450

vorigen Jahres trocken gelegen hatte und die Schlammportion am 14. Februar dieses Jahres entnommen wurde.

Bereits nach circa zehn Tagen zeigten sich an den Glaswänden reichlich Harpacticiden — und nur solche —, welche ausnahmslos der mehr erwähnten Species angehörten und fast alle in Copula betroffen wurden. Die nach so kurzer Zeit erscheinenden Copepoden hatten somit eine den ganzen Winter überdauernde Periode der Trockenstarre, wie Zacharias diesen Zustand irgendwo ganz zutreffend bezeichnet, durchgemacht.

München, im October 1899.

5. A new Function of the Vascular ampullae in the Botryllidae [1].

By Frank W. Bancroft, Parker, Fellow of Harvard University, U. S. A.

(With 2 figs.)

The colonial vessels of the *Botryllidae* are, as has long been known, purely ectodermic structures, and end in large numbers of terminal enlargements — the ampullae. These are found nearly everywhere within the colony, but are situated principally along the edges, which they often line several rows deep. Many functions have been attributed to them. The early authors considered them buds that developed into the zoöids of the colony (Giard '72), but since this was disproved by Metschnikoff ('69) the blastogenic function of the ampullae has been generally given up. Herdman ('86 p. 25) however believes that, in *Sarcobotrylloides Wyvillii* at any rate, buds are formed by the ampullae; and Giard ('91) still thinks that the inability of these structures to produce new zoöids has not been sufficiently demonstrated. At present most authors consider the ampullae purely ectodermic structures having nothing to do with reproduction and acting as storage reservoirs for blood, organs for the secretion of the test matrix (Oka '92, p. 524) and respiration (Herdman '84). In these three respects the ampullae undoubtedly perform important functions, and I have still another to add to the list of their activities; namely that of blood propulsion.

It seemed probable that if, as Herdman and Giard maintain, the ampullae are capable of producing new individuals, severing them

from the rest of the colony would be likely to stimulate whatever latent tendency they possess in this direction. Accordingly in several *Botryllus* colonies that were growing vigorously so that they possessed quite a broad edge containing nothing but vessels and ampullae the greater portion of the colony was removed, leaving only a few pieces of the edge without traces of zoöids or buds. When these pieces were examined on the next day a well defined circulation was seen to be established, the blood travelling for a short distance in one direction and then in the other. The source of this circulation was soon located in the ampullae which were seen to be contracting rythmically, driving the blood out, and then sucking it up again as they expanded. An examination of uninjured colonies showed that all the ampullae normally carry out pulsations, and that the contractions of neighboring ampullae are very well coördinated so that they are all executed at the same time. But, before I proceed to discuss these phenomena, a word about the fate of the isolated pieces.

They never regenerated a colony, none of the ampullae showed the least tendency towards budding, but the vitality of the pieces was remarkable. On the second day the thickened cylindrical epithelium usually present at the tips of the ampullae had been resorbed. Later the ampullae and the blood cells they contained grew more opaque, and the former became progressively smaller until most of them had entirely disappeared. The piece was then a rich reticulum of small blood vessels with an occasional shrivelled ampulla, the whole filled with blood containing a much greater proportion of cells than normal, but still circulating feebly in alternating directions. The ampullae were still pulsating, but not with enough force to drive the cells out into the vessels. This condition obtained from about the 5th day on; then the circulation became feebler and feebler until it finally stopped. The cessation took place on the 9th day in the smallest piece, and in the largest it had not yet occurred on the 15th when the experiment had to be discontinued.

To return to the contractile activity of the ampullae: So far as I know, the only author who has mentioned even the regular flow of blood to and from the periphery of the colony is Pizon ('98, p. 129). He says:

»Dans une même colonie, les différents cœurs se contractent simultanément et tous dans le même sens, chassant, par exemple, les corpuscules sanguins et les éléments d'origine régressive dans les ampoules périphériques; puis, à mesure que la pression augmente dans ces ampoules, les battements se ralentissent progressivement et à un certain moment ils paraissent devoir s'arrêter totalement, mais tout à

coup le renversement de la circulation s'opère; le sang accumulé à la périphérie se précipite en sens inverse dans les parties centrales de la colonie et les cœurs changent le sens de leurs contractions, qui passent subitement par un nouveau maximum.«

Althoug the pulsations of the isolated ampullae show that they have an independent contractility, and that the flow of blood to and from the periphery of the uninjured colony is not entirely due to the coördinated contractions of the hearts, as Pizon thinks, still a series of observations was made to test whether the heart beats have any effect upon the contractions of the ampullae. I will discuss first the coördination of the heart beats and of their reversals, then the influence of the heart beats on the length of the ampullar pulsations and the characters of the latter, and finally the coördination in the contractions of the neighboring ampullae and the means by which it is accomplished.

