THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Madeleine Guillemin final oral examination for the degree of Master of Arts . We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

May 24 1941

W P Larson
Chairman

E C Rosenow

Arthur T. Hamlin

C. H. Bailey

REPORT

of

COMMITTEE ON THESIS

The undersigned, acting as a committee of the Graduate School, have read the accompanying thesis submitted by Madeleine Guillemin for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

W. P. Larson

W. H. Hunter

E. C. Rosenow. E.C.R.

THE RELATION BETWEEN THE STRUCTURAL AND UNBOUND SALTS IN BACTERIA

A Thesis submitted to the
Faculty of the Graduate School of the
University of Minnesota
by
Madeleine Guillemin
In partial fulfillment of the requirements
for the degree of
Master of Arts
June
1921

THE RELATION BETWEEN THE STRUCTURAL AND UNBOUND SALTS IN BACTERIA

The purpose of the work discussed in this paper was first to find the total ash content of bacterium coli as a typical organism and to determine as accurately as possible the nature and amount of the elements in that ash, and secondly to establish the relation between the two types of salts known to be present in bacterial cells. The two types or groups of salts are the structural and what may be termed the unbound, diffusible or physiological salts. These terms will be explained later and their origin will be discussed.

Only a very limited amount of work has been done on the physiology of bacteria and still less on the special subject of salt metabolism in these organisms. In nearly all of the research more attention has been given to the proteim and fat content of the cell than to the inorganic constituents. In some cases the ash content is not considered at all, and in others the amount but not the nature of the ash is given. A few investigations have been made, however, in which the total ash content, as well as the elements in the ash, are determined. Furthermore, some work has been done to establish a relationship between the ash content of bacterial cells and the medium on which they are grown and to find out what other factors influence the amount and nature of the mineral matter in bacteria. The most important of these investigations, as well as those having a more or less direct bearing on the work of this paper, will be cited and briefly discussed here.

One of the earliest investigators named in the literature is Flügge who analysed cells of different microorganisms and merely stated, that they are rich in protein but contain other substances.

Nenki and Schaffner[2] give us some of the first analyses to determine the content of organic and inorganic matter in certain putrefactive organisms. Their organisms were grown on 2% gelatine and the growth was precipitated with acetic acid or by boiling and filtered off. The results of their analyses are:

Water----------83.42%
Protein----------84.20% of the dry matter
Fat---------- 6.04% " " " "
Ash---------- 4.72% " " " ""
Undetermined------ 5.04%.

Another worker, Brieger[3], analysed gelatine plate cultures of the Friedlander bacillus and found the following:

Water----------84.20%
Fat---------- 1.74% of the dry matter
Ash----------30.24% " " " "
Nitrogen in ash free organisms, 9.75%.

Brieger also made a qualitative analysis of the ash and reports $Ca_3(PO_4)_2$ $Mg_3(PO_4)_2$ Na_2SO_4 and NaCL present.

Nägeli and Loew worked with micrococci grown on ammonium tartrate solution and also with mother of vinegar. The results of their analyses as given by Cramer are:

Micrococci-

Nitrogen---------10.65%
Ash---------- 9.94%

Mother of Vinegar-

Dry matter------- 1.70%
Nitorgen in the dry matter---1.82%
Ash--- " " " " ---3.37%

Among the more recent investigations are those of Koch and Kappes[5], who analysed cultures of B. Prodigiosus and B. Xerosis grown on 1.5% meat extract peptone agar. They also worked with yeasts. Their results, as cited by Cramer[6], are given below:

Organisms	%Water	%Ash
B.Prodigiosus	85.45	13.47
B.Xerosis	84.93	9.52
Yeasts	81.40	10.83

By far the greatest amount, as well as the most valuable

work, has been done by Cramer[7-10] on the cholera vibfio and other organisms, and by deSchweinitz and Dorset[11] on the tubercle bacilli.

