ELECTROLYTES IN BIOLOGICAL SYSTEMS

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ABRAHAM M. SHANES, *Editor*

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INTRODUCTION

SEVEN OF THE NINE CONTRIBUTIONS to this monograph were presented in part at a Symposium on "Electrolytes in Biological Systems", organized by Abraham M. Shanes at the request of the Society of General Physiologists, at the Society's ninth annual meeting held at the Marine Biological Laboratory, Woods Hole, Massachusetts, on September 8, 1954. The organizing chairman was also asked to look into the possibility of publication, which led to the present volume. Two additional sections—on the higher plants by Dr. Epstein and on chloride transfer by Dr. Hogben—which could not be presented at the Symposium, have been included to broaden the scope of the volume.

The Symposium and the monograph are dedicated to two investigators—Dr. M. H. Jacobs and Dr. W. J. V. Osterhout—whose extensive work and thought in this field have provided a broad base for current methods and concepts and indeed keynote the papers which follow. A foremost feature of their studies has been the extensive use of comparative physiology. They have thus amply demonstrated how the similarities and differences among organisms can distinguish the special from the general, the superficial from the basic. The variety of organisms represented by this monograph again demonstrates the value of comparative studies. The recent tendency to emphasize the sodium ion in several biological systems as a result of the recent excellent work on frog skin is seen to require careful evaluation in the light of the present evidence for discrete mechanisms for chloride movement in gastric mucosa and for sodium and potassium transfer in Ulva, Valonia and nerve. The demonstration by Cowie and Roberts of the marked permeability of *E. coli* and other microorganisms to solutes known to penetrate cells with difficulty, associated with an ability to retain components to which they are permeable, calls attention to the possibility of mechanisms other than peripheral cell boundaries whereby intracellular components are controlled. The ion selectivity of mitochondria, described by Mudge, represents one property susceptible of study which offers considerable promise for an understanding of the role of protoplasmic constituents in the regulation of the intracellular environment.

The comparative viewpoint is of considerable value in permitting selection of material particularly suitable for the elucidation of specific principles. This is evident in research such as that by Rothstein and Epstein which has been remarkably successful in revealing the importance of surface reactions in the penetration of substances into organisms.

Another important aspect of the approach employed by Dr. Jacobs and Dr. Osterhout has been the application of quantitative methods. Precision is of inestimable importance in sharply delimiting possible mechanisms, for on purely qualitative grounds many more hypotheses must usually be entertained. This is evident throughout the present volume.

The collective work of Dr. Osterhout and Dr. Jacobs emphasizes the two features of electrolyte distribution which current studies are attempting to discriminate—the "passive", governed by electrochemical forces, and the "active", directly dependent on continuous energy expenditure by the cell. The techniques for distinguishing these are still far from fully developed, but Shanes and Tosteson point out that the prospects are promising. Under the circumstances, special terms like "transport", which are now so popular and which are subject to misuse, should be avoided in favor of more general expressions unless indisputable evidence justifies their application.

The Society of General Physiologists is indebted to the National Science Foundation for underwriting this volume. The editor is especially grateful to Dr. Louis Levin and Dr. John Buck for their advice and encouragement, to Professor Wallace O. Fenn and to Dr. Milton O. Lee, whose interest and efforts contributed so substantially to publication of the monograph, and to the authors for their wholehearted cooperation

CONTENTS

Permeability of Microorganisms to Inorganic Ions, Amino Acids and Peptides

DEAN B. COWIE AND RICHARD B. ROBERTS

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DURING THE PAST FIVE YEARS, the group at our laboratory has been using a wide variety of radioactive tracer molecules to study the pathways and rates of biosynthesis in *E. coli*, strain B.[1] In these studies we have repeatedly observed that in addition to material metabolically incorporated into the constituents of the cell, a pellet of cells invariably contains a predictable minimum quantity of the radioactive tracer. Thus, when a gram wet weight of cells is suspended in a radioactive solution and centrifuged, the pellet contains at least the radioactivity of 0.75 ml of the suspending fluid. When such a pellet is dried, it loses approximately 0.75 ml of water. Accordingly, we have come to the conclusion that a wet pellet of *E. coli* cells weighing 1 gram contains 0.75 ml of water, and this water in turn has the same concentration of ions and molecules as the suspending fluid.