Coördination of the Heart Beats and their Reversals.

Pizon writes, in the passage quoted above, that in a single colony the hearts beat simultaneously, but I have been unable to confirm this statement. In some cases it is even impossible for the beats to be simultaneous for there is a constant and characteristic difference in the lengths of the heart beats. Thus in one colony an old zoöid, the greater part of which had degenerated, had an average of 76.2 heart beats per minute (extremes 69 and 80) while the adult zoöid developed from it had an average of 100,5 (extremes 99 and 102) and in the young buds the heart beat faster still [2].

In zoöids of the same generation the rapidity of the heart beats is about the same; but simple inspection in transparent colonies shows that here too the contractions are often not simultaneous, though they may be so in isolated cases.

Secondly Pizon says that the hearts beat all in the same direction and that the reversals in the direction of the heart beats take place at the same time in different zoöids. To test this matter I made 5 series of observations. One on an old zoöid that had partly degenerated and the adult zoöid developed from its bud, one on an adult zoöid and its bud, and 3 on adjacent adult zoöids of the same generation. Of these last one pair was in a colony containing 2 zoöids, one in a colony of 8 zoöids, and the last in a larger colony with 3 systems of zoöids. In each of these series the two zoöids were carefully watched for about 20 consecutive minutes and the escond in which the reversal took place

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[2] The colonies observed were transparent ones growing on glass slides. This allowed an examination of the attached ventral surface and enabled all the details of the heart beats to be seen with the greatest distinctness.
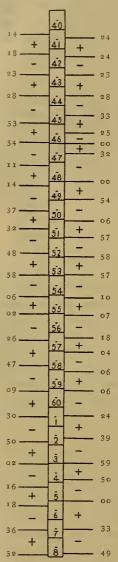
noted. In no case did the two hearts examined regularly beat in the
same direction and reverse at the same time. Just as the heart beats
were slower in the older zooids the frequency of the reversal was also
less. This will be seen from Table 1 which gives the times between
reversals (reversal period) for the 5 pairs of zooids observed. In the

Table 1.

	Older Zooid (in the first 2 lines)		Younger Zooid (in the first 2 lines)	
	Reversal Period		Reversal Period	
	Average	Extremes	Average	Extremes
Degeneration and adult Zooid	91	138,42	56	81,24
Adult Zooid and its bud	80	122,41	66	89,33
Two Zooids of the same generation	68	90,46	65	79,52
- - -	89	152,50	79	108,56
- - -	72	85,55	63	93,32

first two lines the reversal periods of the zooids of the older generation
are placed in the first column, and it will be seen that they are consi-
derably longer than in the corresponding younger generation. Smaller
differences were observed between the zooids of the same generation
though even here they were considerable. The variation expressed by
the extremes in the table appears greater than it really is; for in each
zooid the period during which the blood is propelled in one direction
is usually decidedly longer than when the blood passes in the opposite
direction. Thus there are really two groups of variants from which the
extremes are selected.

But inspite of the variation in the lengths of the reversal periods of
the different zooids a considerable amount of coördination as regards
the moment of the reversal and the direction of the flow of blood is
possible. As a matter of fact, however, it does not occur. Table 2
represents the coördination between the two zooids whose reversal
periods are recorded in the last line of Table 1; these two zooids being
selected because they were better coördinated than any of the others.
The central column represents the time, divided into minutes and
half-minutes. The number of the minute is indicated by the figures
above each line. The times of reversal of the hearts are indicated by
the horizontal lines on both sides of the time column; those on one
side always referring to the same zooid. These lines are inserted on
the time column as nearly as possible at the time when the reversal
occurred, and for additional accuracy the second during which the

Table 2.