In his first work on ash content of bacteria Cramer[7] used B Prodigiosus grown on potato. The organisms were scraped off of the potato by means of a platinum needle, dried at 100° C and ashed. The results are:

Dry matter----------15.87*26.03%
Ash in dry matter-------- 7.76-16.37%
Ash in moist organisms--- 1.60- 3.21%

He also tried growing organisms on old and new potatoes with the following results in ash content:

B.Prodigiosus on old potatoes-----2.8% ash
B.Prodigiosus on new potatoes-----9.85% ash.

The difference in ash content here with the same organism and on media very much alike is certainly very striking. Growing the same organisms on carrots gave a lower ash content than on potato. He furthermore analysed a large number of organisms isolated from drinking water and found variations as high as 30% in dry matter and up to 40% in ash content. He finally came to the conclusion that the percentage of ash as well as that of dry matter in the cell contents of bacteria depends upon the organism and is furthemmore influenced by temperature, time of growth and to a very marked degree by the medium used. Cramer greatly stressed this last factor and made it the object of some of his later investigations. He also found, that at lower temperatures the percentage of ash in the dry matter is higher. Finally, he made the statement, that there is no example in plant or animal kingdom of so great a difference in chemical composition in a group of so closely related organisms as the case in the composition of bacterial cells.

Cramer's[8] next work was with four organisms; a saprophte,

which he calls No 28 from Marburg, B.capsulatus of Pfeiffer, B. pneumoniae of Friedlander and B.rhinoskleromae of Paltauf. These cultures were grown on 5% peptone meat infusion agar, 5% dextrose meat infusion agar, and 1% peptone meat infusion agar in plates. The fourty-eight hour cultures were scraped off with a platinum needle anddried at 105° C. Nitrogen and fat, as well as ash determinations were made. The dry matter in the case of the Pfeiffer bacillus varied from 11.23-13.55%. The results from the ash determinations are given below:

Percentage of ash in the dry matter.

No.28	1% peptone	5% peptone	5% dextrose
	11.42	7.79	9.20
Friedlander bac.	13.94	10.36	7.88
Rhinosklerom	13.45	9.33	9.44
Pfeiffer bac.	12.56	9.10	9.13.

Although there are differences in the ash content for different organisms on the same medium, they are not so marked as the variations for the same organisms on different media. On 1% peptone agar, the medium with the smallest amount of organic matter, the organisms have the highest ash content. Wide variations were also obtained in protein and fat content. In other words, bacteria do not have a typical cell composition or even a composition typical of each species. They adapt themselves, within certain limits to the medium in which they are grown, and their cell composition changes accordingly. Comparative results in analyses of bacteria could be obtained only when equally heavy inoculations of the same organisms are grown on a medium of constant composition and the temperature and period of growth are the same throughout the experiment. The percentages of ash in the media used by Cramer are 1% peptone agar 0.79% ash, 5% peptone agar 0.89% ash, and 5% dextrose agar 0.78% ash.

Following this, Cramer[9] worked with the cholera vibrio grown in a pellicle on broth. The pellicle after three days growth was removed, washed with physiological salt solution, sucked dry with filter paper, and dried further in a vacuum with the addition of chloroform at 20-25°C. The dry matter was analysed for protein, water and ash content. The media used were meat infusion peptone broth with 1% sodium carbonate and Uschinsky's solution. Cramer used five strains of the cholera vibrio and obtained almost identical results for the five organisms when grown on the same medium. (Another evidence of the relation between medium and cell composition.) The results are: 65% protein and 31% ash in the dry matter and 88.3% water. The soda broth contained 25-27% ash in the dry matter. Grown on the Uschinsky's solution, the cholera vibrio yielded 34-60% protein, and 7-15% ash, showing the tremendous difference in ash content in the same organism grown on different media. Especially is this true, when one is an inorganic medium unfavorable to a pathogen. 30.78% is the highest ash content ever found for bacterial cells.

