STUDIES OF THE BLOOD OF *ASCIDIA NIGRA* (SAVIGNY). II. VANADOCYTE AGGLUTINATION AND ITS EFFECT UPON THE HEART.

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Introduction

The agglutination of the blood cells of *Ascidia nigra* was first described by Hecht (1918). He showed that agitation of *A. nigra* caused the blood cells to agglutinate, and that the agglutination process normally reversed itself after fifteen or twenty minutes. He theorized that agglutination was caused by the secretion of some substance into the blood, while Fulton (1920) suggested that this substance was secreted by the blood cells. It was found by the present author that the heart responded to agitation and agglutination in a characteristic manner. In the present paper vanadocyte agglutination, and its effect upon the heart will be described and discussed, especially with regard to the theories put forth to explain the reversal of direction of the heart beat.

It is well known that the tunicate heart undergoes a periodic reversal in the direction of the heart beat, beating for a short time in the advisceral direction, pausing and then beating in the abvisceral direction. The heart of *Ascidia nigra* was studied in some detail by Hecht (1918), who reported that the heart showed a greater number of beats during the advisceral phase than during the abvisceral phase (*i.e.*, 24 abvisceral beats compared to 37 advisceral beats.) There has been a considerable difference of opinion as to the cause of the reversal of the direction of the heart beat. Two theories have been proposed: (1) the pacemaker theory, and (2) the back-pressure theory. Haywood and Moon (1950) support the back-pressure theory, suggesting that the blood is pumped into one side of the circulatory system faster than it can pass through the network of blood sinuses. As a result, a back-pressure is built up causing the heart to stop beating. However, Millar (1952) found that periodic reversals in the direction of the heart beat still took place in the isolated heart of *Ciona intestinalis*, and therefore stated that the back-pressure theory was untenable. Krijgsman (1956) also discounted the back-pressure theory. He felt that there were two myogenic pacemakers, one at each end of the

heart, and proposed a theory to explain reversal. He suggested that a metabolite might stimulate a sensory mechanism associated with the pacemakers, causing them to contract. However, the sensory mechanism would then become fatigued and cease responding to the metabolite. As a result the pacemaker would stop functioning and the pacemaker at the opposite end of the heart would take over. Krijgsman (1956) pointed out that there is as yet no adequate explanation for the refilling of the tunicate heart. He assumed that there must be sufficient blood pressure on the "venous" side of the circulatory system to force blood into the heart.

Materials and Methods

Animals were collected from sea walls on Key Biscayne, Florida, and placed in an aquarium with running sea water.

Blood pressure measurements were made in the visceral vessel. To do this a piece of capillary tubing 1.5 mm in diameter was drawn out to a fine tip and bent 90°. The fine-tipped end of the capillary was gently inserted into the horizontal visceral vessel, and the height to which the blood rose in the vertical part of the capillary was measured (this requires about 1% of the tunicates blood volume). The capillary was calibrated in millimeters, and corrected for the effects of capillarity.

To record the heartbeat a kymograph and ink-writing lever system was used. The lever was pivoted in the center. The pen at one end was counterbalanced by a fire polished glass weight suspended by a thread from the other end. The glass weight rested gently on the pericardium so that each contraction wave passing along the heart caused the glass weight, and thus the pen, to move. The kymograph was run at a speed of 5.181 cm per minute.

Observations and Results

The blood pressure of *Ascidia nigra* is shown in Table 1, the average blood pressure being 4.2 mm of mercury. It should also be pointed out that this blood pressure remained unchanged, even during the short pause in the heartbeat which characterizes the reversal of the heartbeat. The pericardial fluid is also under considerable pressure. When the pericardium is punctured and the pericardial fluid allowed to escape, the heart no longer contracts.

Observation of the heart of *A. nigra* showed that there are a greater number of beats during the advisceral phase than during the

abvisceral phase, as reported by Hecht (1918). For example, during a period of twelve reversals in the direction of the heartbeat there were an average of 20.5 systoles in the abvisceral phase and 32.5 beats in the advisceral phase, at 25°C. However, there is a great deal of variation, and the heart may beat 70 or 80 times before reversal. The pulse rate averaged 24 beats per minute in both directions.

