Name: Mary Ann Prewitt Davis Date of Degree: May, 1967

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Title of Study: THE PROTOZOAN CONTRACTILE VACUOLE

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Major Field: Natural Science

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In most cases the contractile vacuoles serve as osmoregulatory devices which remove excess water from the cell and is thought by some to be an excretory organelle.

ADVISER'S APPROVAL L. Herbert Bruneau

THE PROTOZOAN CONTRACTILE VACUOLE

By

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CHAPTER I

INTRODUCTION

Contractile vacuoles are organelles that collect fluid from the cytoplasm and expel it to the outside of the organism. Since the first description of the contractile vacuole, few structures have received such intensive investigation. They have been extensively studied in protozoa in which they occur in almost all free-living freshwater forms, and in some parasitic and sea water species. The presence of such vacuoles is not, however, limited to protozoa, they have been described in freshwater algae, sponges, and in certain blood cells of the frog, guinea pig, and man, (Rudzinska, 1958). Their occurrence in such diverse forms as protozoa, invertebrates and vertebrates suggests that similar vacuoles are probably present in many other groups, but so far have not been noticed.

To identify the contractile vacuole with certainty it is necessary to see it in operation in the living cell. The rhythmic contraction of this organelle is the feature that distinguishes it from the other vacuoles of the cytoplasm. As is well known, each contraction results in the discharge of the fluid contained within the vacuole and is followed by the reappearance of the structure and its expansion until it is ready for a repeated contraction. The growing phase (diastole) may last for several seconds to several minutes, while the emptying (systole) of the vacuole is usually very rapid and lasts only 2 to 3 seconds.

In spite of the extensive work on the contractile vacuole very little is known about the structural mechanisms involved in its systolic and diastolic movements. Its function is still debated. There is good evidence that it regulates osmotic pressure inside the cell, and there are also suggestions that it may function as an excretory organelle.

Because of its accessibility, the protozoan contractile vacuole offers an elegant experimental material, still largely unexploited, to investigators concerned with intracellular water transport mechanisms, and a considerable amount of information already available on its fine structure provides an added attraction.

CHAPTER II

THE ORIGIN OF CONTRACTILE VACUOLES

Metcalf (1910) noticed in Amoeba proteus that the vacuole is surrounded by a layer of granules. When the vacuole is of moderate size, these granules form a layer on its surface one granule thick; when the vacuole is fully distended, as just before systole, there are spaces between the granules; but when the vacuole is small the layer may be several granules thick. At systole the vacuole usually collapses completely, and the granules may be seen clumped together in the region of the cytoplasm previously occupied by the vacuole. The new vacuole arises in the midst of these granules, and is formed by the fusion of several small vacuoles. According to Metcalf, who reported observations which sometimes lasted for as long as several hours on a single organism, the vacuole never arises in any other part of the body under normal conditions, except among the granules which surrounded it before its last contraction. From these observations he concludes that the granules are associated in some way with the origin and the function of the vacuole, and for this reason calls them "excretory granules". However, he decides that the granules are not essential for life, since most of them, together with the vacuole, may be removed from an amoeba by operation without a fatal result. Under these conditions a new vacuole develops, although there are few if any granules to be surrounding it where it first appears. Metcalf reaffirmed his statement

concerning these observations in 1926.

Mast (1926) also observed the frequent present of granules around the vacuole, but does not interpret this as indicating a physiological association between them. This opinion being based on his having observed vacuoles functioning perfectly normally without the presence of a single granule in the immediate vacinity of the vacuole. To these granules Mast applies the name "beta granules", to distinguish them from others of a different nature which are also present in the cytoplasm. Mast and Doyle (1935) reinvestigated the relationship between granules and vacuoles. By centrifuging amoebae it is possible to cause stratification of various cytoplasmic constituents. Organisms treated in such a manner can be operated on so as to remove all or any desired portion of almost any one of the constituents, including these granules. Mast and Doyle found that removal of all or most of the granules resulted in the death of the organism. Removal of fewer granules caused a decrease in pulsation frequency of the vacuole which was directly proportional to the relative number of granules removed, that is, pulsation frequency was found to be directly proportional to the number of granules remaining. Removal of the contractile vacuole alone resulted in the prompt formation of another.

