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Fish, Charles Austin

Boston University

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NUCLEOPROTEIN CONTENT OF THE "L" STRAIN OF STAPHYLOCOCCUS AUREUS

by

CHARLES AUSTIN FISH

(A.B., Harvard University, 1947)

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1950
The object of the present study has been the determination of the two nucleic acids, desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) at significant stages in the growth cycle of *Staphylococcus aureus*. The nucleic acids of this organism have been determined at a single stage by Vendrely and Lehoult (1946), the somewhat indefinite agar slant culture of twenty hours yet affording some basis for comparison. A more complete series of determinations was carried out by Price (1949) on *Staphylococcus muscae* including material from a twenty-two hour slant. This work is discussed below. Much work has been done in the identification of nucleoproteins in other bacteria, upon which we have drawn to supplement the meagre literature on *Staphylococcus*.

Boor and Miller in 1935 prepared nucleoprotein from *Staphylococcus aureus* for the purpose of investigating possible cross reactions with other microorganisms. They describe the material as a mixture of acid precipitable protein including albumins and globulins as well as nucleoproteins.

The preparation was soluble in alkaline solution (pH 7.6) and gave such protein reactions as biuret, xanthoproteic, Millon, Hopkins-Cole, as well as a Molisch test.

Numerous extraction methods were used to determine their possible effects on antigenicity. Included were extraction with distilled water, maceration with sodium chloride, grinding in the dry state, freezing and thawing, treatment with surface tension depressants such as sodium taurocholate, and treatment with dilute alkali. The authors chose N/100 sodium hydroxide extraction as a satisfactory solvent for an immunological
preparation.

They investigated the toxicity of their material and reported that it was very stable to heat. No details were given.

Among the findings of their immunological study was the fact that staphylococcal nucleoprotein reacted strongly with antiserum to type three pneumococcus.

One fact stands out in the paper; dilute hydroxide extraction yields an immunologically active nucleoprotein presumably undegraded, although the quality of their antiserum to an admittedly impure antigen may well be questioned.

Also of interest is the use of taurocholate since this has recently proved to be a valuable reagent in preparing undegraded nucleic acid.

In the introduction to his publication on the nucleus in Staphylococcus, Knaysi (1942) reviews the progress made in demonstrating the nuclear material in bacteria. By 1933, Petter had noted Feulgen positive granules in Sarcina gigantea. Piekarski in 1938 and 1939 observed Sarcina alba and various staphylococci with the ultraviolet and electron microscopes.

In Sarcina alba which has a cell diameter of 1 to 1.5 μ, Piekarski found a Feulgen positive granule of about .25 to .4 μ in diameter. Similar observations were made on the staphylococci studied.

A recent observation of Vendrely and Lehoult (1946) is of interest here. On the basis of their values for DNA in several bacteria including Staphylococcus aureus, they compute the size of a nucleus necessary to account for the corresponding nucleoprotein as half the linear dimensions of the cell. It can be seen that Piekarski's nucleus is one quarter to one third the linear dimensions of the cell.
In staining the nuclear material, Knaysi used methylene blue at pH 1.8 to 4.8. He found that one per cent sulfuric acid decolorized the cell body and the chromatin. The metachromatin was also decolorized but much more slowly.

The Feulgen nucleal reaction and the Schiff reaction were carried out on the bacteria both intact and after treatment with several solvents. The granules appeared dark red on staining with Schiff's reagent. The Feulgen reaction was positive under varying conditions of hydrolysis. Millon's test indicated a protein content in the granules. Boiling water did not dissolve the granules, nor did treatment with sodium acid carbonate for two hours. The granules did dissolve in normal sodium hydroxide. Some change in staining with methylene blue was noted after twenty-four hours in ten per cent hydrochloric acid when the granules no longer recolored Schiff's reagent. They still gave a positive Millon test however.

Knaysi has built up an ironclad case while reporting in an almost ingenuous tone. One statement may be somewhat misleading. Chargaff and Saidell (1949) have found that nucleoprotein is soluble on prolonged shaking in borate buffer of pH 8 and we infer that it would also be soluble in dilute sodium acid carbonate. Apparently the nucleic acid was hydrolyzed away on twenty-four hour treatment with ten per cent hydrochloric acid leaving the protein in place. It would be of interest to know how the protein was identified as being part of the nucleus after the removal of the nucleic acid.

Knaysi states that the protein of the granules is conjugated with a strong acid since it takes up methylene blue at pH 1.8. It may
be pertinent to record here the dissociation constants of several nucleotide units taken from Everett (1946).