Left sec	Left dir	Min	Right dir	Right sec
14		40		24
18	+	41	+	24
23	−	42	−	23
	+	43	+	
28	−	44	−	28
53		45	+	33
54	+	46	−	25
	−		+	00
11		47	+	32
14	+	48	−	00
37	−	49	+	54
32		50	−	06
48	+	51	+	57
58	−	52	−	58
		53	+	57
06	+	54	−	10
02	−	55	+	07
26		56	−	18
47	+	57	+	04
	−	58	−	06
09		59	+	06
30	+	60	−	24
50	−	1	+	39
02	+	2	−	59
	−	3	−	50
16		4	+	50
18	+	5	−	00
	−	6	+	
36		7	−	33
52	+	8		49

The central column represents the time, divided into half minutes. Each minute is numbered. Each of the lines on the sides represents a heart reversal. The time in which it occurred is indicated by the number at the end of the line. The distances between the lines correspond to the length of the reversal periods; + denotes a posterior and — an anterior direction of the blood in the heart.

heart reversed is added at the end of each line. A plus sign is inserted in those intervals in which the blood flowed posteriorly, the opposite direction being indicated by the opposite sign. The table shows that there is a tendency for the reversal of both hearts to take place at nearly the same time, but this tendency is about as strong when the hearts are beating in the same as in opposite directions. Thus, near the top of the table, the reversals take place nearly as was stated by Pizon, but at the bottom exactly the opposite occurs. During the whole time of the observation the hearts were beating in the same direction for 834 seconds, and in opposite directions for 824 seconds. Accordingly, even in this most favorable case, it is evident that there is no tendency for the hearts of a colony to beat in the same direction.

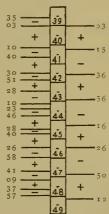### Relation between Heart Beats and Ampullar Pulsations.

Pizon describes the reversal of the hearts as being synchronous with the reversal of the flow of blood in the ampullae, and seems to think that the direction of the flow in the latter depends upon pressure derived from the hearts. Three series of observations to determine the influence of the hearts upon the ampullar contractions were made. In each case a number of ampullae that contracted synchronously were observed and the reversals of the blood flowing in and out of them compared with the heart reversals of the nearest zoöid. In one case this zoöid was connected with the ampullae observed by a complex network of blood vessels having the most various relations whith the rest of the colony, so that probably the greater portion of the blood supply was not derived from the zoöid with which they were compared. In another case the ampullae were in intimate vascular

connection with the zoöid, probably deriving most of their blood from it, but were also connected with the rest of the colony by another vessel. In the third case all vessels connecting the ampullae with other parts of the colony were severed, thus realizing the conditions which would most facilitate any possible influence of the heart beats upon the ampullar contractions. In all three cases the results were the same. The ampullae pulsated in a rythm of their own which was independent of and considerably shorter than the reversal periods of the zoöids. Table 3 represents the coördination between ampullar contractions and reversal period in the third case, when the ampullae were connected only with one zoöid. The vessels were so arranged that when the heart propelled the blood in an anterior direction the tendency was to force it into the ampullae, and vice versa.

Accordingly, in the column for the ampullae the + sign stands for the systole of the ampullae which would tend to drive the blood in the same direction as the + or posterior contractions of the heart. Similarly the ampullar diastole is indicated by the — sign and has the same effect on the blood as the — or anterior contractions of the heart. A glance at Table 3 suffices to show the entire absence of coördination between the ampullar pulsations and the heart reversals, even under these most favorable circumstances. Inspection showed that there was a slight influence of the heart beats upon the ampullar circulation, the latter being less vigorous when opposed by the heart. The opposing pressure also seemed to help determine the exact moment when the ampullar systole or diastole commences, though it does not perceptibly influence the flow of blood at other times.

Table 3.

35 03	− [39]	○3
	+ [40] +	15
10 40	− [41] −	
30 51	+ [42]	36
28	− [43] +	
10 23 46	+ [44] −	56
28 40	− [45] +	16
26 58	+ [46] −	26
41 09	− [47]	50
37 57	+ [48] +	12
	− [49]	

The lines to the left of the time column represent the reversals of the flow of blood in the ampullae; those to the right the reversals of the heart. + indicates the ampullar systole and — its diastole. For other explanations see Table 2.

The contractions of the whole zoöid, however, were so vigorous that the ampullae could not resist them; and thus the blood was occasionally forced back into the ampullae for an instant in the middle of a systole. But as the contractions of the zoöids were usually only a few seconds long the blood very soon flowed back in the original direction. In conclusion then it may be said that the ampullae evidently have an independent power of contraction, and an independent rythm that is influenced but little by the zoöids of the colony.