The only exceptions observed have occurred when very large radioactive molecules (proteins) were used in the suspending fluid. In this case the content of the pellet was equivalent to 0.1 to 0.2 ml of the suspending fluid. We have attributed this smaller quantity to protein dissolved in the intercellular fluid of the pellet leaving .55 to .65 ml of the fluid which must be within the cell wall.

In addition to the radioactive material which appears to be simply dissolved in the water of the cell, further material may be incorporated or absorbed. Fortunately, a clear distinction can be made. The quantity of dissolved material is proportional to the concentration in the suspending fluid; its entrance does not depend on conditions (temperature, composition of medium, etc.) which affect the metabolic activity, and it can be readily washed out. It appears, therefore, that small molecules and ions penetrate the cell wall readily in either direction, and the 'water space' (12) of the cells contains the same concentration of small molecules and ions as the surrounding medium. We prefer to limit the use of the terms 'permeable' and 'permeability' to the simple problem of penetration of the cell wall. In many cases a suspected intermediate

[1] Obtained from the Department of Genetics, Carnegie Institution of Washington.

may not enter into the metabolic activities of the cell. However, we believe that such puzzling effects should not be attributed to 'impermeability' of the cell membrane unless it is shown that the molecule does not penetrate (23).

Radioactive substances ranging from inorganic ions to peptides have been used in the investigations to be described, and with *Escherichia coli* and *Torulopsis utilis* the same water space was found for each of the different compounds tested. Other evidence demonstrating the permeability of *E. coli*, *T. utilis*, and *Neurospora crassa* supports the conclusion that in these organisms passive diffusion is entirely adequate as a mode of transport.

TABLE 1. IMMEDIATE RADIOSULFATE UPTAKE FROM COMPLETE MEDIUM*

Mg S/ml Med.†	Radioactivity Ratio cps	Water Space %
1.0	850/1178	72.2
.10	907/1246	72.8
.01	849/1176	72.2

* Synthetic medium containing glucose and aerated at 37°C.

† The same quantity of radioactivity was added to each flask.

TABLE 2. IMMEDIATE RADIOSULFATE UPTAKE FROM SALINE SOLUTION

Exper.*	Radioactivity Ratio cps	Water Space %
1	29.7/41.4	72
2	34.7/48.3	72
3	27.5/36.4	76

* 0.026 mg sulfur/ml of saline solution (0.85%) was used in each experiment.

RESULTS

Measurement of Water Space. The distinction between metabolized and water space materials appearing within the confines of the cell membrane can be clearly shown through studies of the uptake and incorporation of sulfur by *E. coli*. In this species growth is required for the formation of nondiffusible sulfur compounds (10). Without growth, sulfur uptake can only occur by diffusion into the water space of the cell. This uptake occurs immediately when resting cells are immersed in a complete medium containing radiosulfate, and its measurement serves as a quantitative determination of the water space volume.

The immediate uptake of radiosulfate is shown in table 1. In these experiments 1 ml of resting cells was washed in 0.85% saline solution and then suspended in synthetic medium[2] containing a known quantity of S^{35}-labeled Na_2SO_4. The cells were immediately harvested by centrifugation at 14,000 *g*. The supernatant solution was decanted from the packed cells and the pellet and tube were rinsed with 20 ml of saline solution. This rinse was carried out by pouring the solution into the centrifuge tube containing the packed pellet

[2] 6g Na_2HPO_4, 2g NH_4Cl, 3g KH_2PO_4, 3g NaCl, 10 mg Mg ($MgCl_2$), 26 mg S (Na_2SO_4) and 900 ml water.

of cells and then decanting without disturbing the cell pellet. Aliquots of the cells and supernatant fluid were taken for radioactivity measurements. The time elapsed between the immersion of the cells and the decanting of the supernatant fluid was 8 minutes.

In table 1 the ratio, radioactivity per ml of cells/radioactivity per ml of medium, is shown to be independent of the sulfate concentration in the medium.