Dissociation Constants (pK acid)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytidyllic acid</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanylic acid</td>
<td>0.7</td>
<td>5.9</td>
<td>9.4</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>1.0</td>
<td>5.9</td>
<td>9.4</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>2.0</td>
<td>6.8</td>
<td>12.4</td>
</tr>
</tbody>
</table>

Knaysi rules out metachromatin as soluble in boiling water and .02 per cent sodium acid carbonate. The material, he concludes, is nucleoprotein. The granules demonstrated have been seen in mature or old colonies. Young cells were found to undergo homogeneous staining of a strongly acid nature.

The Bacterial Cell of Dubos (1947) contains a chapter on the cytology of bacteria. It is a discussion of the evidence for the existence and nature of the bacterial nucleus. Bacteria have been shown to contain acidic intracellular granules. Among these, the volutin granules, which may consist of reserve ribonucleic acid, probably have been mistaken for nuclei. The observation that a basophilic structure undergoes division is not proof of a nuclear nature, since other cellular structures such as mitochondria and chloroplasts also divide.

Evidence for a bacterial nuclear structure comes from ultraviolet microscopy although the conclusions are as conflicting as those obtained by the use of basic dyes. The very large content of nucleic acid in bacteria complicates the use of ultraviolet light. Additionally, the two nucleic acids are now thought to be related by interconversion; that is, the DNA is produced by reduction of the ribo compound.
To explain some of the inconsistencies it has been suggested that the state of the nuclear material changes not only from one strain to another but also during the growth cycle of a single culture.

The opinion that Feulgen positive substance occurs in the bacterial cell in minute particles evenly dispersed may have been due to the extreme conditions of hydrolysis. Acid treatment at 60 C or above disrupts the structure of the cell. Well defined structures of Feulgen positive material can be seen when carefully hydrolysis is carried out in the staining.

During the past ten years uniform results of several different observers have provided evidence of a discrete nuclear apparatus in many common bacteria.

Photographs were made by Dubos(1947) with a visible light microscope and enlarged to a final magnification of 4600 times. They show the dumbbell-shaped nucleus of Escherischia coli going through successive divisions in the formation of daughter cells. The nuclear structures are best studied in bacteria withdrawn from cultures a few hours after transfer to new medium.

Knaysi(1942), using the electron microscope, saw nuclear structures in old cultures, although he failed to find a nucleus in young cells. Nuclear structures cannot be seen with visible light in old cells because of their diminished size; however, they have been demonstrated in young cells with the use of Feulgen and Giemsa stains(Dubos,1947).

The work of Delaporte(1936,1939) is quoted to the effect that the nucleus of yeasts recolors Schiff's reagent only after partial hydrolysis. This is in contrast to Knaysi's findings with Staphylococcus and he concludes that the configuration of the yeast nucleic acid differs from that
of the staphylococcal nucleus.

Vendrely and Lehoult (1946) have determined the nucleic acid content of several species of bacteria including *Staphylococcus aureus* in order to implement a theory on the size and appearance of the bacterial cell nucleus.

They report an average value for DNA of three to four per cent of the dry weight and they postulate that the DNA is conjugated with one or two times its weight of protein by analogy with the nucleoprotein of higher organisms. The authors feel that such an amount of nucleoprotein demands a nucleus as large as half the linear dimensions of the cell. A nucleus of these proportions has been reported by Robinow (1942) using Giemsa stain.

The determinations were carried out on bacteria from twenty hour cultures on agar. The high values found for RNA indicate that the bulk of the organisms are in an early stage of the growth cycle. (Mirsky, 1945)

Their values are as follows in per cent of dry *Staphylococcus aureus*:

<table>
<thead>
<tr>
<th>Total N.</th>
<th>Protein Calculated</th>
<th>Total Nucleic Acids</th>
<th>DNA</th>
<th>RNA</th>
<th>RNA NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.95</td>
<td>75.50</td>
<td>11.57</td>
<td>2.82</td>
<td>8.75</td>
<td>.24</td>
</tr>
</tbody>
</table>

Price (1949) has investigated nucleic acid content of *Staphylococcus muscae* during virus formation and has reported the findings for normal cell suspensions. (Virus formation apparently refers to the development and multiplication of the virus within the infected bacteria). In cells from a twenty-two hour slant, he reports equal amounts of RNA and
DNA. In the first hour the content of RNA per cell was twice that of DNA. As the cells enter the logarithmic phase, there is 2½ times as much RNA as DNA. Price states that the Staphylococcus muscae division time is about two hours and the lag period sixty to seventy-five minutes.

Price's nucleic acid values by three methods: color tests, phosphorus, and ultraviolet absorption, agree within ± fifteen per cent. He found that no RNA or DNA was released into the medium by normal cells.