Characteristics of the Ampullar Pulsations.

Table 4 gives the average times of the systole and diastole in 9 series of observations, each continued, on an average, for 17 seconds. It shows that the contraction is very slow, much slower than the contractions of the zoöids and hearts which are accomplished by means of muscles. The systole is usually decidedly longer than the diastole; but the two exceptions, series 5 and 9, seem to show that the time relations are easily modified. Thus in series 4 the pulsations had the usual characters, but in less than one hour (series 5) they had changed so much that the average time of the diastole had increased from 32 to 42 seconds. As the ampullae remained the same it would seem

Table 4.

Series.	Systole.		Diastole.		Remarks.
	Average	Extremes	Average	Extremes	
1	30	37,5	29	48,10	Ampullae connected with zoöid and colony.
2	45	67,13	27	41,12	Ampullae connected with zoöid only.
3	39	51,26	20	25,17	Connections complex.
4	38	48,10	32	43,17	Connections complex.
5	39	51,20	42	66,16	Same ampullae after 50 minutes.
6	48	66,25	28	47,16	Systole against gravity.
7	43	66,10	30	50,14	Systole with gravity.
8	38	64,10	33	57,15	Adjacent ampullae, contracting alternately.
9	20	28,16	55	68,28	
Average	37,6		32,7		

that this change was due to variation in the conditions external to the ampullae, such as the distribution and pressure of the blood. In series 8 and 9, however, adjacent ampullae were observed at the same time, and, as the external conditions must have been nearly the same, the differences in the pulsations must be explained by differences within the ampullae. It must be concluded then that the lengths of systole and diastole are determined both by influences from without and within the ampullae.

Of the external influences that may act upon the ampullae blood pressure has already been mentioned. Several methods were tried to ascertain whether a slight increase in the blood pressure would leng-

then the ampullar systole. The length of the pulsations when assisted by the action of the heart, and when opposed by it were compared; the effect of gravity acting against and with the contractions of the ampullae at the end of a long blood vessel was tested. One half of a colony was also subjected to pressure, thus forcing part of the blood it contained into the other half and the influence of this increased blood supply upon the rythm of the ampullar pulsations was examined. The results of these experiments indicated that increased pressure slightly lengthened the systole; but the changes noticed were so small that they fell within the normal variations of the pulsations, and thus cannot be considered as establishing the influence of a change of blood pressure upon the ampullar contractions.

The extent of the contractions like their rapidity is small. In

Fig. 1. A normal ampulla, contracting a little more vigorously than is usual. The continuous line shows its shape at the beginning of the systole and the broken line at its end. Camera, × 86.

Fig. 2. A small group of ampullae one day after isolation from the rest of the vascular system, showing the alternate nature of the contractions. The continuous line shows the outline of the whole group before the lower ampulla has contracted, and the dotted line after it has contracted. It will be seen that when one end is contracted the other is expanded. Camera, × 86.

many cases the change in shape may be detected by simple inspection, but often an ocular micrometer or a camera drawing is necessary to make sure that a contraction actually occurs. But although the motions of the ampullar walls may be hard to detect, the movements of the blood corpuscles are very evident, and it was by means of them that the data for the ampullae in tables 3 and 4 were obtained. Figures 1 and 2 show the extent of the contraction, the latter especially showing that the contraction may take place in all directions, decreasing the length as well as the diameter of the ampullae. The small extent and extreme slowness of the contraction, and the fact that it is not always limited to one direction agree well with the simple structure of the contracting tissue, which is a thin pavement epithelium without any fibrous differentiations that I have been able to detect.

Coördination of the Pulsations.

The coördination of the ampullar contractions is their most important characteristic, for it is only by means of this working together that they gain enough strength to force the blood rapidly through the larger vessels and thus, of their own accord, maintain a vigorous circulation. This coördination is often very extensive, all the ampullae (perhaps 50 or more) in 4 or 5 square millimeters pulsating together; but it is not usually more extensive than this.

In attempting to ascertain the means by which this coördination is accomplished there are three principal possibilities to be tested. The coördination may be accomplished by:

1. Nervous control.
2. Conduction of the stimulus along the vessels, each contracting cell stimulating its neighbor.
3. A similar blood pressure in the coördinated region, the pressure having an inhibiting or stimulating action or both, according to the circumstances.