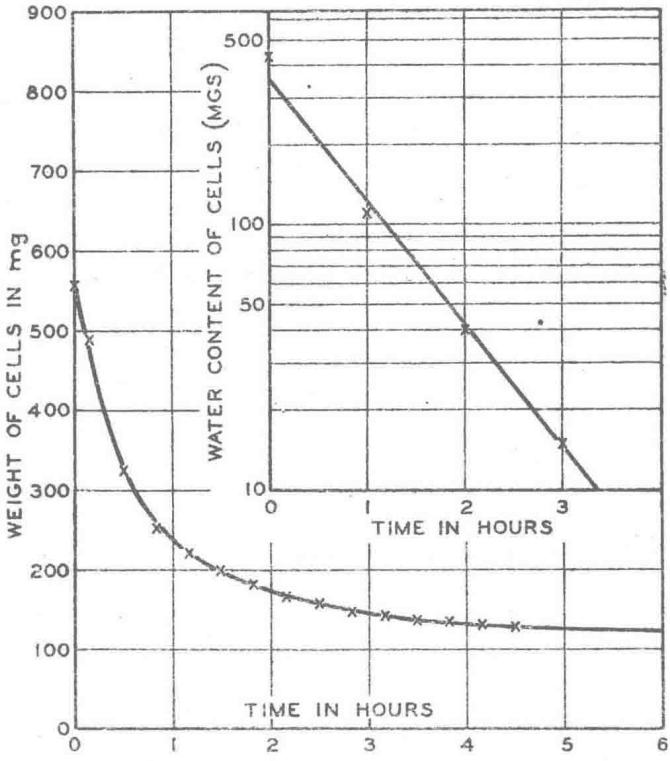

FIG. 1. Fluid content of *E. coli*.

This result demonstrates that a fixed relation exists between the sulfate within the cell and the sulfate of the medium. It is evident that within the 8-minute period of immersion and centrifugation an equilibrium is established across the cell membrane and that in these experiments approximately 72% of the total cell volume is available for the immediate uptake of sulfur. A similar result is obtained when cells are studied in cold saline solution in order to prevent possible metabolic uptake.

Fluid Content of E. Coli. It can be shown that there is a close correlation between the water space volume of the cells and their actual fluid content.

When approximately 1 gm of wet cells was dried at 110°C and then weighed, the fluid loss in three experiments corresponded to 72, 76, and 74% of the wet weight of the cells. These values are in good agreement with those reported for the fluid content of *E. coli* by Nicolle and Aliliare (20), and are not significantly different from the water space volumes reported in tables 1 and 2. Figure 1 shows the fluid loss as a function of time when approximately 0.5 gm of wet cells was dried *in vacuo*. Under these conditions there was a regular loss of water from the cells and in 3 hours 97% of the water of the cell was removed. The final amount of the fluid removed was 77.7% of the initial weight of the cells.

Measurement of Water Space by Analysis of the Supernatant Fluid. Another determination of water space may be made by measuring the loss of radio-

TABLE 3. RADIOSULFATE UPTAKE BY RESTING CELLS MEASURED BY ANALYSIS OF SUPERNATANT FLUID*

Mg S/ml Medium	Temperature of Medium °C	Total Volume of Cells in Medium	Total Radioactivity of Supernatant cps	Water Space Calculated from Supernatant Radioactivity %
.13	37	0.22	3,882	76
.013	20	0.44	3,633	72
.0013	3	0.87	3,184	74

* Total radioactivity of 2 ml immersion fluid, 4,208 counts per second.

activity from the initial immersion fluid. Only two measurements are required: *a*) the radioactivity of the immersion medium prior to the introduction of the cells, and *b*) the radioactivity of the cell-free supernatant after centrifugation of the cells. This method of determination has two advantages. When large volumes of cells are employed, no corrections of the radioactivity measurements for self-absorption by the cells are necessary. Furthermore, losses due to the rinsing procedures (washing pellet and tube with 0.85% NaCl) are not introduced.

Table 3 shows the results of a typical experiment. Carrier-free $S^{35}O_4^{=}$ was added to 7 ml of S-medium containing glucose and thoroughly mixed. Two ml of this radioactive solution were added to each of three centrifuge tubes containing 1 ml of carrier sulfate solution yielding the final sulfur concentrations shown in table 3. Two 0.5-ml samples of the medium were removed from each tube for radioactivity determination. Each tube was maintained at a different temperature. The contents of each tube were added to one of three new tubes, each containing a pellet of washed cells of a known volume. After resuspension of the cells and immediate centrifugation, the supernatant was decanted and two 0.5-ml samples were removed for radioactivity measurements. Table 3

shows that despite the variables introduced (cell volume, temperature, and sulfate concentration) the water space determined by the radioactivity of the supernatant fluid, is correlated with the volume of cells in each tube and is independent of the other variables.