Hall (1930a) studied the cytoplasmic inclusions in Trichamoeba after osmic and silver impregnation. In a few instances he observed the adherence of blackened globules to the outer surface of vacuoles. At first glance these appeared to be vacuoles with heavily walls, but close observation revealed the granular or globular nature of the blackened material.

The cytoplasmic granules associated with the contractile vacuole are not confined to the immediate vicinity of the contractile vacuole, but usually are scattered through out the entire cytoplasm as well. If the origin of the vacuole is associated with and dependent on the presence of these granules, then one would expect other parts of the organism to be at least potentially capable of giving rise to vacuoles, since some granules are present in other parts. This phenomenon has been observed in Amoeba by various authors, among whom are Day (1927), as well as Howland, Mast and Doyle. Dimitrowa (1928) was able to induce formation of extra vacuoles in Paramecium caudatum by interfering mechanically with the normal function of those already present. These extra vacuoles usually appeared to be entirely normal although in a few instances there were no radial canals. The customary number of vacuoles was restored at fission by failure of the organism to form new ones if two extra ones had been induced, or by the formation of one new vacuole if only one had been induced artificially.

Hall (1930b) found that in Menoidum, stained according to the Da Fano silver method, the contractile vacuole is formed by the fusion of several smaller vacuoles arising near the gullet.

The mode of origin of contractile vacuoles has been studied in a greater variety of ciliates than in either rhizopods or flagellates. Taylor (1923) observed in Euplotes that the vacuole (V_1), in its final form immediately before contraction, is the result of the fusion of several smaller vacuoles, and that these smaller vacuoles (designated as group V_2) in turn are formed by the fusion of still smaller vacuoles (group V_3). The smallest vacuoles in the series are thought to arise as the result of the dissolving of granules, or to arise de novo. Thus

Taylor suggests granules as a possible source of vacuolar fluid, and he observed formation of the vacuole by the fusion of several small accessory vacuoles. King (1933), who studies Euplotes after impregnation with osmic acid, found that the smallest visible accessory vacuoles (V_3) have their origin at the distal ends of a very large number of collecting canals, located just under the ectoplasm on the dorsal surface of the ciliate. These canals radiate like a sun-burst from the vicinity of the vacuoles, and seem to end blindly in the protoplasm of the organism. These canals have a diameter of approximately 0.5 micron at their distal ends, and become relatively much narrower as they pass away from the region of the vacuoles. The canals are not visible in living organisms, but may be clearly demonstrated by proper impregnation with osmic acid.

Of particular interest are the observations of MacLennan (1933) on the Ophryoscolecidae, ciliates from the stomachs of cattle. The cycle of the contractile vacuole was studied in both living and fixed material, including the following genera: Ophryoscolex, Epidinium, Ostracodinium, Polyplastron, Endiplodinium, and Metadinium. In all these genera the contractile vacuole is formed by the coalescence of small accessory vacuoles. These accessory vacuoles arise from the dissolving of granules which are found in sharply defined regions around the contractile vacuole in Endiplodinium and Metadinium, in a narrow dorsal strip of the ectoplasm in Ostracodinium, and in the whole ectoplasm in Ophryoscolex and Epidinium.