The difficulties of making comparisons in work using bacterial culture cycles is illustrated by values just given. Staphylococcus muscae grown on agar for twenty-two hours was found to have equal amounts of RNA and DNA and the culture to be a resting stage, while Vendrely and Lehoult report values for the more rapidly dividing Staphylococcus aureus from a twenty-hour slant as having three times as much RNA as DNA and apparently in a logarithmic growth stage.

In the work of Vendrely and Lipardy (1946) concerned with the bacterial nucleus, several strains of bacteria including a Staphylococcus were extracted successively with normal HCl at 60 C and with trichloracetic acid at 90 C and the nucleic acid content of the extracts determined.

For the Staphylococcus, only 7.5 per cent of the DNA was in the HCl extract. The remainder was in the trichloracetic acid, while almost all of the RNA was in the HCl extract. The HCl extract contained purines in excess of its nucleic acid content. The authors conclude that the purines of the DNA have been partially hydrolyzed by the HCl. They state this as explaining why HCl-extracted cells stain intensely with basic dyes.

Krampitz and Werkman (1947) have investigated the mode of peni-
cillin action using cultures of *Staphylococcus aureus*. They present evidence that the ribose of RNA is metabolized as an energy source by this organism. The RNA source may be the bacterial cytoplasm or material supplied in the culture medium. Penicillin apparently blocks a step in the release of ribose from RNA.

The authors used the method of Schneider (1945) in extracting nucleic acids. They found it necessary to modify the procedure, however, to include two extractions with hot five per cent trichloracetic acid instead of one.

Their RNA values are expressed as ribose. In cultures with no penicillin, they found 2.4 per cent ribose in early stages. At the end of twenty-four hours the ribose had dropped to one per cent. Penicillin did not interfere with the metabolism of ribose as such already present in the culture.

Petrik (1946) has investigated nucleic acids in atypical acid-fast bacilli with the purpose of finding a means to correlate these bacteria with tubercle bacilli and thus aid in evaluating their significance in sputa and other human excreta. Three strains of atypical acid-fast bacilli were grown on Long’s or Henley’s medium for four to eight weeks. The harvest was washed with distilled water and for extended periods with alcohol, ether and chloroform.

Petrik investigated several methods of nucleoprotein extraction since evidence has accumulated to indicate that the nucleic acid structure is disrupted by some of the methods in use and moreover that the bonds in the two nucleoproteins are of a different nature. Specifically, the DNA to protein link appeared to be salt-like in nature, whereas the RNA of a hemolytic streptococcus was more firmly bound to the protein. The linkage
of nucleoprotein of streptococcus has also been referred to as non-polar and requiring acid, alkali, or organic solvents to split the linkage.

The methods compared for extraction of nucleoprotein were that of Coghill (1931) for Timothy bacillus; Levene’s method for DNA (Levene and Bass, 1931); extraction with molar sodium chloride. Methods used to split the protein bond were: (a) five per cent hydroxide for two hours, (b) five per cent sodium hydroxide at 0°C for one hour, and (c) 0.1 normal sodium chloride at pH 5 to 6 for one minute at 100°C. The last extraction method was that of Bang (Mirsky, 1943) using a saturated sodium chloride solution.

Petrik stated that DNA was obtained from all the organisms assayed and that RNA was absent in at least one strain. The author cites the age of culture as a possible reason for this lack, since it is known that old cultures are low in RNA (Mirsky, 1943). No free nucleic acid could be recovered using 0.1 normal sodium chloride at 100°C, nor by the method of Bang which uses saturated sodium chloride to split the nucleic acid-protein bond. Petrik found acceptable the extraction method of Levene and the method of alkali hydrolysis (five per cent NaOH for one hour) in splitting the bond.

Chargaff and Saidell (1949) are concerned with the preparation of undegraded nucleic acid from avian tubercle bacilli. The organisms were lyophilized and extracted with ice cold ether and ground with powdered glass. The result was shaken for two days in the cold with borate buffer at pH 8.5. Glycogen was removed by centrifugation and the solution dialyzed and again lyophilized.
The desoxypentose nucleoprotein, the principal fraction present, was insoluble at pH 4 and was not precipitated by half saturation with ammonium sulfate which fact enabled a separation from pentose nucleoprotein. The presence of basic proteins as protamine or histone could not be demonstrated. The intact nucleoprotein in the crude state was precipitated as the lanthanum salt at pH 7.7.

Gold cleavage was achieved in saturated sodium chloride solution yielding 28 per cent of the original phosphorus. However, 0.5 per cent sodium desoxycholate gave better results, yielding 72 per cent of the original phosphorus.