1. The meagre development of the nerves in *Botryllus*, and the fact that they have never been observed to extend far from the ganglion or into the common test makes it improbable that these should be the cause of the coördination, and the behavior of the ampullae when severed from the rest of the colony conclusively proves it. Thus when a mass of ampullae is severed from the colony their contractions become coördinated in a few hours, at most, but not in the same way that they were before. They were all acting together then, driving the blood towards the zoöids or drawing it away, while now they have divided into groups which contract alternately so that the blood is kept circulating between them (Fig. 2). When first separated from the colony many of the ampullae are separated from each other. They send out processes which fuse and form small groups of ampullae whose contractions are alternately coördinated. Later these groups fuse to form larger ones and again we have another coördination supplanting the old one, and better adapted to the purpose of keeping up the most vigorous circulation. The rapidity with which this readjustment may take place is surprising. Thus in isolating several pairs of ampullae, which previously contracted together, the first alternate contractions were observed 7 minutes after isolation, and in another case well coördinated alternate contractions following each other at the usual intervals were established 18 minutes after isolation. These facts, especially the change of coördination when isolated groups fuse, and

the rapidity with which the change is accomplished, make it certain that the coördination is not accomplished by means of nerves.

2. The apriori argument in favor of the stimulus to contraction being transmitted along the contracting walls of the vessels is quite strong. From what we know about contraction in muscles it seems hard to believe that the cells of one region could contract without stimulating the neighboring ones. As a matter of fact a contraction of the blood vessels between the ampullae has also been observed, thus showing that the contractility is present throughout the whole vascular system. This makes it possible for the stimulus to be conveyed from one ampulla to the next, and if it originated in some of the larger vessels near the zoöids and travelled peripherally we can see how it might bring about a coördination. In testing this hypothesis, however, I have been unable to find any evidence in its favor. Thus in observing the beginnings of the systole of a number of ampullae connected with the zoöids by only one vessel a regular sequence could not be observed. When the blood pressure was strong the ampullae usually began to contract strictly together, and when they were connected with the rest of the colony only by long narrow vessels, so that the pressure was comparatively weak, there were usually irregularities in the sequence of the contracting ampullae that could not be correlated with their distances from the colony. The rapid readjustment of the coördination in isolated groups of ampullae also tells against this explanation, though not so strongly, as against the nerve hypothesis. The strongest evidence against the conduction theory is the occasional occurrence of adjacent ampullae that are freely connected with the rest of the vascular system but which do not contract together. This is but rarely encountered and has been usually seen in parts of the vascular system where the changes in blood pressure were comparatively small. In the most conspicuous instance seen the two ampullae were almost touching each other but they regularly contracted alternately. In this case, hen, conduction cannot explain the phenomena; and this, together with the other evidence, shows that, in general, the importance of the conduction of the stimulus along the contracting vessels is small when compared with other coördinating agencies.

3. There remains then the variations in blood pressure as the principal, if not the only cause bringing about the coördination of the contractions; and when examined in detail it appears possible to explain the whole of the normal phenomena, as well as most of the variations, entirely by this principle.

In the first place we have seen that the blood pressure does influence the ampullar contractions somewhat though not enough to dis-

guise their own characteristic rythm. Thus the rush of blood caused by the contractions of the zoöids is so strong that the ampullae cannot withstand it, even during the most vigorous part of their systole. The effect of the opposed heart beats is only exceptionally strong enough to prevent the emptying of the ampullae but it can often be seen to be strong enough to delay the systole for 5 seconds. Accordingly its effect on the coördination must be pronounced, for any ampulla that might otherwise have contracted a little ahead of the others will be retarded, and the rest can catch up, so that all will empty their contents together. The blood pressure theory is in perfect harmony with the fact already mentioned that the ampullar contractions are less rigidly coördinated in those parts of the vascular system that are considerably removed from the zoöids and connected with them by few vessels; for here the variations of pressure are less, as they depend mostly upon the weaker contractions of the ampullae.

