

obsessive-compulsives showed characteristic behavior in spending a considerably longer time in the selection of "pleasing" rhythms than members of other groups. These experiments were not intended to demonstrate that this method is capable of discriminating between clinical groups. However, the indications are that further research is justified on the basis of the interesting responses to audio-visual-tactile stimulation.

References and Notes

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Ionic Permeability and Osmotic Swelling of Cells

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Many mammalian tissues when studied under unnatural conditions outside the body have been observed to swell in salt solutions isosmotic with blood and tissue fluids. One suggested explanation of this behavior is that the contents of the cells of these tissues *in vivo* are hypertonic to the surrounding media and the water that tends to enter them by osmosis is normally removed by a process of active water secretion (1). When this process fails because of anaerobic conditions, the action of metabolic poisons, or low temperature, the cell swells.

Little is definitely known at present about mechanisms, other than contractile vacuoles, for primary active transport of water, but much information has recently been gained concerning processes of active ionic transport in a great variety of cells. Such processes are known to be frequently accompanied by passive osmotic movement of water—for example, the movement of water associated with secretion of NaCl by a frog's skin (2).

The influence of colloid osmotic pressure on the movements of ions and water is well accepted in the case of capillary walls, but it is not generally recognized in the case of most tissue cells. A known instance in which this is involved is the type of hemolysis that Wilbrandt (3) calls "colloid-osmotic." Jacobs and Stewart (4) have discussed in some detail

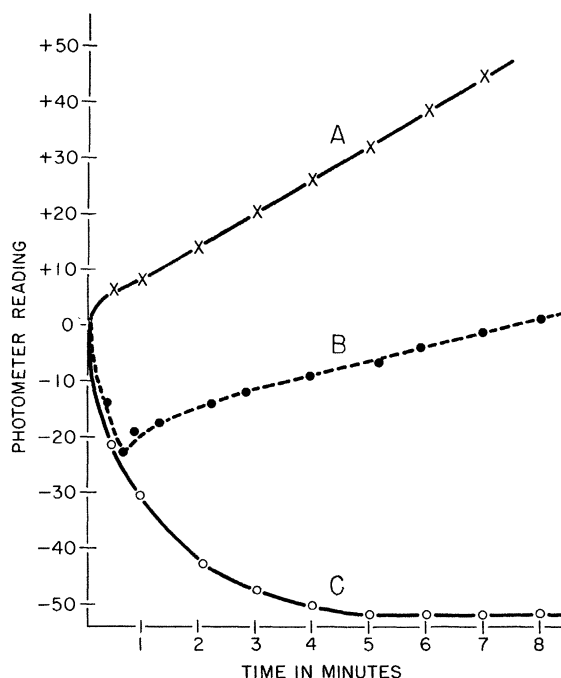


Fig. 1. Volume changes of butyl alcohol-treated beef lymphocytes in various isosmotic solutions. Beef lymphocytes were placed in a mixture of 1 part 0.3M sucrose and 5 parts 0.15M NaCl (pH adjusted to 7.4 with phosphate buffer) containing 5 vol percent n-butyl alcohol. After exposure for 5 min, the cells were diluted five fold with the same sucrose-NaCl mixture without butyl alcohol. One milliliter of the treated cell suspension was added to 10 ml of the following solutions: (A) 0.15M NaCl; (B) 1 part 0.3M sucrose and 5 parts 0.15M NaCl; at zero time 3 drops of saturated NaCl solution was added; (C) 0.3M sucrose. Similar results were obtained with polymorphonuclear leucocytes from rabbits.

the osmotic consequences of a variety of types of ionic and molecular permeability, including the abnormally high cation permeability of the erythrocyte that gives rise to hemolysis by swelling. A very convenient agent for producing different easily controlled degrees of cation permeability and of swelling in salt solution is n-butyl alcohol (5, 6).

The erythrocyte is a highly specialized cell whose behavior may or may not throw light on the mechanism of volume changes in typical mammalian tissue cells. Experiments were, therefore, made with leucocytes, whose general properties are more closely related to other animal cells. Polymorphonuclear leucocytes were prepared from rabbits by lavage of the peritoneal cavity with saline. A mixture of lymphocytes, monocytes, macrophages, and erythrocytes was also prepared from spleen by mincing the tissue suspended in isosmotic NH_4Cl . Erythrocytes were hemolyzed in this solution, leaving intact the leucocytes, which were then centrifuged and resuspended in buffered saline. Volume changes of these cells were measured by changes in optical density in a sensitive photometer.