In his last paper on this work, Cramer[10] gives the results of a qualitative, as well as a quantitative analysis of the cholera vibrio grown and treated as described above. The three kinds of media used were meat infusion peptone broth with 1% sodium carbonate and same broth with additions of 4% sodium phosphate and 3% sodium chloride respectively. These media contained the following amounts of ash:

1% soda broth, 1.23% total ash, 0.467% Na Cl, 0.283% Cl, 0.096% P_2O_8.
4% sodium phosphate broth, 2.48% total ash, 0.467% Na Cl, 0.283% Cl, 0.987% P_2O_8
3% sodium chloride broth, 4.1% total ash, 3.356% Na Cl, 2.034% Cl, 0.088% P_2O_8.

Cramer found, that the organisms in each case used up about 0.49% of the ash in the medium.

Results of the quantitative analysis of the Ash of Cholera Vibrio in Percent.

Cholera	Medium	Cl	P_2O_8	SO_4	K	Na	Ca	Mg	Sum.
	1% Soda	17.02	20.48	8.55	6.32	32.06	0.98	Trace	85.41
Shanghai	+3% NaCl	41.15	9.64	1.02	5.26	33.79	---	---	90.86
	+4% Na_3PO_4	9.99	34.3	2.24	4.97	31.82	1.29	0.12	84.73
Hamburg	1% Soda	15.42	31.18	7.59	8.02	31.19	0.3	0.64	94.34
Winter	+3% NaCl	43.69	9.6	1.59	9.01	31.88	---	---	95.68
	+4% Na_3PO_4	8.89	35.36	2.33	4.32	27.50	0.79	Trace	79.7
	1% Soda	18.34	34.69	8.07	6.32	32.06	---	---	99.38
Bürgeln	+3% NaCl	37.36	13.58	1.31	6.32	32.06	---	---	90.63
	+4% Na_3PO_4	5.05	45.42	2.29	6.32	32.06	---	----	91.14

The results again show, that up to a certain limit there is a direct relation between the ash content of the organisms and that of the medium. The sodium remains more or less constant showing, that even in media poor in sodium, there is always enough of this element to satisfy the requirements of the organism. Bacteria do, however, according to Cramer, show a certain selective action toward some elements. For instance, even in a medium poor in phosphate they will select phosphate in preference to chloride, and the phosphate content is higher in the organism than in the medium. The concentration of calcium, magnesium, and sulphate are often higher in the organism than in the medium.

Schweinitz and Dorset[11] in their work on the mineral matter of the tubercle bacilli grew their organisms on neutral broth containing 1% peptone 0.5% salt and 7% glycerine. The cultures were heated,

filtered and washed with boiling water, dried over concentrated sulphuric acid and extracted thoroughly with pure ether and 98% alcohol. They were then dried and ashed and the ash analysed. Results calculated upon the dry ash:

Na_2O---------- ----------13.62%
K_2O---------- 6.35%
CaO----------12.64%
MgO----------11.55%
$C+SiO_2$---------- ------ 0.57%
P_2O_5----------55.23%

These investigators found no sulphate or chloride, but state that these elements may have been washed out in the preparation of the sample for analysis. This may be true in this, as well as in some of the other analyses discussed here.

Two other names that may be mentioned in connection with ash analysis are Lyon[12] and Nishimura[13]. Lyon grew his organisms on media of varying concentrations of dextrose. The conclusions drawn from his work are, that an increase of dextrose tends toward an increase of fat content and up to a certain point to a decrease in ash content in the organisms. The ash content, however, reaches a minimum beyond which the amount of dextrose in the medium has no further effect.

In the work of Nishimura the organisms were grown on potato, dried over sulphuric acid, extracted with ether and alcohol and ashed. The results are 84.43% water, 11.01% ash in the dry matter and 8.02% fat.

Beside the work on the schizomycetes some research has been carried out on the ash of yeasts and molds.

Fluegge[14] found that some of the higher molds contain about 88% water and 1% ash. Zopf[15] found from 1.94-0.46% ash in molds. Stern[16] analysed the ash of yeasts and reported K, $Mg_3(PO_4)_2$, Ca, Si,

S and Fe. Nägeli and Loew[17] state, that the weight of inorganic matter in yeasts is approximately equal to the weight of nitrogen. Zieber[18] worked with a mixture of aspergillus and penicillium and found 84.7% water and 4.89% ash. Penicillium alone was grown on dextrose tartaric broth and analysed. The mineral matter ran from 7.67-9.41%. Molds grown on bread contained more dry matter and more ash, 16.32-16.91%. These organisms have about the same water content, but usually a lower percentage of ash than bacteria.

