CHANGES IN THE APPARENT CYTOPLASMIC HYDROGEN ION CONCENTRATION OF AMEBA DUBIA ON INJECTION OF EGG ALBUMIN

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INTRODUCTION

At the time this work was done more than ten years ago, no satisfactory explanation of the results could be found. The observations required that protein introduced into the medium containing the amebae, rapidly enter these cells, and this was considered unlikely. Recent work with entirely different materials and methods has indicated that this is not at all improbable (7). The results are therefore being presented with an interpretation based on the assumption that the protein used does enter the cell. The experiments to be described furnish evidence of the presence of a bicarbonate buffer system in the cytoplasm and show that the nucleus is functionally associated with the maintenance of the intracellular pH.

The early work of Chambers and his coworkers (4–6, 9–11) has been interpreted as indicating that the cytoplasm of various kinds of cells is uniformly at pH 6.9 ± 0.1. This pH was maintained as long as the cell remained alive even in the presence of acids or alkalies in the surrounding medium. They explained their observations by assuming that the cytoplasm is a buffered system and as such tends to maintain a uniform characteristic pH. Ammonia and carbon dioxide were striking exceptions to the behavior of other reagents in that they made the apparent pH of the cytoplasm respectively more alkaline or more acid.

More recently Spek (14) and Spek and Chambers (15) have described two components of the cytoplasm which give different staining reactions, the hyaloplasm which shows the color of the dye at about pH 7.6, and granuloplasm which has a more acid-staining reaction at about pH 5.0.

Materials and Methods

Egg albumin was crystallized with ammonium sulfate and sulfuric acid by the method of Sørensen (12) and then three times recrystallized. The crystals were dialyzed against distilled water for 48 hours by the method of Abramson and Grossman (1, 2) and then dried under reduced pressure over sulfuric acid. A 2 per cent solution of this dried product in glass-distilled water was prepared and kept at 2 to 6°C. Small samples were colored with a few drops of phenol red before injection. The pH of the injected solution was about 5.0.

All injections were made with a Chambers' micromanipulator, using a 43 × objective and 10 × ocular. pH determinations were made by comparing the color of the cytoplasm under a 10 × objective and 10 × ocular with a set of Clark standard tubes. The light source was a ribbon filament lamp with a white opal glass screen. Pipettes for handling amebae were made of pyrex glass, as were the micropipettes used for injection. All the amebae used were grown in Pace's medium (8). While making the injections the amebae were mounted in Pace's medium (without the wheat) and in glass-distilled water. The phenol red solutions used were made up with 28.2 cc. of 0.01 N NaOH per 0.1 gm. of the dye and diluted to 25 cc. with distilled water to give the 0.4 per cent reagent. The descriptions to follow, unless otherwise indicated, will be summaries of individual protocols.

OBSERVATIONS

A. Changes in the Streaming and Configuration of the Cytoplasm

1. Amebae Mounted in Pace's Solution and Injected with 2 Per Cent Albumin Colored with Phenol Red.—Immediately after injection of amounts varying from two to six or seven times the volume of the nucleus, the plasmalemma is raised to form one or two hyaline "blisters" equal in volume to one-half or the whole of the ameba. The hyaline area rapidly spreads to include the entire periphery of the ameba. Rotary streaming of the hyaline and granular cytoplasm, as described by Chambers and Reznikoff (4) for distilled water injections, then sets in. The hyaline area then becomes reduced to approximately normal proportions, the animal becomes elongate and monopodial, with streaming of cytoplasm in a central column toward the anterior (see Figs. 1–3).

With repeated injections the broad hyaline and granular regions remain distinct for a longer time, streaming movements are less frequent. When streaming occurs it proceeds as follows: The hyaline region of the anterior end broadens (Fig. 4); the more finely granular cytoplasm then streams into this broadened area from one or both sides and occasionally through the middle (Fig. 5), but the border between the granular and hyaline cytoplasm remains distinct; this border is then suddenly swept posteriorly and becomes lost as a definite boundary in the posterior end of the animal (Fig. 6).