Residual protein was removed by extraction with a chloroform-octanol mixture. The removal of protein and glycogen was the principal obstacle in preparing pure undegraded nucleic acid.

Electrophoretic purification was accompanied by considerable loss but gave the best results; nitrogen, 12.9 per cent and phosphorus, 7.6 per cent with a ratio of 1.7.

Theoretical per cents of nitrogen and phosphorus in DNA are 17 and 10 respectively. Here also the ratio is 1.7. It is likely that the impurity in the nucleic acid preparation contains neither nitrogen nor phosphorus, e.g. glycogen.

The work of Sevag, Smolens and Lackman (1940) is concerned with the nucleic acid content of *Streptococcus pyogenes*. In analyzing the bacteria for total phosphorus, purine nitrogen, and total nitrogen, their results were variable when carried out on material washed with distilled water. Results were in agreement after washing the organisms with 95 per cent alcohol containing 0.2 per cent by volume of concentrated HCl with shaking for 30 minutes.
The bacteria were extracted with ether and dried at 56° C. and then at 110° C. Extraction of nucleic acid was carried out by hydrolyzing with 0.1 normal HCl on a boiling water bath for 15 minutes.

Total nucleic acid was 14.8 to 22.5 per cent determined by purine nitrogen using the method of Graff and Maculla (1935) which employs formic acid-hydrochloric acid hydrolysis. The purines are precipitated with cuprous oxide. DNA values by the method of Dische (1950) were reported on smooth and rough variants ranging from 2 to 6 per cent of dry weight.

The work of Morse and Carter (1949) deals with the nucleic acid content of *Escherichia coli* throughout the growth cycle. Growth curves were followed by means of plate counts.

The procedure consisted of adding trichloracetic acid to ten per cent of volume of aliquots and centrifuging at 2500 r.p.m. Without further washing the cells were extracted once with five per cent trichloracetic acid on a boiling water bath for 30 minutes. Nucleic acids were determined colorimetrically.

They conclude that considerable amounts of nucleic acids are synthesized before cell multiplication has commenced and that the amounts of RNA and DNA per cell reach a maximum as or just before cell multiplication can be detected. The increase of RNA was found to be between five and ten times the initial amount, and the increase of DNA approximately twice. The duration of the lag period was 40 to 60 minutes.

The paper notes that increase in the size of bacteria is characteristic of the lag period. If we assume that the cells double in size over the lag phase, the values reported based on cell count should be
FIG. 1. CHANGES IN AVERAGE NUCLEIC ACID CONTENT OF E. COLE STRAIN B DURING GROWTH IN SYNTHETIC MEDIUM

FIG. 2. SYNTHESIS OF NUCLEIC ACID BY E. COLE AFTER CELLS ARE TRANSFERRED TO SYNTHETIC MEDIUM
halved to render them comparable to values based on weight or total nitrogen. By this token, the content of DNA remains unchanged and the RNA increases \(2^{3/2}\) to 5 times. Figures 1 and 2 show the data of Morse and Carter as relative amounts of nucleic acids per cell and per milliliter.

The work of M.J. Moses (unpublished data) on nucleoprotein distribution in Paramecium nuclei brings to light a contrast with other cell nuclei. The Paramecium studied shows a large somatic nucleus functioning in vegetative needs and a micronucleus active at conjugation or autogamy, both of which contain RNA and DNA.

Nucleic acids were identified by the Feulgen nucleol reaction and by trichloracetic acid hydrolysis (total nucleic acid). RNA was determined by difference after digestion with ribonuclease. Protein was 85 per cent of the nucleus. RNA was present to nearly twice the amount of DNA in the nucleus.

Alper and Sterne (1955) have reported on the use of photometry in following bacterial growth curves. Their work includes a comparison of growth curves by haemocytometer count and by the opacity method working with Salmonella gallinarum. Several citations state that bacteria change in size during growth cycles, and that an increase in size takes place before cell division begins.

The authors show that when a growth curve is followed by opacity the lag phase appears to be shorter than when followed by actual count. They go on to state that an interdependence of opacity and cell size would seem likely. Their data is illustrated in a graph of curves. The curve secured by cell count has a lag period showing no multiplication.
Over the same period the opacity curve shows a gradual increase.

Mestre (1955) reports the statement of Von Anger (1924) that the intensity of the light scattered laterally by suspensions of bacteria could be expected to be a function of their number per unit volume, their size, shape, and index of refraction. As the result of an experimental study of mastic particles of various sizes approximating those of bacteria, he found that the intensity of the light scattered at right angles to the incident beam appeared to be directly proportional to the number of particles per unit volume and to the square of the radius of the particle. This relation in the scattering of light has been demonstrated for spherical microorganisms by Liese (1926) and Muntner (1926).