In the papers reviewed here, where ash analyses are made, the investigators do not in any case outline their methods of analysis.

The object of the work of this thesis is not only to determine the amount and nature of the ash of bacterium coli, but to establish a relationship between the elements in two types or groups of salts known to be present in these and other bacterial cells. In a thesis by Robert G. Green for the Master's degree, University of Minnesota 1920, the author states and proves that bacterial cells suspended in distilled water and killed by heat will after several hours standing give off a certain portion of their salt content. The salts which upon the death of the cells aregiven off and diffuse into the surrounding medium are here called unbound, diffusable or physiological salts. In counterdistinction to these, the salts which are bound up chemically in the protoplasm of the cell, so that they are not given off until the cell disintegrates, are called structural salts.

The bacterial cell, although very minute may nevertheless be a complex and well organized system. We cannot determine any particular specific structures in the cell, but we know, that it is composed of solid particles of protoplasm suspended in liquid medium.

This liquid, beside keeping the cell turgid against the pressure of the surrounding medium, serves as a carrier for the particles of food from the inside of the cell wall to the cell substance, and also as a carrier for the waste products of the cell back to the cell wall to pass from there into the surrounding medium. No regular circulatory system has ever been demonstrated in bacteria, but there must be some means of carrying the ions of salts, as well as other particles in solution, which have passed through

the cell wall, to those parts of the cell where they are built up into new protoplasm. Whether or not the liquid passes through the cell in a regular path carrying with it particles of food or carrying away waste materials, or whether the substances in solution simply move about in the liquid as a dispersion medium, from which they are adsorbed to the cell structures, has never been determined.

It has frequently been pointed out, that the membrane of bacteria may be lipoidal in nature. Lipoids being surface tension depressors cause the cell membrane to collect or adsorb particles in solution at its surface. These particles then pass into the cell wall due to the pressure of the solution on the outside and are released from the inner cell wall by the reduced pressure of the liquid inside of the cell. Whether the cell membrane shows any selective action to particles passing through it, or whether the whole process is simply a physical one of adsorption and osmosis, regardless of the specific nature of the particles, is still a matter of doubt. Some evidence for the former theory has been presented by Cramer[10] when he pointed out, that organisms show a selective action toward phosphate as against chloride. Again the fact that a certain amount of chloride is taken up by the cell and apparently, as will be pointed out later, is not utilized in building up the cell substance, is evidence of little or no selective action on the part of the cell membrane. Chloride may, however, have a purely physiological function, which is not yet clearly understood.

There are then two distinct groups of salts present in the living bacterial cell. The structural salts are those which are

tied up chemically or form a part of the protoplasm of the cell. It would seem, if there is any definite or constant composition of this cell protoplasm, that the nature and amount of the structural salts should remain relatively constant, at least for the same organism, regardless of the conditions of growth. The unbound salts, however, performing as we think of them purely physical functions as equalizing the osmotic pressure and preserving the turgor of the cell or being connected with the physiological elements of the cell, must vary in amount and to a certain extent in composition with the concentration and composition of salts in the medium. An increase of the total concentration of salts in the medium would bring about an increase of salts in the cell liquid. An increase of either sodium, potassium, chloride, or phosphate in the medium would cause an increase of the corresponding substance in the cell contents. The osmotic pressure, for example, of sodium on one side of the membrane would be equalized by an equal pressure of sodium on the other side. Whether this equality of pressure for each element is maintained is not known. We know from data of former investigators, that cells do pile up concentrations of certain elements above those of the same element in the medium. Especially is this the case where a particular element is essential to the life process or structure of the organism and is present in comparatively low concentration in the medium. All that this may mean, however, is that the cell structure requires relatively constant amounts of certain essential elements and extracts these from the medium, no matter how low the concentration may be. Such elements then being bound up in the cell structure have little or no effect on the osmotic pressure. The excess concentration of certain salts in the cell over that in the medium may be entirely in the structural salts, and there may

not conflict in the least with our theory of osmotice pressure balance.