There are several limitations on such a determination. When small volumes of cells are used, the loss of radioactivity from the original medium is small and the method is inaccurate. Even more important, however, is the fact that this method measures the total uptake of materials by the cell. If metabolic uptake occurs in the experiment, an erroneous estimate of water space will be made since such bound material is not distinguished from that retained passively in the water space of the cell. Since in the experiments shown in table 3 the highest specific radioactivity was used with the largest volume of cells, any metabolic uptake would be emphasized. Therefore, the results shown in table 3 indicate that little metabolic incorporation occurred. In addition, the uniformity of the results obtained over the wide range of sulfur concentration rules out any large amount of metabolic binding. Any sulfur metabolically bound can, moreover, be detected by measuring the radioactivity contained in the cells. For example, the pellets of cells in the experiment of table 3 were rinsed with 20 ml of NaCl and then resuspended in 20 ml of NaCl solution. After centrifuging, aliquots of the washed cells were measured. Less than 1% of the sulfur initially taken up by the cells was retained showing that these resting cells did not bind the sulfur.

Measurement of the rinse solution obtained from this experiment showed that the radioactivity lost in the rinse corresponded to a volume of approximately 0.1 ml of the original medium. The three rinse values obtained were 0.1, 0.08, and 0.09 ml. If the rinse procedure were effective in removing trapped intercellular medium from the pellets, a correlation with cell volume would be expected. Since no correlation was observed, it is concluded that the radioactivity of the rinsing solution is due to residual medium adhering to the walls of the centrifuge tube or to the surface of the pellet.

Intercellular Fluid Volume. What has been defined as 'water space' might be considered to be nothing more than medium trapped in the interstices between the packed cells. This intercellular volume has been measured, using Fe^{55} and I^{131} labeled proteins as the tracer materials. Fe^{55} was used to label ferric-beta-1-globulinate (30) and I^{131} was used to label serum albumin. Cells were suspended in media containing these tracer compounds of high molecular weight and immediately centrifuged at 14,000 *g*. After centrifuging, the supernatant was decanted, and the centrifuge tube and pellet were not rinsed; the radioactivity retained in the tube corresponded to a water space of 20%. In a parallel experiment rinsing removed half of this retained radioactivity. The residual radioactivity thus corresponded to a water space of 10%. As the previous experiments showed that the rinse loss corresponded mainly to the

amount of the medium adhering to the walls of the centrifuge tube, the intercellular fluid volume contributes between 10 and 20% of the water space of the cell.

Wash Losses From Resting Cells. If the radiosulfate immediately taken into the cell is contained passively within a freely permeable membrane, then the radioactivity should be readily washed out by immersion of the cells in a saline solution. Such is the case as is shown in table 4. Cells immersed in saline solution containing radiosulfate were harvested and reimmersed in saline solution. The sulfur taken up by the cells from the first solution was then found to be distributed between the cells and the second fluid, the washing fluid gaining in radioactivity and the cells corresponding losing radioactivity. A subsequent washing further reduced the remaining radioactivity of the

TABLE 4. WASH LOSSES OF RADIOSULFATE FROM CELLS INTO SODIUM CHLORIDE MEDIUM

Mg S/ml Medium*	Radioactivity, cpst			Water Space, %		
	Total uptake by cells	Radioactivity removed by 1st wash	Radioactivity removed by 2nd wash	From supernatant analysis	From 1st washout	From total washout
1.0	345	289	35	75.1	63	70
.10	344	274	25	74.7	59	65
.01	348	278	26	75.5	60	66
.001	364	293	35	79.8	64	72

* 0.49 ml cells suspended in 2 ml saline solution containing carrier sulfate at the concentration indicated for each tube. Wash volumes 5 ml in each case.