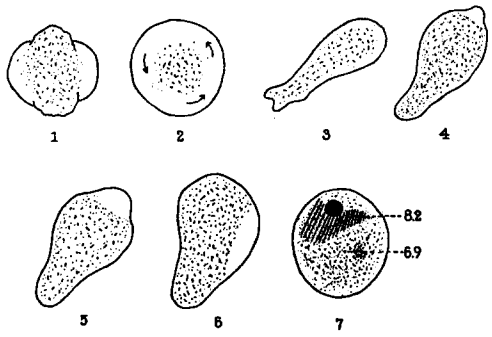
After the later injections (sixth, seventh) marked Brownian movement appears in the more hyaline regions and continues until the "boundary" between the heavily granular and more hyaline cytoplasm passes through them.

After about five injections there is also a noticeable change in the surface layer of the ameba. It seems to be much more easily penetrated by the pipette than in the first or second injections, and apparently adheres more firmly to the coverslip, since it is not as readily dislodged upon removal of the pipette as in the previous injections.

When the injections are made with the amebae mounted in distilled water the behavior is the same as described above. Such distilled water cannot

be considered free of salt, for when the animals are placed in a large volume of the same water or washed repeatedly with distilled water they become detached from the substrate, the pseudopodia become long and slender, and eventually all movement ceases. We must consider therefore that small volumes of distilled water dissolve enough salt from the container or the coverslip to maintain the osmotic balance.

II. Amebae Mounted in Pace Solution and Injected with 0.4 Per cent Phenol Red.—After injection of phenol red the plasmalemma is not raised as in the albumin injections and the animal retains its polypodial form. Sometimes there are one or two aggregations of crystals, apparently held together by



FIGS. 1 to 6. Successive changes in the form of an ameba injected with 2 per cent albumin in distilled water.

FIG. 7. An ameba which received one injection of phenol red followed by eight injections of 2 per cent albumin, 10 hours after the last injection. A portion of the cytoplasm has a color indicating a pH of about 8.2, while the rest of the cytoplasm is at pH 6.9.

a localized gelation of the cytoplasm. Streaming of the cytoplasm may be slow or may stop completely, but is resumed again at the usual rate. In the former case the normal rate is resumed in about 3 minutes, in the latter in half an hour. Coincident with this change is the shift back to the normal pH as described below. The first injection is very easily made, but the third and subsequent injections are increasingly more difficult. An interfacial boundary is formed about the fluid being injected. The droplet so formed is usually expelled from the cytoplasm. When the droplet is retained, it has a distinct boundary about it. These droplets lose their color completely in a few seconds. After a few minutes the droplets themselves disappear.

B. Color Changes

The color observed immediately upon injection of 0.4 per cent phenol red is similar to that of a standard tube at pH 6.6 or 6.7. Within 5 seconds the

color shifts to that characteristic for pH 7.0 to 7.2. When normal streaming is resumed (the time will vary depending upon the extent of the apparent gelation induced by the injection) the color is shifted back to pH 6.9. This color appears uniform throughout the cytoplasm except in the posterior regions where there are minute spherules that are definitely pink; *i.e.*, at a pH of about 7.4 to 7.6

I. Amebae Mounted in Pace's Medium and Injected First with a Solution Made up of Equal Parts 0.4 Per Cent Phenol Red and M/208 Calcium Chloride.[1] *Succeeding Injections Were of 2 Per Cent Albumin Solution.*—The albumin injections were made 2 hours after the injections of the dye. Following a momentary shift to the acid range, there was a shift to the more alkaline color. (This preliminary acid shift was not always observed.) For example, at the second injection two amebae shifted from pH 6.9 to pH 7.1. Another ameba shifted from pH 7.1 to pH 7.4 Four minutes after the injection the nucleus was slowly extruded. When this enucleated protoplast was injected 3 minutes later there was a shift to the more acid pH 7.1 and then to the more alkaline again, pH 7.4 to 7.6.