A very plausible conclusion to draw from all this is first, that the structural salts remain constant under different conditions of growth, at least with the same organism, and secondly, that the wide variations found by Cramer and others in the ash content of an organism grown on media of different salt concentrations, are variations in the unbound or physiological salts only.

All of this work on diffusible salts in cells is comparatively new and as yet, there is no available data to prove or disprove the statement just made. It remains, therefore, for the present a very plausible conclusion and nothing more. The problem is to be investigated in the near future.

In most animals and plants certain cells or groups of cells have purely structural, others purely physiological, functions. In bacteria in a very general way certain elements compose the cell structure, while others take part in metabolism. The salts combined in the former are the structural, those connected with the latter, the physiological salts.

Another question still to be answered about the diffusible salt is the one concerning the manner in which these salts are held by the cell during life. They may be adsorbed to the cell wall, prior to passing into or out of the cell, they may be adsorbed to the cell substance within before actual chemical combination, or they may be in solution in the cell liquid. The broadest assumption and the most logical one would be that they occupy not one, but all three of these places, and fulfill all three functions.

Previous investigators giving no heed to the fact, that cells

give off salts when killed, may have lost at least a part of these losely held salts in the preparation of their samples for analysis through a series of washings and extractions. Schweinitz and Dorset state as much in their paper on the ash analysis of the tubercle bacilli. It is significant for our purpose, that these two investigators find no chloride in their samples, which in the present analysis does not appear in the ash but in the diffusate. It is easily seen, that a part of the salts, and with that the chlorides, might have been lost by numerous washings with hot water and by extracting the fats with alcohol and ether. Also the sulphate, absent in the analyses of Schweinitz and Dorset,[11] was found in this work mainly in the unbound salts.

In planning the technique for this work one of the first problems was to find a good method for separating the organisms from the broth without injuring the cells in any way or having them contaminated with the medium on which they were growing. Centrifuging the organisms from a liquid medium seemed from all points of view the best method. The next factor was the choosing of the organisms and the medium. Bacterium coli was used, because it is a luxuriant grower, and not being a pathogen, it was more easily and safely handled in the method in which this experiment was conducted. For the medium ordinary meat extract peptone broth was used, which as far as possible was constant in composition and reaction throughout the experiment. This broth, after the first sterilization, was centrifuged to remove particles still in suspension, and resterilized. It is evident, that if this substance had been left in the broth, it would have been mixed with the organisms later and would ultimately have effected the results.

After centrifuging the organisms had to be washed free of the

broth adhering to them. The sticky mass was ground up in a mortar and mixed very gradually with distilled water. The purpose was to get an even suspension and have the organisms dispersed as single cells in the water. The washing had to be done as quickly and carefully as possible, in order to prevent injuring the cells.

The method of analysis used in this work was compiled from several sources among them texts on quantitative analysis in general, soil and mineral analysis and articles on plant ash analysis. The main sources of information were a pamphlet by Hillebrand on the analysis of silicate and carbonate rocks and also the general quantitative analysis by the same author. Hillebrand discusses some of the most accurate methods to be used in cases where the amount of material available for analysis is small. It is evident, that in an ash determination of bacteria, the accumulation of the sample is a long tedious process and the yield is small compared with the amount of material used. No single scheme of analysis by any one author could be used. Even the original plan compiled from all of the above named sources had to be revised and augmented during the analysis as the occasion demanded.

Some previous work had been done on a rapid qualitative analysis of ash of bacterium coli. Ca, Mg, Na, K, Fe, Cl, SO_4 and P_2O_5 were found present.

The entire method of procedure used in carrying out the work of this paper is as follows. The organisms, a strain of bacterium coli, were grown on ordinary meat extract peptone broth containing 0.5% salt, in liter flasks. About fifty-five flasks were inoculated at one time and incubated forty-eight hours at 37°C. The organisms were then separated from the medium by centrifuging and weighed.