The behavior of isolated ampullae is also explained; for, given their inherent tendency to execute rythmical contractions, all will tend to contract together, as before the isolation. But, this being impossible, the stronger ones will force the blood into the weaker ones whose systole will be delayed until the diastole of the stronger ampullae. The rare cases of adjacent ampullae executing alternate contractions may be explained in the same way. For here, though the ampullae are not isolated, the differences of pressure coming from a distance are small, so that it might happen occasionally that an exceptionally weak ampulla would be influenced more by a neighboring strong one than by the general pressure caused by more distant ampullae. In this case it would contract alternately with the adjacent rather than with the distant ampullae.

In one respect, however, this theory encounters a difficulty. This is in the case of isolated portions of the vascular system which become functionally divided into several regions, the ampullae of each region contracting together and forcing the blood into other regions. The apparent »purpose« of the apparatus is to produce the most vigorous circulation possible, and it is accomplished with wonderful success. But if the path of least resistance were followed adjacent ampullae would contract alternately instead of adjacent regions; for it would be easier for the contracting ampullae to force the blood to the next ampulla than to another region.

There are two ways to explain this discrepancy. It may be said that it is only the weakest ampullae that have the tendency to yield to all pressures, that normally a slight pressure acts as a stimulus to contraction, and that therefore the ampullae do not seek the path of least

resistance though they cannot make headway against a strong pressure. On the other hand it may be maintained that here we have an effect of the conduction of a stimulus by means of the contracting cells along the walls of the vessels. This stimulus is not strong enough to prevent alternate contractions in different regions, but is too powerful to allow a d j a c e n t ampullae to follow the paths of least resistance, and thus makes them contract together. Both explanations are probable, and I shall not attempt to decide between them, for no matter which one we accept, it must be confessed that the chief explanatory principle for coördination in normal and isolated ampullae is the variation in blood pressure.

In conclusion I will mention two cases which serve to emphasize the importance of the coördinated ampullar contractions and their power to maintain a circulation independently of the activity of the heart. In narcotizing the *Botryllus* colony with cocaine the voluntary muscles of the zoöids are first affected, then the heart is stopped, and finally the contractions of the ampullae. Accordingly there is a stage when the hearts have stopped beating but the ampullae still contract, though not always with their full vigor. During this stage it is seen that the ampullar contractions can not only maintain a circulation in the common vascular system, but can also force the blood though some of the zoöids, and through there inactive hearts, though the current is very slow and weak.

In a colony of *Botrylloides Gascoi* which I have kept for over 6 months in the aquarium at Naples a case of aestivation, or partial lying down in summer, was observed. A more explicit account will he published later, at present we are concerned only with the ampullae. While the colony was still apparently in good health a large lobe grew out containing no zoöids, but only ampullae, so far as could be observed in the living colony. Later most of the colony died leaving only this lobe in which a vigorous circulation was maintained by means of the ampullae. These conditions prevailed for about two weeks when the first buds appeared. These were very few in number, and for several generations degenerated without arriving at maturity, often without even opening their siphons. For about a month they did not cover more than about one tenth of the area of the colony, and during part of that time they were so small that they could not be detected with certainty. During this month their hearts cannot have helped much in keeping up the vigorous circulation that was maintained. Later the buds began to grow faster and gradually recovered their vitality, the part of the colony that contained only ampullae dying. We thus see that the ampullae had to maintain the entire

circulation for two weeks and the greater part of it for a month longer so that this mode of aestivation would be entirely impossible were it not for the ampullar circulation[3].

Summary.

1) The ampullae in *Botryllus* and *Botrylloides* normally execute coördinated pulsations.

2) These pulsations continue after the ampullae are separated from the rest of the colony, but the nature of the coördination changes.

3) In the various zoöids of the same colony the hearts do not beat synchronously or in the same direction at the same time. As a rule the reversals of the hearts take place at different times.

4) The rythm of the ampullar pulsations is not affected by the reversals of the hearts of the zoöids.

5) The pulsations are very slow, the systole averaging 38 seconds and the diastole 33.

6) The contracting tissue is a thin pavement epithelium composed of polygonal cells in which no fibrous differentiations could be detected.

7) The coördination of the ampullae is accomplished principally by means of variations in blood pressure.

8) In the aestivating colony of *Botrylloides Gascoi* the circulation is kept up almost entirely by means of the ampullae.

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Naples, Oct. 22, 1899.

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[3] It is probable that in hibernation and aestivation in other genera having a common vascular system the circulation may be maintained by some such means.