CHAPTER III

THE STRUCTURE OF CONTRACTILE VACUOLES

In amebae, contractile vacuoles move about through the protoplasm and presumably have no morphologically determined discharge pore. Light microscopy indicates a rather thick vacuolar membrane and surrounding this a layer of gelated protoplasm and a cloud of beta granules. In the electron microscope the vacuole in Amoeba proteus, Pelomyxa carolinensis, and Hartmannella rhysodes (Greider, Koster, and Frajola, 1958; Pappas, 1959; Mercer, 1959) is limited by a typical unit membrane. The protoplasm surrounding it is filled with tubules and vesicles, 20 to 200 millimicrons in diameter. This vesicular zone varies from 0.5 to 2 microns in thickness, probably depending on the stage in the vacuolar cycle. Beyond it is a halo of mitochondria (the beta granules of the light microscopist), irregularly crowded while the growing vacuole is small but becoming aligned in a compact ring as the vacuole enlarges. Rather frequently, individual vesicles appear to open into the vacuole, and Mercer and Pappas agree in their interpretation of this picture as one suggesting segregation of fluid into membrane-bounded vesicles and tubules, and the emptying of this fluid into the main vacuole by coalescence. The mitochondria presumably provide energy for the segregation, and may in addition be involved in the water transport itself (Pitelka 1963).

In Tokophrya the vesicles do not appear to be particularly numerous; in the peritrichs and the astomes, the thick cortical zone is permeated by distinctly tubular elements that branch and anastomose and occasionally dilate to form larger vesicles. In all cases, occasional images suggest the opening of tubules or vesicles into the main vacuole. Around the periphery of the spongy zone, membranes of the tubules occasionally appear to be continuous with membranes of the endoplasmic reticulum. Mitochondria are variably abundant in the surrounding cytoplasm (Pitelka 1963).

Relatively long discharge canals lead from the vacuole to the exterior in the two peritrichs; these have thick, apparently homogeneous walls and appear during diastole to be closed at both proximal and distal ends by membranes. In Tokophrya a permanently open external channel leads to the body surface from a small papilla projecting into the contractile vacuole; in the papilla the lumen of the canal narrows to a very fine tubule during diastole but is widely expanded during systole. Fine fibrils, about 18 millimicrons in diameter, radiate from the channel to the adjacent vacuole membrane and might, if they are contractile, be responsible for changes in the diameter of the excurrent tubule.

In the astome Metaradiophrys gigas the discharge canal consists of a distal invagination of the cell surface with annular fibers in one side of its wall and a proximal cone-shaped part ending in a papilla in in the main vacuole wall. This proximal end is closed off by a septum from the vacuole cavity. Radially arranged fibrils that look tubular in section pass from the canal wall to the vacuole's cortex. Some other astomes have a contractile vacuole apparatus that is a permanent long tube. This undergoes rhythmic waves of contraction, and discharges

by a series of well-marked pores. The latter have longitudinal fibers in their walls, and again, radial fibers leading from their proximal parts toward the main vacuole wall.

Rudzinska (1958) found in Tokophrya several structures of possible importance for the mechanisms of systole. The permanent vacuolar outlet or canal has a complex structure which involves differentiation along its axis and the presence of numerous orderly disposed fibrils. The canal consist of a pore, a broad channel, and a narrow tubule located in a papilla that protrudes into the cavity of the contractile vacuole while the pore and channel have relatively stable dimensions and are permanently widely open, the diameter of the tubule changes over a wide range. During diastole its diameter is so small that it might be regarded as being closed. At systole the tubule opens so widely that its identification as a distinct segment of the channel becomes rather difficult.

Rudzinska assumes that the broading and narrowing of the tubule is accomplished by the contraction and relaxation of the numerous fibrils located around the canal. If, in addition to the radial array, circular fibrils are a regular feature of the outlet, then the presence of two sets of fibrils would suggest that they act like a combined dilator and constrictor in opening and closing the tubule.

Noirot-Timothee (1960) observed contractile vacuoles of an unusual sort in several entodiniomorph ciliates. These intestinal symbiotes of ungulates have vacuoles that pulsate at rather infrequent intervals, with a prolonged resting stage interrupting diastole. Light microscopy shows a basophilic and osmiophilic cortex about the vacuoles. In electron micrographs this zone during the resting stage appears as a sparse halo of tiny, clear, spherical vesicles.