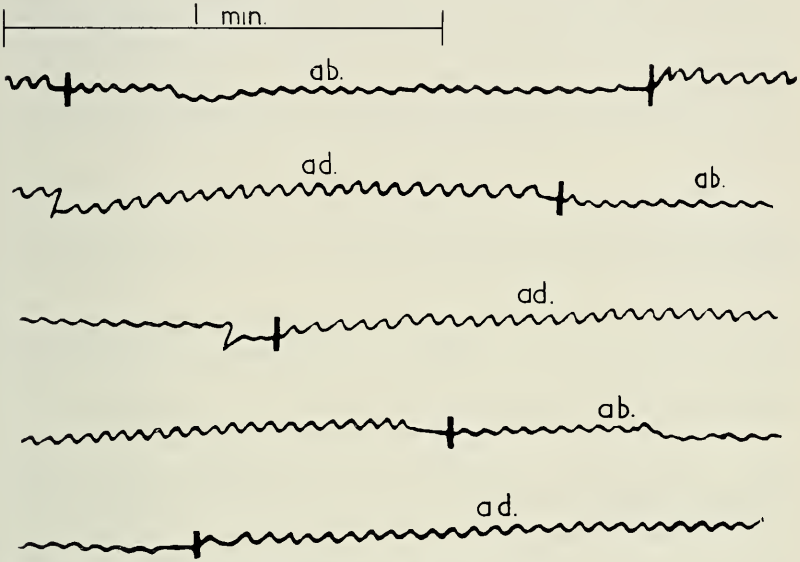

Figure 1. Kymograph record of the heart of *Ascidia nigra.*

Figure 1 is a kymograph recording of the normal heartbeat. The vertical bars were added to indicate the reversal in the direction of the heartbeat. It can be seen that there is normally a two- to four-second pause between the time the heart stops beating in one direction and starts beating in the other. It is also interesting to note that the shape of the recording of the advisceral heartbeat differs from that of the abvisceral heartbeat. This is because the contraction wave of the advisceral heartbeat approaches the kymograph weight from a direction opposite that of the abvisceral heartbeat. Figure 2 is a kymograph recording of the heartbeat of a tunicate just after the animal has been agitated by grasping its siphons with a pair of forceps. This causes the pulse rate to decrease considerably, only five beats being recorded during the first minute of the record. However, the reversal of the direction of the heartbeat takes place much more

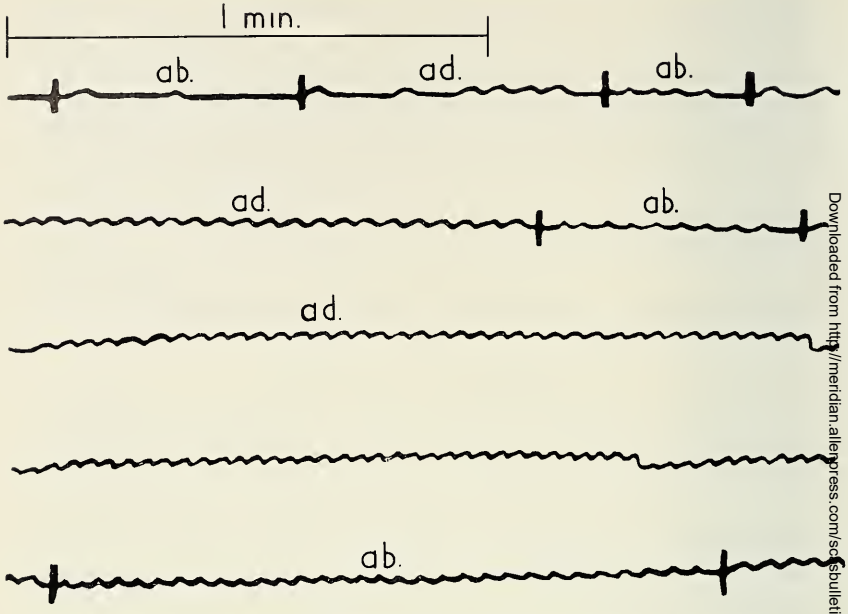

Figure 2. Kymograph record of the heart of an agitated animal (*Ascidia nigra*).