They were then washed carefully and quickly by suspending them in about six liters of water to remove the broth still adhering to them, and centrifuged from the wash water. After suspending the cells again in about six liters of distilled water, they were killed by heating to from 60-80° C for thirty minutes. Immediately after heating the suspension was cooled and diluted to from twelve to eighteen liters and allowed to stand for several hours for the salts to diffuse out. Again the liquid was separated from the organisms by centrifuging. The diffusate was concentrated and finally evaporated to dryness by boiling and weighed. The organisms left after diffusion were suspended in alcohol, dried, and weighed. About one hundred and sixty-five flasks were inoculated and treated in the manner just described. The yields were combined and the total dry matter of the organisms and of the diffusate were ashed in platimum in an electric muffle and the dry ash weighed. It was impossible, especially in the case of the organisms themselves, to burn off all of the carbon at this point without danger of volatilizing some of the salts. The excess of carbon was removed later as described below. The first recorded weight of the ash, therefore, is somewhat high. In the diffusate, due to the small amount of organic matter present, the difficulty of removing the carbon was not so great. The ash and diffusate were analysed quantitatively. Cl, SiO_2, Fe, Ca, P_2O_5, Mg, SO_4, Na and K were determined in the order just named.

In each case the sample was ground up to a fine powder and digested with water for several hours. The water soluble portion of the ash was then filtered off and the chloride precipitated with silver nitrate. The remainder of the ash was then dried and weighed. It was ignited to remove the remaining carbon and reweighed. The practically pure ash was then treated with concentrated hydrochloric acid

and evaporated to dryness several times to dehydrate the silica. After taking up the mass with water the silica was filtered off, ignited and weighed. The hydrochloric acid solution was added to the filtrate from the chloride precipitation, the excess of hydrochloric acid present precipitating the small excess of silver. After filtering again, the solution was diluted, heated to boiling and made alkaline with ammonium hydroxide. The flocculent precipitate which came down at this point was weighed and analysed. It was found to consist almost entirely of calcium phosphate with a small amount of iron. This separate analysis was made by fusing the precipitate with sodium carbonate, taking up the melt with water and filtering. The phosphoric acid in the filtrate was precipitated with ammonium molybdate. The water insoluble portion of the melt was dissolved in hydrochloric acid and the calcium thrown down with ammonium oxalate. The presence of a trace of iron was indicated by the reddish color of the precipitate.

After precipitating as much of the calcium and phosphate together by ammonia as possible, a few drops of the filtrate were tested for the further presence of these two radicals with ammonium oxalate and ammonium molybdate respectively. In all cases the calcium had been completely removed. An excess of phosphate was still in solution, however, and was taken out in the following manner. The filtrate was made slightly acid with hydrochloric, and a few c.c. of a solution of chemically pure aluminum chloride were added. Methyl red was used as an indicator, the solution was heated to boiling and carefully neutralized with ammonia. The precipitate formed was filtered off, dissolved in dilute nitric acid and the phosphate thrown out with ammonium molybdate.

In the filtrate from the calcium phosphate precipitation magnesium was determined by adding ammonium phosphate. This method was used in order to avoid adding sodium, as this element had to be determined later in the same solution. The method was tested on a known solution of magnesium chloride, and proved to be very satisfactory, if enough time, 12-24 hours, was allowed for precipitation.

The next substance to be determined was sulphate, and this was done in the usual manner by adding barium chloride, the slight excess being removed by dilute sulphuric acid.

The remaining filtrate was then evaporated to dryness and heated to drive off the ammonium salts. The residue was weighed and the weight recorded as NaCl + K Cl.

Potassium was determined by evaporating the mixture down several times with perchloric acid and heating in the fumes of perchloric acid, and finally taking up the salts with absolute alcohol containing a small amount of perchloric acid. The potassium perchlorate was then brought on to a gooch crucible, washed with absolute alcohol saturated with potassium perchlorate and containing some perchloric acid, dried at 110°C and weighed. Sodium was determined by difference.