Paramecia possess the most complex contractile vacuole apparatus ever examined in protozoans. In most species of this genus the contractile vacuole is a reservoir fed by pulsating radial canals. Schneider (1960a, 1960b), in a thorough electron-microscope study observed a network of fine canaliculi, 15 to 20 millimicrons in diameter, forms a cylindrical field around each radial canal. At the periphery of the field these nephridial tubules appear in places to be continuous with endoplasmic reticulum. The radial canal has a round cross-section in diastole and apparently collapses like a ballon at systole. At this time, a narrow layer of homogeneous substance is present between the walls of the deflated radial canal and the adjacent tubules, but during diastole this layer disappears and some of the tubules appear to open into the canal. Around the swollen medial ends, or ampullae, of the radial canals, the surrounding sponge of nephridial tubules is less dense. A narrow injector canal leads from each ampulla obliquely into the main contractile vacuole, nephridial tubules are lacking around the injector canal and around the vacuole. Fine fibrils, about 20 millimicrons in diameter and appearing tubular in cross-section, are seen singly on the outer surface of the membrane of the ampullae. They pass along the walls of the injector canals, joining to form increasingly wider ribbons, and continue, in tracts of ten to forty fibrils each, along the outer, or pellicle, side of the contractile vacuole. The vacuole itself has a membrane indestinguishable from that of the radial and injector canals. In diastole, the vacuole is round; in systole the inner wall of the vacuole is flattened but smooth while the outer wall is deeply folded in regular waves, with the bands of fibrils occurring on one slope of each wave. The fibril

bands continue and describe a spiral about the discharge canal, which is formed of invaginated cell membrane. It is open externally and, during diastole of the vacuole, closed at its inner end by a double septum consisting of cell membrane on the outside and vacuole membrane on the inside.

In the cytoplasm surrounding the zone of nephridial tubules are mitochondria in moderate numbers and clusters of tube-like elements of unknown significance.

In most studies, the cytoplasmic zone in which segregation of water and resorption of solutes almost certainly takes place is seen to be occupied by tubules or vesicles providing a large, and in the more extreme cases enormous, membrane surface area. Linkage of these tubules with endoplasmic reticulum may be significant if the latter system can be demonstrated to function in water transport elsewhere in the cell; the contractile vacuole apparatus occupies, after all, only a relatively small part of the total cell volume (Pitelka 1963).

Contractile vacuoles in flagellates have been seen occasionally in electron micrographs; in several instances, simple membranes with no apparent cortical differentiations have been described.

The rate of pulsation of a contractile vacuole is dependent on such factors as temperature, age, physiological state, food, salt concentration, etc. In some protozoa a volume of water equivalent to the volume of the entire cell may be voided in as short a time as two minutes; under other conditions and in other species an equivalent amount of water may be voided in 24 to 48 hours, (Pennak 1953).

CHAPTER IV

TYPE AND NUMBER OF CONTRACTILE VACUOLES

Roving vacuoles, which may discharge anywhere on the body surface, are found in Rhizopoda, which have a changeable body form, and stationary vacuoles, with fixed outlet, are found in Flagellata and Ciliate, organisms with a more or less fixed body form which only changes within the limits set by the elasticity of the body surface. This correspondence between body form and type of vacuole is not surprising. Whereas roving vacuoles are formed by the fusion of contributory vacuoles, the origin of which in turn is obscure, fixed vacuoles either originate in contributory vacuoles, or are replenished by canals. In some cases small contributory vacuoles are formed in the surface membrane of the main vacuole. The distinction between the various types may be less clear than appears if contributory vacuoles are also fed by canals.

In most protozoa with contractile vacuoles one or two of these occur in each individual; sometimes there are three or four; and more rarely there is a greater number; up to fifty or a hundred. There is no correlation between the possession of numerous contractile vacuoles and body size. A few of these protozoa with numerous contractile vacuoles are usually large; most are of normal size. Some of these protozoa live in fresh water, some are marine, and a certain number are endoparasitic. However, size and body shape can be correlated with one particular type of contractile vacuole. Elongated ciliates of large

size are likely to have a canal-like contractile vacuole running the whole length of the body (Kitching 1938).