† Original radioactivity per ml of saline solution before immersion of cells, 1,112 counts per second.

cells, a new equilibrium being established between the radioactivity of the water space and that of the medium. It can be seen from the calculations of the water space volume, both by the supernatant analysis method and from the wash losses observed, that the sulfur concentration of the cells was always equal to about 75% of the sulfur concentration of the medium. Little metabolic binding occurred as shown by the ease of removing the sulfur by washing and by the similarity of the results obtained over the wide range of sulfur concentrations. If metabolic uptake occurred, its effects would be emphasized at the concentration where the specific radioactivity was highest. It is evident that metabolic uptake did not occur.

Uptake During Growth. The uptake of radiosulfate during growth contrasts sharply with that observed in resting cells. Radiosulfate uptake is directly proportional to growth provided the water space sulfur has been removed by washing the cells. This uptake as a function of growth is shown in figure 2. A direct correlation between growth and the incorporation of sulfur into both

the trichloroacetic acid soluble and insoluble fractions of the cell is also evident in figure 2. Glucose is required for this metabolic uptake of sulfate (10). Cells immersed in synthetic medium, complete with the exception of glucose, do not incorporate sulfur metabolically. Upon the addition of glucose, growth begins, and there is a direct correlation among growth, nitrogen content, and sulfate uptake. Except at extremely low concentrations of sulfate, the quantity of sulfur metabolically bound is independent of the concentration in the medium.

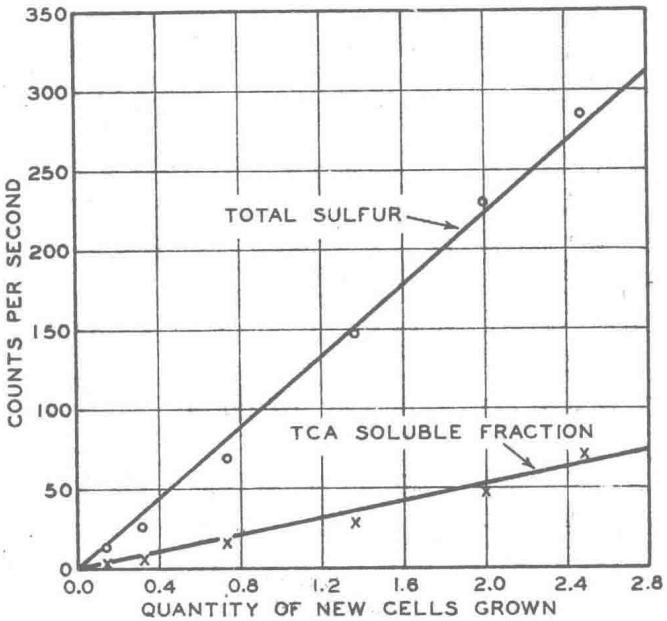

FIG. 2. Sulfate uptake during growth (*E. coli*). The upper curve shows the total radiosulfate uptake as a function of the quantity of new cells grown. The lower curve shows the radiosulfur of the TCA soluble fraction as a function of growth.

It is obvious from the preceding experiments that if the wash procedures were not carried out, large quantities of nonmetabolized sulfur passively held by these cells could constitute a potential source of error in the study of cell metabolism.

Exchange and Wash Losses During Growth. Further comparison of the uptake of radiosulfate by resting and by growing cells may be made using wash-loss measurements. Cells grown in radiosulfate from a light inoculum were washed to remove water space sulfur and immersed in complete media containing various nonradioactive sulfur sources. It can be seen in table 5 that little of the labeled sulfur exchanged with the sulfur of the medium despite continuous cellular growth. The fact that no large losses of metabolically bound

sulfur occurred in these experiments also indicates that the sulfur removed from resting cells (tables 1, 2) by washing did not contain large quantities of metabolically incorporated sulfur.