As a great deal of time and material wasconsumed in preparing the samples for analysis, the whole ash analysis had to be done on one sample. It was absolutely imperative, therefore, that the chemicals used be perfectly pure, and that in all cases large excess in adding chemicals be avoided. It was often necessary to purify chemicals by recrystallizing. Whenever large flocculent precipitates were formed, it was necessary to dissolve and reprecipitate to prevent mechanical occlusion of other elements in the solution to be de-

termined later. Some of the methods were tried out as described above, on known salts or combinations to test their accuracy.

In the case of the organisms, the ash contained a considerable portion, which was insoluble in water and hydrochloric acid. This portion, on treatment with sulphuric and hydrofluoric acids, subsequent fusion with sodium and potassium carbonate and further analysis as described above, was found to contain silica, phosphate and calcium and a small amount of iron and magnesium.

Data as obtained by Analysis.

Total weight of moist organisms---------- 114 grams

Weight of dry matter in organisms---------- 12.9260 grams

Structural ash + carbon---------- 0.8392 "

Structural ash---------- 0.7050 "

Amounts of different elements present in the structural ash.

Cl---------- 0.0000 grams

SiO_2---------- 0.0021 "

$Ca_3(PO_4)_2$---------- 0.2440 "

Ca---------- 0.0936 "

PO_4---------- 0.1504 "

$Mg_2P_2O_7$---------- 0.1935 "

Mg---------- 0.0417 "

$BaSO_4$---------- 0.0302 "

SO_4---------- 0.0125 "

Fe_2O_3---------- 0.0236 "

NaCl + K Cl---------- 0.2157 "

K Cl O_4---------- 0.3175 "

K---------- 0.0913 "

Na---------- 0.0184 "

$Mg_2P_2O_7$---------- 0.2642 "

PO_4---------- 0.2251 "

Weight of diffusate dried----------3.5318 grams

Unbound salts + carbon----------0.9425 "

Amounts of different elements present in the unbound salts.

Ag Cl----------0.2857 grams

Cl----------0.0700 "

Si O_2----------0.0079 "

$Ca_3(PO_4)_2$----------0.2223 "

Ca----------0.0864 "

PO_4----------0.1359 "

$Mg_2P_2O_7$----------0.0882 "

Mg----------0.0192 "

Ba SO_4----------0.1162 "

SO_4----------0.0410 "

Fe_2O_3----------Trace

Na Cl + K Cl----------0.6470 "

K Cl O_4----------0.3375 "

K----------0.0936 "

Na----------0.1863 "

$Mg_2P_2O_7$----------0.2944 "

PO_4----------0.2520 "

Carbon----------about----------0.01 "

Data in Percent.

Total dry matter in organisms----------11.34%

Total ash in the dry matter----------12.75%

Structural salts in the ash----------42.79%

Unbound salts in the ash----------57.21%

Percentage of elements calculated upon the dry weight of the structural ash.

Cl----------	0.00%
$Ca(PO_4)_2$----------	35.61%
(Ca O 13.77%, P_2O_5	21.84%)
MgO----------	5.92%
SO_4----------	1.78%
Fe_2O_3----------	3.35%
$Si\ O_2$----------	0.30%
K----------	12.95%
Na----------	2.61%
P_2O_5----------	33.99%
Total----------	96.51%

Percentage of elements calculated upon the dry weight of the unbound salts.

Cl	7.40%
SiO_2	0.82%
$Ca_3(PO_4)_2$	23.59%
	(CaO 9.13%, P_2O_5 14.46%)
Mg O	2.04%
SO_4	4.36%
Fe_2O_3	Trace
K	9.94%
Na	19.77%
P_2O_5	26.84%
Carbon	1+ %
Total	97.76%

A rough estimate was made also of the mineral constituents of the broth used for the cultivation of the organisms. The results of this analysis are as follows:

Percentage of ash in the broth---------- 0.9%

Percentage of constituents of the ash in broth.