With further injections of albumin into non-enucleated amebae, *i.e.* 3rd, 4th, etc., there are similar successive shifts with each injection; for example, from 7.0 to 7.4, 7.6 to 8.0, 8.0 to 8.4 In a total of eleven ambae injected in this manner, ten showed a shift to a more alkaline condition. The one ameba which did not, changed from 8.0 to 7.1. It then rounded up and became colorless and motionless in 15 minutes.

II. Amebae Mounted in Pace's Solution and Injected with 2 Per Cent Albumin Colored with Phenol Red.—The effect of the first few injections cannot be followed because the color of the cytoplasm is too faint. Later injections show the alkaline shifts similar to those described above. Six such cases were observed. One animal showed no alkaline shift. It ruptured suddenly after the fifth injection.

With animals mounted in distilled water and injected with albumin colored with phenol red the behavior is similar. Four showed alkaline shifts. Two showed apparent acid shifts but are not considered significant since these were only second injections and the color was rather faint.

C. Enucleated Protoplasts

During the course of some violent injections the nucleus along with a considerable amount of cytoplasm was forced out of the cell. In one case, the membrane reformed about the enucleated protoplast which had a pH of about 7.2. No further observations were made on this animal. In other cases, however, the nucleus was slowly extruded through the plasma membrane

[1] The dye solution had been stored in a soft glass bottle. The calcium chloride was added in order to counteract any possible effect of Na^+ and K^+ dissolved from the glass.

some time after the injection was made. Four such cases were observed and are described below:

The ameba was mounted in distilled water and injected with 2 per cent albumin colored with phenol red. After the second injection, the cytoplasm showed a pH of 6.9. When pierced with the pipette there was a monentary acid shift, then a change to about 7.1. When punctured after the third injection, there was a change to about pH 7.3. Albumin was then discharged into the medium about the ameba and the cytoplasm took on a violet color (about pH 8.6). An hour and a half later the color was red (about pH 7.2 to pH 7.4). A volume of albumin about two times that of the nucleus was then injected. The color of the cytoplasm indicated a pH of 8.2 to 8.4. The plasma membrane then became crenulate and streaming of the cytoplasm set in. The nucleus was then slowly extruded through the plasma membrane. The enucleated protoplast then streamed away in an apparently normal manner with the cytoplasm at pH 8.2 to 8.4. When albumin was discharged about the enucleated ameba the pH shifted to 8.6. Another injection of albumin was then made, and the color became violet (8.6 or higher). When the animal was now pierced the color became bluer.

Another ameba mounted in the same medium extruded its nucleus after the second injection of albumin when its cytoplasm was at a pH of about 7.4. The enucleated protoplast was then injected and a shift to pH 7.5 or 7.6 was noticed. 12 hours later the pH was about 8.2 and the cytoplasm was still actively streaming.

A third ameba was first injected with phenol red and then given injections of albumin, colored with the same dye. After the third injection the pH shifted from 8.0 to 7.7 locally in the region of the injection and then to 8.2 throughout the cytoplasm. The nucleus was then slowly extruded. One hour later the cytoplasm was actively streaming and had a color comparable to pH 8.2 to 8.4. Three hours later streaming was still active with the cytoplasm at pH 8.6 or higher.

A fourth ameba was injected under conditions similar to the previous one. After the fourth injection the pH shifted from 7.6 to 8.3. When albumin was then discharged about the animal there was a shift to pH 8.6. The nucleus was then slowly extruded and the enucleated protoplast streamed away with the cytoplasm about pH 8.2. It then rounded up and the crystals aggregated at one end. There was no color change on piercing. Twelve hours later the animal was colorless and apparently dead.

It was noted that all the enucleated animals which remained alive showed more alkaline pH values on standing whereas those with the nuclei retained, either shifted back to pH 6.9 completely or, where the injections had been numerous and large, contained localized regions of about $\frac{1}{4}$ to $\frac{1}{3}$ the size of the animal with a pH of 8.2 or higher while the rest of the cytoplasm was at pH 6.9 (Fig.7).