Rates of Uptake of Radiosulfate by E. Coli. The methods used for the measurement of the water space are too slow to show the rate of diffusion across the cell membrane. Some lower limits of the rate can be deduced from the rate at which the cells assimilate sulfur. During exponential growth the quantity of sulfur incorporated per gram dry weight of cells per second is 8.1×10^{-2} micromoles. This corresponds to an uptake of 1.2×10^{4} atoms of sulfur per cell per second assuming an average cell volume of 10^{-12} ml. When the sulfur content of the medium is 0.1 μg/ml, every 0.1 second the cell will metabolize

TABLE 5. SULFUR EXCHANGE IN GROWING E. COLI

Supplements	Time, hr.	Mɪ Cells/Tube	Radio-activity in Cells cps	Supplements	Time, hr.	Mɪ Cells/Tube	Radio-activity in Cells cps
None	0	0.284	1.17	Cysteine	0	0.284	1.17
	1	0.441	1.06		1	0.327	1.14
	2	0.753	1.14		2	0.439	1.07
	3	0.880	1.14		3	0.774	1.14
Cystine	0	0.284	1.16	Methionine	0	0.284	1.19
	1	0.456	1.05		1	0.447	1.13
	2	0.882	1.06		2	0.860	1.11
	3	0.998	1.10		3	0.946	1.19

.01 mg/ml sulfur as sulfate in all tubes; .01 mg/ml sulfur additional from indicated supplements.

enough sulfur to deplete the water space volume, and, therefore, it must rely chiefly on exogenous sulfur to maintain growth. Since this concentration is sufficient to support growth at optimal rates, it is obvious that the rate of transport of sulfate across the cell boundary is very rapid.

Though this rate of penetration is rapid, even faster rates are required to supply the sulfur to cells previously grown in sulfur-deficient media as shown by Cowie, Roberts and Bolton (11). Immersion of these cells in a complete medium containing radiosulfate at a concentration of 0.1 μg/ml produces an initial uptake rate five times as fast. At this rate the cell would sweep out the sulfur of 50 cell volumes of medium per second. The calculated diffusion rate in water would permit 100 times this rate of penetration. It therefore appears that the membrane of *E. coli* is sufficiently permeable to sulfate ions so that the rate of penetration causes no limitation on the biochemical activities of the cell. Such rates are far too high to be measured by ordinary kinetic methods.

Sulfate Summary. It is evident that the sulfur content of *E. coli* is distrib-

uted among two classes of sulfur compounds which can be clearly distinguished from each other. The first is the bound sulfur which includes the protein sulfur of the cell together with sulfur found in smaller molecules such as glutathione (25). This sulfur content is characteristic of the cells and is, to a limited extent at least, independent of the chemical form and concentration of the sulfur in the medium. In addition to the bound sulfur, the cells are permeated by sulfate from the medium. The concentration of this 'water space' sulfate is always directly proportional to that of the medium; consequently, it is readily removed by washing.

The incorporation of sulfate from the medium into the bound sulfur of the cells shows all the characteristics of a synthetic process. The quantity incorporated is proportional to the increase in cell mass, i.e. to the increase of protein. It is, therefore, sensitive to all the factors which influence protein synthesis, e.g. the presence of glucose and nitrogen in the medium, temperature, aeration and so on.

In contrast, the penetration of sulfate into the water space of the cells is independent of: *1*) the presence of glucose in the medium (tables 1, 2); *2*) the presence of a nitrogen source (tables 1, 2); *3*) temperature variations (tables 1, 2, 3); *4*) degree of aeration (tables 1, 2); and *5*) time. These characteristics make it reasonable to believe that no metabolic 'active transport' is involved; diffusion through a permeable membrane is apparently an adequate description.

Furthermore, independently of the concentration of sulfate in the medium, the pellet of cells contains 75% as much sulfate per ml as is present in the medium. As this amount corresponds roughly to the fluid content of the pellet measured by the loss of weight on drying, the concept of a 'water space' which assumes the sulfate concentration of the medium appears satisfactory.

The permeability of *E. coli* to sulfate has been discussed at the onset of this paper because with these cells the distinction between water space sulfur and that metabolically bound is easily demonstrable. Very little sulfate is bound unless the conditions permit growth and, once it is bound, very little sulfur is lost or exchanged. The same methods have been used to study the permeability of *E. coli* and other microorganisms to a variety of materials. In some cases there is more difficulty in separating the water space penetration from metabolic binding and the results are more difficult to interpret.

Permeability of Torulopsis Utilis to Sulfate Ions. While the results of the studies with *E. coli* provide a foundation for the design and interpretation of experiments with other microorganisms, species variations often introduce factors which must be taken into account. *Torulopsis utilis* was chosen to determine whether or not the concept of permeability by passive diffusion could be extended to include other microorganisms besides *E. coli*.

