Reports

Some Physical Aspects of the Bacterial Cell

The volume and weight fractions of protoplasm and wall, and the water content, are, in the case of the bacterial cell, interlocking elements of knowledge. When the water content of the cell, and the weight fraction and the volume fraction of either the protoplasm or the wall, are known, the remaining elements can be calculated. The information obtained not only provides additional knowledge concerning the structure of the organism but also tests the self-consistency of experimental results. The information necessary for the calculation has, within the past few years, become available for several organisms.

On the basis of direct isolation procedures, Cooper, Rowley, and Dawson (*1*) concluded that the cell wall of *Staphylococcus aureus* accounted for 20 percent of the dry weight of the organism. McCarty (*2*) has reported, also on the basis of direct isolation of cell-wall materials, that the cell walls of group A streptococci make up at least 10 to 15 percent of the dry weight of these cells. More recently, Barkulis and Jones (*3*) concluded that cell walls make up 23 to 25 percent of the dry weight of group A streptococci. The thickness of the wall of both *S. aureus* and *Streptococcus faecalis* has been estimated by means of the electron microscope to be 150 to 200 A (*4*). Electron diffraction studies of group A streptococci (*3, 5*) suggest that the wall of this organism may be somewhat thicker than that of *Staphylococcus aureus*. Since the electron diffraction studies pertain to desiccated structures in which shrinkage has occurred, the thickness of the walls of the wet cells may be considerably greater than 200 A. The volume fraction of the protoplasm of cocci amounts to 0.87 if one uses 4500 A for the radius of the cell and 200 A for the thickness of the wall, and 0.81 if one uses 300 A for the thickness of the wall.

From the data of Weibull (*6*), the *Bacillus megaterium* cell contains 80 percent water and the volume fraction of the protoplasm amounts to 0.54. Weibull (*7*) has estimated that the wall of *B. megaterium* represents 20 percent of the dry weight of the cell.

In this report (*8*) an attempt is made to consider the structural features of the cell which are implied as a consequence of the limitations imposed by the data reported above.

Assumptions and calculations. The water content of the cell is an important parameter in the calculations which follow. Group A streptococcal cells, when removed from a water suspension by centrifuging for 15 minutes at 20,000 $\times g$, yield a tightly packed pellet which, after being lyophilized, has amounted repeatedly to 17 percent of the wet weight (*9*). The interspace volume for close-packed rigid spheres is 26 percent. If the above interspace volume is taken into account, the water content of the cell becomes 77 percent. In the case of cocci, however, chains of cells are usually present in the culture which would cause the interspace volume of the centrifuged pellet to be greater than 26 percent. When an arbitrarily selected interspace volume correction of 33 percent is applied, the water content of the cell becomes 75 percent. It is apparent that even a considerable error in the correction for interspace volume does not greatly influence a calculation of the water content of the cell.

Mitchell and Moyle (*10*), employing osmotic methods, found an effective phosphate-impermeable cell volume of 2.42 ml/g of cell dry weight for *Staphylococcus aureus*. If we assume the partial specific volume of the "cell solids" (*11*) to be 0.72, the water content of *S. aureus*, according to Mitchell's data, becomes 63 percent by weight. The method used by Mitchell, however, may provide the volume of the protoplast rather than that of the whole cell. We have adopted a value of 0.70 as representing a reasonable approximation of the weight fraction of water in the cells of cocci.

The average specific volume of the cellular constituents, other than water, is assumed to be 0.72. The partial specific volumes for most simple proteins, nucleoproteins, and glycoproteins lie between 0.68 and 0.75. The quantity of lipids and dialyzable material (*9, 12*) represents such a small fraction of the cellular dry weight that the value of 0.72 seems reasonable. Weibull (*6*) reported a value of 0.70 for the average partial specific volume of the "cell solids" of *Bacillus megaterium*. The factor of electrostriction can be ignored as not seriously affecting density and volume (*13*).

For simplification the cell is considered to consist of a protoplast surrounded by a wall. It is assumed that the coccus can be represented as a rigid sphere. It seems reasonable to assume, also, that the portion of volume of wall not occupied by "solids" is fully occupied by water.

With volume and weight fractions established, the proportion of water to solids in both wall and protoplast can be readily calculated in terms of an average partial specific volume of the solids.