D. Gas Exchange

The rapidity with which the color changes are produced by injection or discharge makes it seem likely that ionic interchanges rather than digestive processes are responsible for the observed behavior. If there are carbonates

and bicarbonates in the cytoplasm, the albumin introduced inside the cell may replace the bicarbonate associated with base resulting in the liberation of free CO_2. Since the color changes observed with phenol red are similar when the albumin is brought in contact with the cell surface to when it is injected one might expect CO_2 to be given off when albumin is added to the medium containing the cells. To determine whether the bicarbonate in the cytoplasm might be involved in producing the change in the color reaction of the cell an experiment was performed, designed to measure changes in O_2 and CO_2 pressure when amebae were brought in contact with albumin.

It was found that by agitating cultures, and then allowing them to settle, the amebae soon became adherent to the bottom of the container and the debris along with foreign organisms could be removed by pouring off the culture medium. Fresh Pace solution (with no wheat or other organic material) was then added and the process repeated about ten times. The cultures were then allowed to stand overnight and the washings repeated on the following day. Examinations under the microscope showed the cultures to be free of all foreign material. The amebae were then concentrated (without centrifuging). A sample of the concentrated suspension was then centrifuged at 3300 R.P.M. for 45 minutes in a Hopkins vaccine tube. The concentration of amebae was found to be approximately 0.005 cc. per cc. of suspension. Three cc. of this suspension were then placed in the chamber of each of two small conical Warburg vessels, one of which had 0.1 cc. of 2 N KOH in the central well, the other 0.2 cc. Pace solution. Half a cc. of 2 per cent albumin in glass-distilled H_2O was placed in the side arm of these vessels. Control vessels contained (1) 0.4 cc. Pace solution in the chamber, 0.5 cc. 2 per cent albumin in the side arm, nothing in the well; (2) 3 cc. Pace solution, 0.5 cc. 2 per cent albumin in the side arm, and 0.1 cc. 2 N KOH in the well; (3) 6 cc. distilled H_2O in the chamber, no KOH, no albumin. Vessels 1 and 2 were used as barometers and checked against 3. They were tipped to bring the albumin into the chamber at the same time that the experimental vessels were. Readings were taken every 5 minutes for 2 hours, the vessels then tipped to bring the albumin into the chamber and readings continued for 2 more hours. Prior to the introduction of albumin, the amebae consumed 14.4 $mm.^3$ of oxygen per hour, during the same period they produced 17.0 $mm.^3$ CO_2 per hour. After the albumin was introduced into the chamber they consumed 17.1 $mm.^3$ O_2 per hour and produced 35.9 $mm.^3$ CO_2 per hour.

Before the amebae were in contact with albumin they produced CO_2 at a rate of 1.4 $mm.^3$ per 5 minutes. In the 4 minutes immediately following tipping of the vessel 9.7 $mm.^3$ CO_2 was produced, in the next 5 minutes 8.5, then 3.7, 3.0, 1.7 $mm.^3$ CO_2. The rate then became uniform as it had been prior to the introduction of albumin and averaged 1.2 $mm.^3$ per 5 minutes. There was no rise in the rate of O_2 consumption during the period when the CO_2 evolution was very high. The slight increase for the 1 hour period in albumin (3 $mm.^3$) was due to a slightly high rate throughout the hour and not to a marked increase immediately after the introduction of albumin as in the case of CO_2. Rates per 5 minutes of O_2 consumption after albumin were: 2.7, 0, 1.8, 0.9, 2.7, 0.9 $mm.^3$. Microscopic examination of the amebae at the end of the experiment showed them to be motile and apparently normal.

The results are shown graphically in Figs. 8–10. They may be taken to indicate that the bicarbonates of the cell are involved in the reaction, the free CO_2 being produced by replacement of bicarbonate ions by the albumin.