often, reversing four times in the first 1.5 minutes of the record, twice the normal rate of reversal. The prolonged advisceral phase has no special significance, since such prolonged phases were found to occur occasionally under both normal and experimental conditions. To obtain the kymograph record shown in Figure 3, the pressure of the glass weight resting on the heart was increased until the heart beating in the advisceral direction could just force blood past this block. Although this block was about three cm from the advisceral pacemaker, it resulted in a marked decrease in the pulse rate of the heart in the advisceral phase. The first three or four advisceral heartbeats occur much less frequently. The advisceral phase is also shorter than the abvisceral phase of a normal heart, although not as short as that of an agitated animal. The abvisceral heart rate was normal because in this direction the stronger beat was able to pump blood past the block without difficulty.

In addition to its effect upon the heart, agitation of the animal also caused the agglutination of the blood cells. When the test of the animal is scraped or rubbed, clumps of blood cells become apparent in the blood vessels of the test. Unagglutinated blood is almost colorless, but after agglutination the blood appears to be bright yellow, the

yellow color of the vanadocytes being much more apparent when the cells are clumped together in small aggregates. As this "agglutinated blood" is carried from the test to the heart, by way of the test vessel, the blood cells in the heart immediately agglutinate, and within one or two minutes the blood cells of the entire circulatory system agglutinate. The large aggregates of blood cells, thus formed, occlude many of the smaller blood sinuses, interfering with the circulation. Often agglutinated blood cells completely occlude these sinuses over a wide area. However, if the animal is left undisturbed for a period of twenty to thirty minutes the agglutinated cell clusters dissociate, liberating trapped cells, and free circulation is re-established.

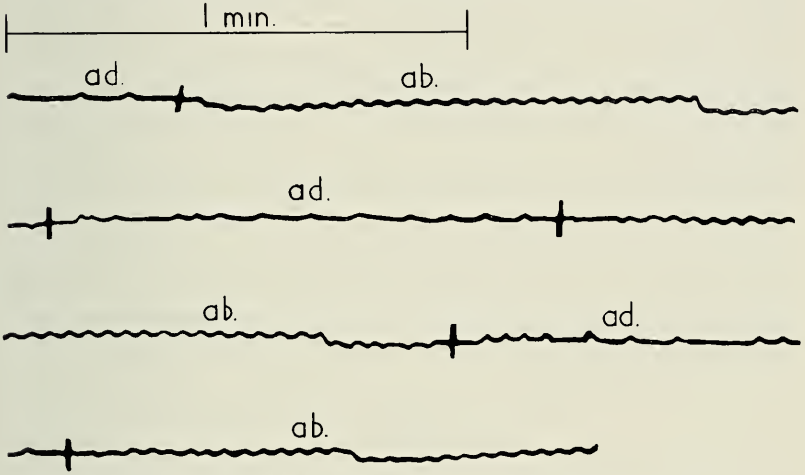

Figure 3. Kymograph record of mechanically blocked heart of *Ascidia nigra.*

It should be emphasized that the test of *Ascidia nigra* is a complex, living structure, containing cells and supplied with an extensive network of blood vessels. Blood vessels approach the free surface of the test. Thus, even gentle stroking of the surface of the test causes the blood vessels to rupture, resulting in the appearance of many spots of yellow blood on the surface of the test.

Thus, it may be stated that agitation of the animal results first in the rupturing of the blood vessels of the test, followed by the agglutination of the blood cells throughout the circulatory system, which is in turn followed by a reduced pulse rate and an increased frequency of the reversal of the direction of the heartbeat.

Observations of agglutinated blood under the microscope showed that not all of the blood cells take part in the agglutination process.

Only the vanadocytes were seen actively to agglutinate. A few colorless cells were trapped in the clumps of vanadocytes. However, most of the colorless cells, orange cells, and blue cells remained free and unagglutinated while almost all of the vanadocytes were agglutinated.