Results. In the case of cocci, the volume fraction of the protoplast has been calculated to be 0.87, as mentioned above. If the relative weight of the cell is taken as 100, and the weight fraction of water in the cell is 0.70, the cell contains 30 weight units of solids. If six weight units—that is, 20 percent—of the solids are in the wall, the relative volume of the cell is

$$30 \times 0.72 + 70 = 91.6.$$

The relative volume of water in the protoplast becomes

$$0.87 \times 91.6 - 24 \times 0.72 = 62.5 \text{ units.}$$

The wall, therefore contains 7.5 weight units of water, and the weight fraction of water in the wall is 0.56. If a weight fraction of water in the cell of 0.75 is used, the fraction of water in the wall is increased to 0.63. It can be seen in Fig. 1 that the thickness of the wall, in the case of cocci, is not especially sensitive to either the radius or the water content of the whole cell. As is shown in Fig. 2 the water content of the wall is, however, for the parameters stated, sharply dependent upon the thickness of the wall. Since the thickness of the wall is related to the structural organization of the solids constituting the wall, the water content probably becomes invariant if the thickness can be established.

In the case of *Bacillus megaterium*, it can be shown that approximately 10 g of water must be present for each gram of "solids" in the wall, for the relationships reported by Weibull (*6, 7*) to obtain. The protoplasm of this organism, on the other hand, would, according to Weibull's data, contain only 2.5 g of water for each gram of solids. As calculated, the wall of *B. megaterium* contains 91

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percent water, whereas the water content of the protoplast is only 71 percent. The result seems bizarre. It also follows, on a wet weight basis, that the wall represents 45 percent by weight of the total cell, which agrees with the experimental findings of Weibull (*14*). The weight fraction of water in the protoplast, 0.71, obtained from the calculation also agrees quite well with that required by the phosphate-impermeable volume found by Weibull (*6*) to be 2.9 ml/g dry weight. The phosphate-permeable volume accounts for a volume fraction of the cell of 0.54 (*6*). A volume fraction of 0.54 for the protoplasm is consistent, furthermore, with dimensions for the protoplasm and the cell listed by Weibull (*6*), for *B. megaterium*.

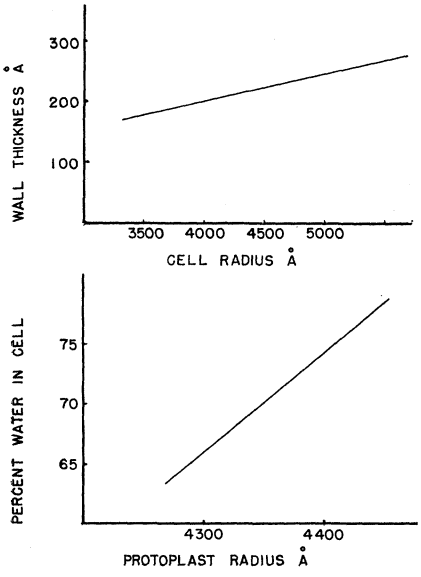

Fig. 1. (Top) Relationship for cocci between the cell radius and thickness of the wall in terms of the parameters listed. Water content of the cell, 0.70; wall dry-weight fraction, 0.20; wall 60 percent water by weight. (Bottom) Relationship for cocci between the radius of the protoplast and the water content of the cell in terms of the parameters listed. Radius of cell, 4500 A; wall dry weight fraction, 0.20; wall 50 percent water by weight.

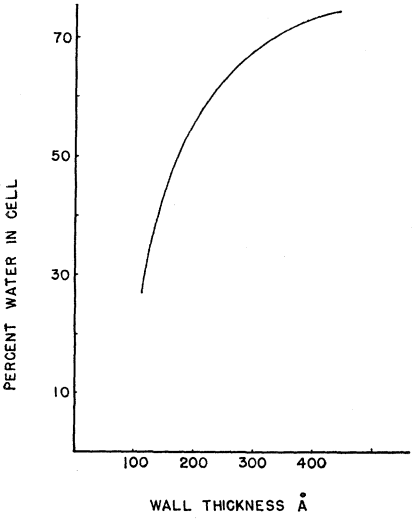

Fig. 2. Thickness of the wall of cocci as a function of water in the wall in terms of the parameters listed. Radius of cell, 4500 A; wall dry weight fraction, 0.20; weight fraction of water in cell, 0.70.