NaCl	0.5%
P_2O_5	0.2%
Ca + Mg	0.1%

Small amounts and traces of K, SO_4 and Fe.

In looking over the results of this work wefind, that the contents of dry matter and total ash for bacterium coli come within the range found by other investigators for other organisms. The water content seems to be relatively constant for all organisms analysed up to the present time.

A very significant factor for our purpose is, that the weight of the unbound salts is greater than that of the structural salts. This shows, that not only do salts come out of bacterial cells when the latter are killed by heat and suspended in distilled water, but the greater part, 57.21%, of the total salts in the cells diffuse out on the death of the organisms. The total absence of chloride in the structural salts and its presence in the unbound salts shows, according to our theory, that chloride is not essential in the cell structure but is used to equalize the pressure of chloride in the medium or in some other unknown function. Sulphates are present to a much greater extent in the diffusate than in the ash. Iron is present almost entirely in the structural salts. The function of iron in the cell is not known. If it serves, as in the cells of higher animals, as an oxygen carrier, its function is a biological one. Phosphate is by far the most abundant element. It seems to be the essential element in the cell structure, as well as in other functions of the cell and is very often found in higher concentrations in the organisms than in the medium.

Sodium and potassium show very interesting results. Both are found in the unbound salts, but sodium predominates. This was to be expected. With a concentration of 0.5% NaCl in the broth the organisms would have to take up a considerable quantity of sodium to equalize the osmotic pressure caused by the sodium ions in the broth.

The fact, that this element appears almost entirely in the diffusible salts shows, that its action in the cell is almost entirely physiological or physical. The results for sodium and potassium obtained here agree very well with analyses of cells of higher plants and animals, where potassium but no sodium is found present. Potassium is invariably the constituent of cell substance while sodium is present in the fluids, that have purely physiological functions in the bodies of animals and in plants. In red blood cells, for instance, potassium is always present, but sodium has never been found. The large amount of sodium found by Cramer[10] and others in bacteria must be almost entirely in the unbound salts. It is not improbable that the small amount of sodium in the structural salts could be replaced by potassium by a little higher concentration of that element in the medium. The amount of potassium present in the diffusate is almost four times as large as the amount of sodium in the ash. This would show, that potassium is utilized by the cell in its structure and also has a biological function.

In the work of this paper it has again been proven conclusively that salts diffuse out of bacterial cells when they are killed by heat and suspended in distilled water. Moreover, it has been shown that the amount of unbound salts in bacteria is greater than the amount of structural salts. The two groups of salts contain about the same constituents in somewhat different proportions. Chloride and iron are the exceptions, occurring only in the diffusate and only in the ash respectively. The results for sodium and potassium agree well with those found, in the past, in analyses for the same elements in higher plant and animal cells.

The results obtained in this investigation may be typical of

other organisms cultivated under similar or different conditions. This fact would, however, have to be determined by added experiments. The results can be regarded as established only for bacterium coli cultivated and treated as described in this paper.

Bibliography.

1. Fluegge, Du Mikroorganismen p.427
2. Journal für praktische Chemie No.5, Vol 20, p.448
3. Zeitchr für phys. Chemie Vol 9 p.7 1885
4. Archiv für Hygiene Vol 16 p.151
5. Massenculturen einiger Spaltpilze und der Soorheefe. Leipzig Dissertat
6. Archiv für Hygiene Vol 16 p.151
7. " " " Vol 13 p.71
8. " " " Vol 16 p.171
9. " " " Vol 22 p.167
10. " " " Vol 28 p.1
11. Centrablatt für Bacteriologie Vol 33 p.993-1898
12. Archiv für Hygiene Vol 28 p.30
13. " " " Vol 18 p.318-1893
14. Fluegge. Die Mikroorganismen p.407
15. Zopf. Die Pilze in morphologischer, physiologischer, biologischer und systematischer Beziehung p.119.
16. Stern.Jour Chem Society 1899 and 1907
17. Zeitschr.für das zesammte Brauwesen 1878
18. Sieber.Jour für praktische Chemie Vol 23 p.412.