagus in the curarized animal does not produce the usual contractions of the gill muscles nor of the constrictor cardiacæ, yet direct stimulation of these muscles is followed by contractions. In the curarized hagfish, stimulation of the vagus produces no visible cardiac effects. Records taken under these conditions continue with uninterrupted rhythm, force, and sequence.

The experiments outlined in this paper were performed first during the summer of 1899, but the results were so exceptional that no report was published until further verification could be had. The experiments were repeated at intervals during the late summer, the early autumn, the following Christmas holidays; in March and September, 1900; and in July, 1901.

The animals were taken directly from the aquarium, and were in good condition. They gave unquestioned evidence of the physiological activity of the muscular and of the nervous tissues for hours after being experimented upon. The experiments were performed during the four seasons of the year. The results are uniform throughout the entire series of experiments, and lead to the conclusion, which may now be announced with confidence, that the California hagfish, Polistotrema stouti, does not possess regulative nerves for the heart.

Since the demonstration of the inhibitory nerves for the heart by the Weber brothers, this class of nerves has been shown to be present in numerous species throughout the vertebrate series. I can find no exception whatever in the literature available. It never occurred to me that an exception was possible until I attempted to use the hagfish for demonstrating the vagus influence upon the heart to my class in physiology at the seashore.

Harrington found a certain amount of cardiac resistance to vagus inhibition in guinea pigs during October and January. At this season, stimulation of the vagus nerves produced only a certain amount of cardiac slowing with fall of blood-pressure, but never heart standstill, no matter how strong the stimulus. From February to April, Harrington secured the usual complete standstill of the heart with a sudden and pronounced fall of blood-pressure. He suggested that the difference noted in his experiments was due to a variation in vitality associated with the season, possibly due to a diminution of fresh air and light during the winter months. But in Harrington’s

1 Harrington: This journal, 1898, i, pp. 383-394.
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experiments there was not a total absence of vagus influence on the heart.

A large number of bony fishes and several sharks and rays have been shown to possess depressor nerves for the heart. Fishes are mentioned in the original list of animals given by the Weber brothers in which cardiac inhibition was produced by vagus stimulation. I have myself demonstrated the presence of such nerves in eleven different species of bony fishes and one shark, common in Monterey Bay.

Among the invertebrates investigated, some have been shown to possess complex cardiac regulative nervous mechanisms. Perhaps the best known of the invertebrate cardiac nervous systems, is that of the cephalopod molluscs. In the octopus and in the squid, both inhibitory and accelerator nerves are distributed to the heart. The cardiac fibres pass to the heart by way of the visceral nerve. The fibres to the branchial heart are exclusively motor, i.e., accelerator.

Conant and Clark, in an excellent research, demonstrated that the American edible crab, Calinectes hastatus, possesses a cardiac nervous mechanism. From the anterior part of the thoracic ganglion, are given off two pairs of accelerator, and one pair of inhibitory nerves, which run to a pericardial plexus. Illustrative tracings are presented, showing cardiac acceleration and inhibition following the stimulation of these nerves.

The land snail, Helix, possesses cardiac inhibitory nerves, as proven by the papers of Young, Foster, Biedermann, and Ransom. On the other hand, the sea snail, Aplysia, possesses a cardiac accelerator nerve, but no inhibitory nerve. Ransom stimulated the "visceral nerve" and always obtained cardiac acceleration. More recently, Bottazzi and Enrique have demonstrated accelerator nerves for Aplysia depilans and A. limacina. They also failed to find inhibitory nerves.

The presence of cardiac regulative nerves in so many invertebrates has tended to strengthen the assumption that such nerves were present in all of the lower vertebrates, an assumption which has not before been questioned.

3 Ransom: Loc. cit., p. 261. Other literature is cited in Ransom’s article.
The present experiments show that in the hagfish, the lowest of the craniata, there are no cardiac regulative nerves. In this fact we have a striking illustration of the automaticity of cardiac muscular tissue. The hagfish heart is comparable to the heart of an embryo before nerves have entered. Any regulation of the heart's action must depend upon the conditions which affect the muscle directly, i.e., tension, nutrition, etc. The volume and pressure of the blood coming to the heart and the changes in the pressure upon the internal organs produced by the ever-varying movements of the plastic body of the animal are the factors that must have a decided influence on the hagfish heart. Questions as to the influence of nutrition I wish to discuss at another time.

Among zoologists there is some discussion as to whether or not the apparent primitive structure of the hagfish may be, in reality, a retrograde or degenerate condition. This question may be raised concerning the physiology of the heart. It is, indeed, a question that cannot be arbitrarily settled, and one that must be taken into consideration in any discussion of the acquirement of a cardiac-regulative nervous mechanism in the vertebrate series.