The result of one experiment is shown in Fig. 1. Following treatment with butyl alcohol, the leucocytes maintain their normal volumes for a long time in a mixture of 1 part isosmotic sucrose and 5 parts isosmotic saline; but the cells swell rapidly in isosmotic NaCl solution alone, shrink rapidly in isosmotic sucrose solution alone, and shrink with rapidity and then less rapidly recover, or slightly surpass, their original volume when a little concentrated NaCl is added to the surrounding medium. Shrinkage of cells in isosmotic sucrose is due to the diffusion of salt out of the cells followed by a movement of water. Such cells have enormously increased permeability to anions, as well as to cations, but retain their normal impermeability to sucrose and protein. These results illustrate an essential agreement in the behavior of leucocytes and erythrocytes.

It should be remembered that, although an active transport of cations is essential to the continued existence of the erythrocyte, its absence would be expected to produce swelling only very slowly. It is now known that the physical permeability of this cell to cations is so low that, despite its extremely favorable surface-volume relationship, rates of exchange of potassium across the surface of the human erythrocyte at body temperature are less than 2 percent of the cell potassium per hour (7, 8). By way of contrast, Davies and Galston (9) have reported for kidney cells exchanges of the order of 15 percent per minute. It follows, therefore, that, while a considerable degree of surface injury by butyl alcohol or other agents is needed to produce rapid swelling of the erythrocyte, a mere cessation of the normal ionic transport process might soon bring about the same result in the kidney cells.

Perhaps the strongest reason at present for believing that ionic movements may be involved in the volume changes of tissue cells outside the body is the recent observation by Deyrup (10) that, while kidney slices swell in solutions of NaCl or monosaccharides isosmotic with blood, they shrink in similar solutions of disaccharides. This behavior, which strongly resembles that of the erythrocyte and the leucocyte after treatment with butyl alcohol, cannot be explained by a simple initial hypertonicity of the cells themselves. Preliminary evidence has also been obtained that the mammalian intestine behaves in the same way. In six experiments, rat intestine increased in water content 17 percent over the control when placed in 0.15M NaCl at 0°C, increased 7.6 percent in a solution composed of 1 part 0.3M lactose and 5 parts 0.15N NaCl, and decreased 13.2 percent in a 0.3M lactose solution.

The data presented are consistent with the hypothesis that the tendency of salts and water to enter cells owing to the intracellular colloid osmotic pressure is, under normal conditions of oxygenation, temperature, and so forth, exactly balanced by the active transport of ions and passive movement of water out of the cell.

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Ascomycete Spore Mutants and Their Use in Genetic Studies

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Ascobolus stercorarius is a heterothallic ascomycete which in its life-cycle resembles in many ways the eight-spored species of *Neurospora*. In wild-type crosses of the two mating types, every fruiting body produces hundreds of asci, each of which contains eight haploid, uninucleate ascospores. These spores become purple, then brown, as they mature.

In the course of our genetic studies, two interesting cultures have been obtained, both of which appear to be single-gene mutants. The first of these carries a factor for ascospore abortion. Thus, in a cross between this mutant and a wild-type isolate, the resultant asci contain four viable, brown-colored spores and four abortive, colorless spores (Fig. 1). This particular mutation apparently occurs quite frequently in this species, and it has been found by at least two previous workers. Dowding (1) reported that in wild-type crosses many of the asci contained these two types of spores. Ingold (2) pictures a fruiting body of *A. stercorarius* showing an ascus with these two spore types.

The second mutant apparently carries a factor influencing only spore color. When it is crossed with a wild-type strain of appropriate mating type, the asci produced contain four wild-type, brown-colored spores and four mutant, tan-colored spores (Fig. 2). In this case, however, all eight spores are viable. In the case of both mutants, crosses with wild-type cultures always produce the six expected ascus segregation patterns, and a preliminary scoring of asci gives a second-division segregation frequency of approximately 20 percent for the spore abortion factor and about 62 percent for the tan-spored factor. Therefore, the two are not alleles, but whether they are linked has not yet been determined.

Figure 3 shows one type of ascus resulting from a cross between the two mutants. The spore abortion locus has segregated in the first division, while the tan-spored locus has segregated in the second. All of the four expected ascospore phenotypes cannot be distinguished, since the four spores carrying the abor-