Discussion

It was pointed out above that the blood pressure within the visceral vessel remains the same (about 4.2 mm Hg) while the heart is beating in the advisceral direction, during the short pause in the heartbeat that occurs when the heartbeat reverses direction, and while the heart is beating in the abvisceral direction. There is no detectable increase in the blood pressure at each contraction of the heart, and there is no detectable drop in the blood pressure when the heart stops beating. The method used was sensitive to a change in pressure of 0.1 mm of Hg. Furthermore, when the blood in the visceral vessel is flowing toward the heart, it has the same blood pressure that it has when it is flowing away from the heart. Thus, the "venous" pressure is nearly equal to the "arterial" pressure. It should also be recalled that the heart of *A. nigra* is a very thin muscular membrane. It is so weak, that if the pericardium is punctured, releasing the pressure of the pericardial fluid on the heart, the heart is unable to contract against the pressure of the blood within it.

TABLE 1

Blood Pressure in *Ascidia nigra*

Tunicate	mm Blood	mm Hg
1	58	4.4
2	57	4.3
3	54	4.1
Average	56	4.2

In vertebrates the blood pressure is determined, among other factors, by the degree of constriction or dilation of the arterioles, and by the cardiac output. Tunicates lack arterioles, and, as seen above, the blood pressure is unaffected by cardiac output. Therefore, it seems most likely that the blood pressure observed is caused by the general muscle tone of the animal. That is, by contracting the muscles of the mantle, etc., the pressure within the circulatory system is brought up

to 4.2 mm Hg. Since the circulatory system lacks valves, the pressure is transmitted throughout the hydrodynamic system. This explains why the "venous" and "arterial" pressures are nearly equal. This also explains why the pressure is maintained when the heart is not beating. The heart, in the relaxed state, is "inflated" with blood under this 4.2 mm Hg pressure. The heart cannot contract against this pressure on its own. However, with the aid of the pressure of the pericardial fluid the heart can contract. The pressure of the pericardial fluid is obviously less than 4.2 mm Hg, since the blood "inflates" the heart against the pressure of the pericardial fluid. However, it was not possible to measure this pressure directly.

The contraction of the heart apparently provides only enough pressure to overcome the frictional resistance of the blood against the walls of the blood vessels and sinuses. This requires very little pressure. Even in vertebrates the resistance to flow is negligible until the arterioles are reached (Fulton, 1950). Since ascidians lack arterioles and capillaries, it is readily understandable that the weak peristaltic wave of the heart provides enough pressure to circulate the blood through the open circulatory system. The blood pressure of 4.2 mm Hg probably serves to keep the blood sinuses open, thus insuring the free circulation of the blood. It also keeps the heart dilated, except where the contraction wave occurs. Thus the filling portion of the cardiac cycle is due to the general muscle tone forcing blood into the heart, not to the residual blood pressure resulting from the previous contraction, as suggested by Krijgsman (1956).

It should also be pointed out that the absence of any pressure drop in the visceral vessel during the reversal in the direction of the heartbeat would seem to be evidence against the "backpressure" theory of heartbeat reversal. The theory implies that the blood pressure in the visceral vessel should increase during the advisceral phase and drop after reversal of the heartbeat. However, no such increase or subsequent drop was observed.

The kymograph records show that when the animal is agitated, thus causing the agglutination of the blood cells, the pulse rate slows markedly, while the rate of heartbeat reversal increases. Supporters of the backpressure theory would state that the agglutinated vanadocytes, which occlude the smaller blood sinuses, cause a more rapid build up of the backpressure, which in turn inhibits the pulse rate and causes more frequent reversals of the heart. Furthermore, when the heart is blocked by additional weight on it the pulse rate slows. All this would seem to be evidence for the backpressure theory, and

it may well be that the theory does apply in animals with agglutinated vanadocytes. If pressure on the pacemakers stimulates them to fire, the increase in the blood pressure resulting from the pumping of blood against occluded blood sinuses might fatigue the sensory mechanism of the pacemakers more rapidly, resulting in a slower pulse and more frequent heart reversals. That is, a more rapid accommodation of the sensory mechanism of the pacemaker may take place. However, it must be emphasized that no such increase in blood pressure occurs in the normal animal during the course of either the advisceral or abvisceral phase. Thus, if the backpressure theory applies at all, it would seem to be limited to tunicates with vanadocytes in the agglutinated condition.

Summary

(1) Kymograph records of the heart of normal and agitated *A. nigra* were obtained. Agitation caused the pulse rate to decrease and the frequency of reversal to increase.
(2) The blood pressure of *A. nigra* is 4.2 mm Hg.
(3) The heart and circulatory system are discussed.

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