Unlike *E. coli*, *T. utilis* metabolically incorporates sulfate sulfur without growth. This uptake is rapid, the sulfur being found mainly in the trichloro-

acetic acid soluble fraction of the cells. In addition to this metabolic uptake, further incorporation is observed when the cells grow. This later uptake (into the TCA precipitable fraction) corresponds to protein synthesis and is proportional to the quantity of new cells grown.

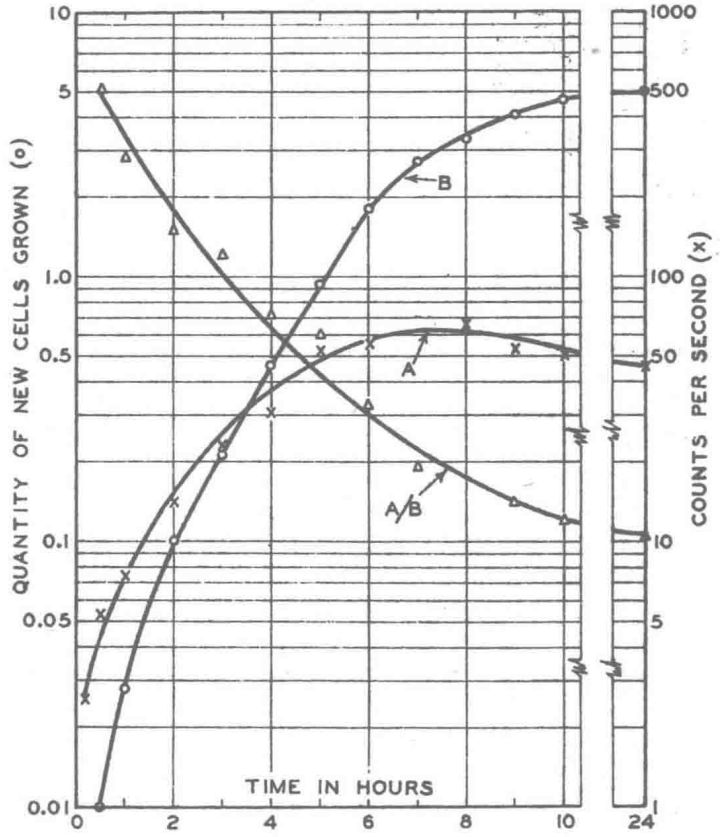

FIG. 3. Radiosulfate uptake by *T. utilis*. Curve A represents radioactivity of TCA soluble fraction observed during the growth of the cells, shown by Curve B. The ratio A/B indicates that no fixed quantity of sulfur is maintained in this fraction during growth.

Figures 3 and 4 show these relations. A comparison of immediate uptake of sulfur in *T. utilis* and in *E. coli* cells is shown in table 6. In this experiment approximately equal quantities of washed *T. utilis* and *E. coli* cells were suspended in 2 ml of glucose-free, chilled synthetic medium containing radiosulfate. The immediate sulfate uptake by each type of cell is indicated by the loss of radioactivity in the supernatant fluid. *E. coli* removed a total of 16% of the original radioactivity which corresponds to a water space of 75%.

T. utilis on the other hand removed 76% of the original radioactivity. From this loss of radioactivity from the immersion fluid it can be calculated that the water space would exceed the total cell volume by more than a factor of 3. This absurd result clearly indicates that the supernatant analysis method, though