CHAPTER V

THE FUNCTION OF CONTRACTILE VACUOLES

Of the various functions assigned to the contractile vacuole those of excretion of metabolic waste products and regulation of hydrostatic pressure within the cell have received most frequent support. Some authors prefer to limit "metabolic waste products" to nitrogenous substances, although others include carbon dioxide as well.

Osmoregulation

In the freshwater protozoa the body of which is hypertonic to surrounding water, the water diffuses through the body surface and so increases the water content of the body protoplasm and interfere with its normal function. The contractile vacuole, which is invariably present in all freshwater forms, is the means of getting rid of this excess water from the body. Most marine or parasitic protozoa live in nearly isotonic media and there is no excess of water entering the body, hence the contractile vacuoles are not usually found in them. Just exactly why all ciliates and suctorians possess the contractile vacuole regardless of habitat, has not fully been explained. It is assumed that the pellicle of the ciliate is impermeable to salts and slowly permeable to water (Kitching, 1936). Tartar (1954) showed that in fragments of Paramecium lacking mouth and gullet, pulsation of the contractile vacuole continued, though it was slower than normal rate, which indicates

that some water passes through the pellicle.

That the elimination of excess amount of water from the body is one of the functions of the contractile vacuole appears to be beyond doubt judging from the observations of Zuelzer (1907), and others, on Amoeba verrucosa which lost gradually its contractile vacuole as sodium chloride was added to the water, losing the organelle completely in the sea water concentration. Furthermore, marine amoebae develop contractile vacuoles do novo when they are transplanted to freshwater as in the case of Vahlkampfa calkinsi (Hogue, 1923) and Amoeba biddulphiae (Zuelzer, 1927). After studying an x-ray induced mutant of Chlamydomonas moewusii without contractile vacuoles, Guillard (1960) concluded that water elimination is the sole essential function of the contractile vacuole in this organism.

The number of the contractile vacuoles present in a species is constant under normal conditions. The contraction period varies from a few seconds to several minutes in freshwater inhabitants, and is, as a rule, considerably longer in marine protozoa. Kitching (1938a) estimated that a quantity of water equivalent to the body volume is eliminated by freshwater protozoa in four to 45 minutes and by marine forms in about three to four hours.

How much water enters through the body surface of protozoa is difficult to determine. In Pelomyxa carolinensis, 2 to 4 per cent of the total volume per hour of water enters through the body surface (Lovtrup and Pigon, 1951). Water also enters the protozoan body in food vacuoles. In Vampyrella lateritia which feed on the cell contents of Spirogyra in a single feeding, many contractile vacuoles appear within the cytoplasm and evacuate the water that has come in with the food (Lloyd,

1926) and the members of Ophryoscolecidae show an increased number and activity of contractile vacuoles while feeding (MacLennan, 1933). The amount of water contained in food vacuoles seems, however, to be far smaller than the amount evacuated by contractile vacuoles (Gelei, 1925). Other evidences such as the contractile vacuole continues to pulsate when cytostome-bearing protozoa are not feeding and its occurrence in astomatous ciliates, would indicate also that the water entering through this avenue is not of a large quantity. In Suctoria, the contractile vacuole pulsates faster at the time of ingesting the protoplasm of the prey and thus apparently aid in feeding by eliminating water from the body (Kitching, 1956). How much water is produced during the metabolic activity of the organisms is unknown, but it is considered to be very small amount (Kitching 1938). The mechanism by which the difference in osmotic pressure can be maintained at the body surface is unknown. It may be, as suggested by Kitching (1934), that the contractile vacuole extrudes water but retains the solutes or some osmotically active substances must be continuously produced within the body.