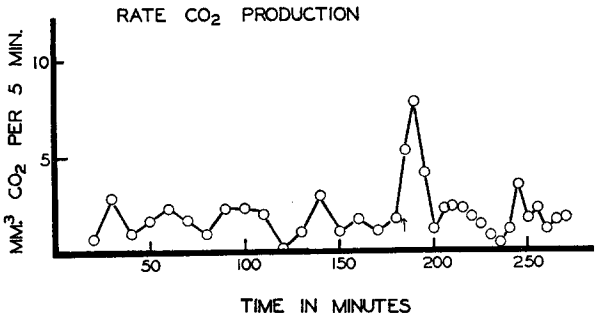


FIG. 8

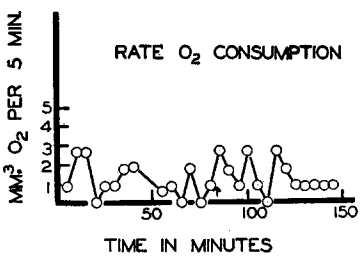


FIG. 9

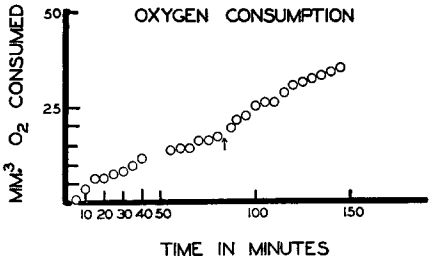


FIG. 10

FIGS. 8 to 10. Oxygen consumption and carbon dioxide production before and after contact with 2 per cent albumin. The arrow indicates the time the albumin was brought in contact with the amebae.

The more or less cyclic changes in the apparent rate of O_2 consumption and CO_2 production are probably due to inadequate shaking of the vessels. The shaking frequency was kept low (8 times per 10 seconds) to avoid injury to the cells.

DISCUSSION

The results observed cannot be explained solely on the basis of a Donnan membrane equilibrium. It is difficult to understand how evolution of CO_2 can be produced by the albumin unless the latter actually enters the cells. The experiments with phenol red point to the same conclusion since the color changes are similar whether the albumin be injected or merely brought to the cell surface. However, this alone does not adequately explain the results since egg albumin added to a bicarbonate solution causes a drop in pH whereas an apparent rise was observed. It seems probable therefore that although both the dye and the albumin readily permeate the cytoplasm when injected they nevertheless become rapidly distributed to different phases of it. Sim-

ilarly, if the albumin enters the cell through the surface membrane it passes into a phase of the cytoplasm other than the one containing the phenol red. Evidence of the existence of at least two phases in the cytoplasm has been obtained by different methods and for various types of cells by Spek and Chambers (15), Baas-Becking and his coworkers (3), and by Seifriz (13). Apparently, albumin when it first enters the cell, displaces HCO_3^- but then becomes bound to a micellar surface and effectively removed from the medium containing the phenol red, resulting in a more alkaline coloration of the latter. When the nucleus is present enough CO_2 is produced to bring the pH back to 6.9. In the absence of the nucleus, however, this process proceeds at too slow a pace to restore the original pH within 12 hours.

SUMMARY

1. Egg albumin when injected into an ameba or discharged into the solution about it raises the apparent pH of the cytoplasm of the ameba.

2. With time the cytoplasm returns to the original pH 6.9 if the nucleus is present. Amebae that have received repeated injections of albumin in some cases extrude their nuclei. In these cells the cytoplasm remains at the more alkaline pH induced by the albumin for at least 12 hours.

3. When a 2 per cent solution of albumin is introduced into a suspension of amebae there is a temporary marked rise in the rate at which CO_2 is given off with no corresponding rise in O_2 uptake.

4. The results observed can be explained if the albumin discharged onto the surface of the ameba rapidly enters the cell and there becomes distributed in a phase of the cytoplasm other than the one which contains the phenol red.

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