Discussion. In the case of cocci, if all the cellular water and only 50 percent of the cell solids (dry weight) were present in the protoplast and if the protoplasmic constituents were dissolved in the water, the concentration of solids would be greater than 17 percent by weight—a quite unlikely situation. Available evidence indicates that only about 10 percent of the cellular constituents are dialyzable materials of low molecular weight (*9, 12*). The remaining 90 percent solids (dry weight) appear to be complex proteins and polysaccharides of high molecular weight (*9, 12*). Few of these substances are likely to be soluble in water to an extent greater than 2 or 3 percent. It seems necessary to conclude that a large fraction of the materials in the protoplast must be present in an undissolved although probably heavily hydrated form. If the weight fraction of the protoplast (dry weight) is substantially greater than 50 percent, the quantity of undissolved matter must increase accordingly.

An alternative possibility would be a protoplast existing as a thick gel. The gel hypothesis has been considered by Mitchell and Moyle (*10*), and there is evidence against this concept. A third possibility, that the solids present in the wall amount to a much larger weight fraction of the cell than has been reported, should not be ignored.

The above considerations suggest that a quantity of water amounting to more than the weight of the solids is present in the walls of bacteria. In order to accommodate such a quantity of water, the wall could be pictured as a fenestrated three-dimensional network of solid material in which the openings are filled with water. Such a structure would confer the rigidity noted in bacterial walls (*15*). In addition, the structure would readily permit the physical passage of molecules of some size, which from a metabolic viewpoint is a necessary quality of the wall.

It can be shown by the method indicated above that an increase in the weight fraction of solids in the wall, the volume fraction of protoplasm, or the water content of the cell either independently or in unison, requires an increase in the water content of the protoplasm. Weibull's data are self-consistent in that the calculated water content of the protoplasm agrees well with that found from phosphate-impermeable volume. Other relationships can be found which will provide a protoplasmic water content agreeing with that obtained from the phosphate-impermeable volume. Such relationships require a decrease in one of the variables when another is increased. For example, if the volume fraction of protoplast is arbitrarily increased to 0.70, the water content of the cell must be decreased to 0.70 for the protoplasmic water content to remain constant. Under these conditions the wall would still contain 4 g of water for each gram of solids, an amount quite inconsistent however, with experiment (*14*).

Irrespective of variations in the parameters, the conclusion appears inescapable that the wall of *Bacillus megaterium* contains far more water than solids. Furthermore, the self-consistency of Weibull's data lends credence to their correctness.

In order to account for 10 g of water for each gram of solids in the wall of the cell, an open meshwork in which the wall solids form the structural elements seems a feasible model. In the meshwork the relatively large open spaces could be filled with water. The proposed model is consistent with permeability behavior (*6, 10*) and electron diffraction studies (*16*). Bacterial cell walls examined with the electron microscope are relatively transparent (*3, 5, 16*). An electron micrograph of the wall from *Spirillum* indicates a fenestrated structure with a hexagonal pattern (*16*).

While it may be coincidental, it is of interest that the thickness of the desiccated wall of *Bacillus megaterium* was found by Piekarski and Giesbrecht (*17*) to be 150 to 300 A in thickness, which is approximately one-tenth the thickness observed with wet organisms (*6, 18*).

The factors considered in this report suggest the usefulness of experimentally determined values for the weight fraction of the dry weight of the cell forming the protoplasm and also for the weight fraction of the total cellular water present in the protoplasm. The present information presents a picture of the bacterial cell as a rigid water-filled mesh inside of which there is a heavy suspension of particulate material. While the concept is not new (*10*), it was arrived at by reasoning to the best of our knowledge not heretofore applied to microorganisms. The analytical principle utilized is applicable to other bacterial cells and provides a test for self-consistency of assembled physical data.

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On the Relationship of Serotonin to Schizophrenia

In 1954, Woolley and Shaw (*1*) formulated the working hypothesis that a cerebral serotonin deficiency or serotonin excess might be a cause of mental disturbances such as those characteristic of schizophrenia. The evidence available at that time which suggested that serotonin played a vital part in the functioning of the central nervous system was of a twofold nature: (i) serotonin occurs in the brain, especially in the hypothalamus (*2, 3*), and (ii) some drugs which act as antimetabolites of serotonin on smooth muscle preparations also cause mental aberrations (*4*). This is especially true of the powerful psychotomimetic agent lysergic acid diethylamide (LSD). Additional evidence has since been presented by Woolley (*5*). Further, Sano (*6*) has reported that the administration of reserpine to psychotic patients causes a temporary increase in urinary 5-hydroxyindoleacetic acid (5-HIAA).