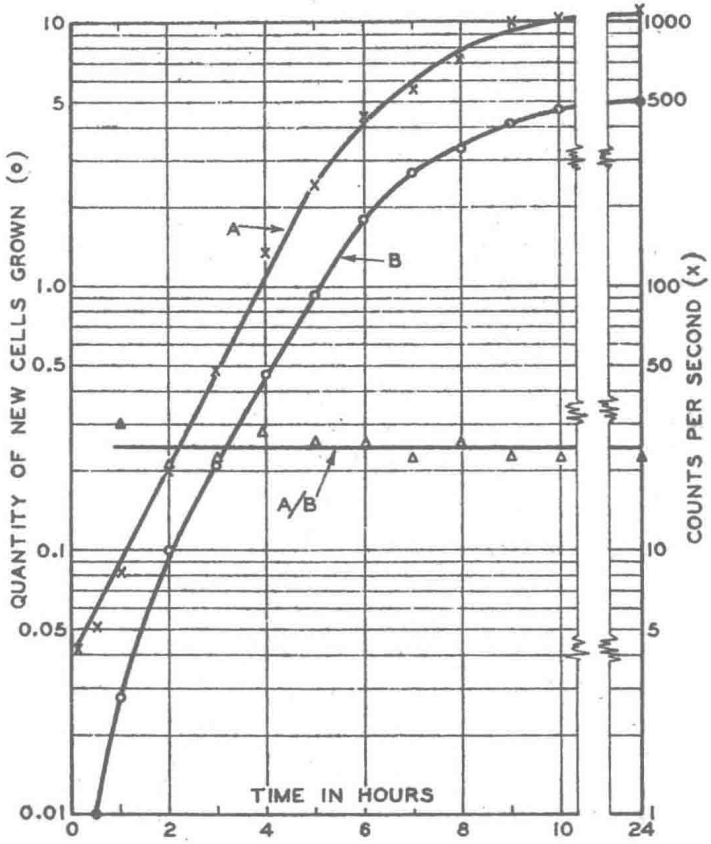

FIG. 4. Radiosulfate uptake by *T. utilis*. Curve A represents radioactivity of TCA precipitable fraction observed during the growth of the cells shown in Curve B. The ratio A/B shows the direct correlation between growth and sulfur uptake of the TCA precipitable fraction (proteins).

satisfactory for studies of *E. coli* permeability, is not necessarily adequate when other organisms are investigated. For *T. utilis* it is also necessary to measure the radioactivity retained by the cells through metabolic incorporation. Table 6 shows that even when the quantity of sulfur in the medium was low only a slight amount of radioactivity was retained by *E. coli*. In contrast, *T. utilis* retained 61% of the total sulfur originally available. When this meta-

bolically bound radioactivity is subtracted from the total quantity of radioactivity removed by the cells, it is found that the quantity of diffusible sulfate was 15.4% of the original radioactivity of the medium. This corresponds to a water space of 64% of the total cell volume.

Since 61% of the initial radioactivity is lost from the immersion fluid by metabolic incorporation, only 39% is left to equilibrate by diffusion between cell and environment. In addition, continuing metabolism of the cells during the wash procedures may markedly reduce the diffusible sulfate content observed in wash analysis. This may account for the lower value (64%) determined for the water space in this experiment.

TABLE 6. IMMEDIATE RADIOSULFATE UPTAKE BY T. UTILIS AND E. COLI

	Radioactivity, cps*	
	E. coli	*T. utilis*
Original medium (2 ml)	162	162
Supernatant fluid	136	39
Cells after washing (0.5 ml)	1	98

* Sulfur concentration of original medium was 0.1 μg/ml.

TABLE 7. IMMEDIATE SULFATE UPTAKE BY T. UTILIS

	Radioactivity cps
Original medium* (2 ml)	136
Supernatant fluid	88
Wash solution (5 ml)	41
Washed cells (1.3 ml)	5
Percentage accounted for	98.5

* Sulfur concentration of original medium, 1.0 mg/ml.

These difficulties may be reduced by several precautions, as the results of table 7 show. In this experiment yeast cells kept in the stationary phase for two days were used. Carrier sulfate at a high concentration of 1.0 mg sulfur/ml of 0.85% saline solution was used in the original medium. Glucose and other nutrients were excluded and the saline and the cells were maintained at 4°C during the experiment. The results shown in table 7 indicate that metabolic incorporation of the labeled sulfate was small as compared to the quantity of radiosulfate in the water space. From the loss of radioactivity of the immersion fluid, the water space volume was calculated to be 84.7%.

This is not the true water space since about 4% of the original radioactivity in the medium was retained in the cells after washing. If this quantity is deducted from the total cellular uptake, the water space corresponds to 75%. The wash loss measurements give a water space of 72%.

Permeability of Escherichia Coli to Phosphate Ions. Experiments carried out on the permeability of *E. coli* to phosphate ions quickly revealed significant differences between the responses of the cell to exogenous phosphate and to sulfate ions. The metabolic incorporation of phosphorus by cells suspended in sodium phosphate buffer is rapid, and it is consequently more difficult to dis-