Excretion

Weatherby (1927) found that urea is excreted by Paramecium caudatum, but was unable to detect urea in the fluid of the contractile vacuole by means of the micro-injection of his own modification of the xanthydrol reagent of Fosse. This reagent yields positive results with dilutions of urea as great as one part in 12,000. Calculations based on the volume of fluid eliminated by vacuoles and the quantity of urea excreted by known numbers of organisms in mass cultures indicated that the concentration in fluid of the vacuole would be of the order of one

part in 2,000 or 3,000, if all the urea were excreted via this route. It therefore appears that at most only a small part of the total urea is excreted in this manner. After removal of the fluid from the contractile vacuole of Spirostomum by means of micro-manipulation apparatus, and subsequent hydrolysis with urease, Weatherby (1929) found urea to be present in the vacuolar fluid in a concentration of about one part in 100,000. Calculations of the rate of excretion of urea by known numbers of Spirostomum in mass cultures indicate that this amount of urea accounts for only about one percent of the total urea excreted.

Parnas (1926) concludes from observed differences in pulsation frequency that the vacuole is mainly excretory in marine protozoa, and both excretory and osmotic-pressure-regulatory in freshwater forms. The excretory function is accepted apparently without reservations by von Gelei (1925), who homologizes the various parts of the vacuole system in Paramecium with the vertebrate kidney, ureter, bladder, and urethra, although he admits the possibility that this system may aid in removing excess water from within the organism. In Paramecium, von Gelei states that the vacuole removes approximately ten times as much water as is taken in with food, a fact which he fails to correlate with his claim of a predominantly excretory function. Day (1927) suggests that vacuoles in Amoeba originate in the fusion and coalescence of ultramicroscopic droplets of soluble katabolic waste which may include water of osmosis. He observed that conductivity water increases size, number, and pulsation frequency of vacuoles. Essentially the same observations and conclusions were extended by him to Paramecium and Spirostomum (1930).

If the contractile vacuole is active in excretion of nitrogenous wastes, as is frequently maintained, then one would expect it to be able to excrete certain dyes which had been injected into the cytoplasm. Many attempts doubtless have been made to demonstrate such a phenomenon, but few accounts of such experiments are to be found (Calkins 1941).

CHAPTER VI

CONCLUSIONS

Although contractile vacuoles occur in various phyla of living organisms, almost all information on these organelles is derived from studies on protozoa, for the simple reason that the latter are the most convenient material for observations and experiments. The contractile vacuole does not seem to be an organelle of uniform structure, even among protozoa. There are great differences as to its shape, size, number, and presence or absence of a permanent canal. However, in spite of these variations all contractile vacuoles have some common features; they form by the fusion of smaller contributory vacuoles, and they have a remarkably fast contraction at systole. The contraction which results always in the emptying of the content lasts in almost all contractile vacuoles from 2 to 3 seconds. The usual speed of contraction and the fact that it occurs at regular intervals when the general conditions remain the same, suggest that a very precise mechanism might be involved.

Contractile vacuoles originate as a result of the activity of certain cytoplasmic inclusions, which may be aggregated in the immediate vicinity of the vacuole in some species, or distributed more or less generally throughout the cytoplasm in others. Temporary contractile vacuoles are formed by the fusion of coalescence of small accessory vacuoles, which in turn originate by the fusion of still smaller

accessory vacuoles, the last and smallest vacuoles being formed in or associated with the cytoplasmic inclusions mentioned above. More or less permanent contractile vacuoles receive fluid as small droplets, or accessory vacuoles which fuse with some portion of the filling canals; these droplets originate in or on cytoplasmic inclusions in the same manner as those mentioned above.

There is a physiological membrane surrounding each contractile vacuole. In some organisms, particularly those possessing more or less permanent vacuole systems, these organelles appear to be surrounded by morphological membranes.

Direct evidence concerning the function of contractile vacuoles is almost entirely lacking. Indirect evidence indicates that in freshwater forms the vacuole protects the organism against excessive dilution of its cytoplasm. In marine and parasitic forms such a function would seem to be largely superfluous, although even in these the elimination of at least a small quantity of water by some mechanism appears to be necessary. Direct evidence indicating the presence of waste products of metabolism in the vacuolar fluid is very scant, although, in those forms possessing relatively impermeable surface structures, the vacuole is the only visible means by which such wastes may be passed to the exterior.

The outstanding features of contractile vacuoles, taken collectively do not lie in differences among them, but rather in similarities, both morphological and physiological.

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