Whether serotonin is involved in the etiology of schizophrenia might be decided by establishing whether serotonin metabolism differs in nonpsychotic well subjects and in schizophrenic patients. We therefore investigated the urinary excretion of its principal metabolite, 5-HIAA, in both groups (*7*). Untreated male chronic schizophrenic patients (median age, 42 years; range, 24 to 63) were selected from the research wards of the Worcester State Hospital and maintained on the normal hospital diet. Acutely ill male schizophrenic patients (median age, 33 years; range, 16 to 57) were selected from the admission wards, and urine samples were obtained before therapy was instituted. All urine samples were collected in the morning and analyzed by the Udenfriend colorimetric procedure for 5-HIAA (*8*). The results are shown in Table 1. Statistical analysis shows that there is no significant difference in the excretion of 5-HIAA between chronic or acute schizophrenic patients and nonpsychotic well subjects. These findings agree with those of others (*6, 9*). The output of 5-HIAA in normal subjects ranged from 0.7 to 13.2 mg/day and in schizophrenic patients from 0.3 to 31.6 mg/day. Bellak (*10*) has commented on the greater variability of values for almost any factor investigated.

Table 1. Urinary excretion of 5-hydroxyindoleacetic acid in male subjects. S.E., standard error.

Diagnosis	Subjects (No.)	Output of 5-HIAA (μg/ml) ± S.E.	Output of 5-HIAA (mg/day) ± S.E.
Chronic schizophrenia*	30†	4.7 ± 0.4	5.3 ± 0.4
Paranoid	7	4.6	4.3
Catatonic	3	3.3	5.5
Hebephrenic	5	3.6	6.2
Simple	3	6.9	6.2
Undifferentiated	12	4.9	5.4
Acute schizophrenia	23†	4.7 ± 0.6	5.5 ± 0.9
Normal controls	29†	4.4 ± 0.3	5.2 ± 0.3

* No. of years hospitalized: median, 9; range, 2 to 27.
† No. of urine collections: chronic schizophrenia, 173; acute schizophrenia, 23; normal controls, 126.

In order to determine the effect of diet on the rate of excretion of 5-HIAA, three normal subjects were maintained for 3 days on each of the following diets with suitable control periods before and between the diets: (i) high carbohydrate, (ii) high fat, (iii) high protein, and (iv) 750 mg/day of L-tryptophan. No unusual effects were found, and all 5-HIAA values were well within the normal range.

In a longitudinal study carried on for 5 weeks, 20 urine samples were collected from each of two chronic schizophrenic patients and from one normal subject, a hospital attendant who ate approximately the same food as the patients. Patient No. 1 excreted an average of 11.0 mg of 5-HIAA per day with a range of 0.7 to 18.9 mg/day. Patient No. 2 excreted 8.6 mg/day with a range of 2.2 to 16.9 mg/day. No correlation between the patients' psychiatric behavior and urinary excretion of 5-HIAA could be demonstrated. The normal subject excreted an average of 5.9 mg/day with a range of 3.1 to 9.8 mg/day.

We also studied the effect of LSD on 5-HIAA excretion in four normal subjects, each of whom received 75 μg of LSD orally. Urine samples were collected immediately before the administration of LSD and 2 hours after. The typical symptoms of the LSD psychosis were evident in less than 2 hours. No significant change in 5-HIAA excretion was observed.

The evidence based on urinary 5-HIAA excretion in chronic and acute schizophrenic patients does not indicate a causal relationship between serotonin and schizophrenia. Preliminary results indicate also that blood serotonin values of chronic schizophrenic patients, measured by the fluorimetric method of Udenfriend and coworkers (*11*), do not differ significantly from those of normal subjects; the values of both groups ranged between 0.1 and 0.3 μg/ml of blood. However, serotonin metabolism, as measured by serotonin levels in blood and by 5-HIAA in urine, reflects serotonin primarily from the larger body stores. A hypothetical defect in mechanisms involving serotonin would most likely exist in the brain and might not be detectable by the methods employed.

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