Ebbecke and Knückel (1959) studied factors influencing coagulation and optical properties of the clot. Using both light transmittance and the Tyndall effect, they found that the changes manifested by the two methods paralleled each other.

Schneider (1945) reports a method for determining phosphorus compounds in animal tissue. He classifies the compounds as acid soluble, lipid, nucleic acid and protein phosphorus.

His procedure of heating tissue with five per cent trichloracetic acid at 90 °C. for 15 minutes removed all nucleic acid. The yield was not increased by repeating the extraction nor by hydrolizing with two per cent sodium hydroxide at 100 °C.

DNA was determined by the diphenylamine and carbazole reactions and RNA by the orcinol reaction. Since orcinol reacts with both RNA and DNA a correction was made in determining RNA with this reagent.

The author cites Dicke (1950) to the effect that diphenylamine reacts only with purine-bound deoxyribose while carbazole reacts only
with pyrimidine-bound desoxyribose. These facts must be taken into account in setting up standards for the reactions since there is not sufficient data on the purine and pyrimidine content of nucleic acids to permit precise estimation of nucleic acids on the basis of ribose or desoxyribose standards. It must be noted, however, that the only improvement at present is to use nucleic acid of the type being determined. (See Vischer and Chargaff below). The author found that all the phosphorus in the trichloracetic acid extracts could be accounted for by nucleic acids determined. This was not the case with nitrogen as calculated from the tetranucleotide structure.

Schneider (1946) has undertaken to evaluate by comparison two recent methods for the determination of nucleic acids: the method of Schneider (1945), (reported above), and that of Schmidt and Tannhauser (1945).

Determinations were carried out on rat tissue after removal of acid soluble and fat-soluble compounds. The tissue is then dissolved in normal KOH (Schmidt and Thannhauser). The DNA and protein are precipitated with acid and the RNA is contained in the filtrate. Schneider has modified the method the Schmidt and Thannhauser by extracting the DNA from the protein-DNA precipitate with trichloracetic acid. In the original method, RNA phosphorus and total phosphorus are determined and DNA evaluated by difference. Schneider states that all the phosphorus present in trichloracetic acid extracts of tissue was accounted for by nucleic acids as measured by pentose determinations.

For the most part good agreement between the two methods was found. The discrepancies noted were values for DNA in spleen and also DNA values on liver and brain. Of six tissues analyzed, two were not
in agreement. It is of interest that the values for DNA in the two procedures were in complete agreement when both hydrolysates were analyzed with diphenylamine.

The structure of nucleic acids is an important aspect of standardizing quantitative determinations based on pentose or phosphorus content and especially purine or pyrimidine content. Vischer and Chargaff (1948) have carried out determinations for the purine and pyrimidine content of yeast and pancreas pentose nucleic acids. They point out the possibility of intricate and specific nucleic acids comparable to the composition and structure of proteins. This is in contrast to the tetranucleotide hypothesis which postulates a unit with four different bases, but having two purines and two pyrimidines. The bearing which their values have on the interchangeability of nucleic acids as standards is illustrated by computing a nucleotide weight of the two ribonucleic acids.

Pancreas and yeast pentose nucleic acids are reported by Vischer and Chargaff to have the following mole per cents of purines and pyrimidines:

<table>
<thead>
<tr>
<th></th>
<th>Yeast</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>3.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Guanine</td>
<td>5.1</td>
<td>8.8</td>
</tr>
<tr>
<td>Cytosine</td>
<td>3.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Uracil</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Totals</td>
<td>10.3</td>
<td>17.9</td>
</tr>
</tbody>
</table>

The average nucleotide weight of pancreas pentose nucleic acid is 330., while that of yeast is 324.6. The two values have a 1.66 per cent difference.

A more impressive figure is the purine mole per cent of total
purines and pyrimidines, which for pancreas pentose nucleic acid is 69 per cent and for yeast 61 per cent, a difference of 11.5 per cent and a divergence from the tetranucleotide theory of 19 per cent.

**Methods**

In addition to presenting historical material, we have attempted to round out a background of theory and method to aid in setting up a procedure for the study at hand. Since we are interested in a quantitative determination for nucleic acids and are not concerned with preserving the intact structure, the methods of Schneider (1945) and Schmidt and Thannhauser (1945) are acceptable. The former method has been widely used in bacterial determinations and has been selected here. The method is usually modified for use with bacteria to include prolonged trichloracetic acid extraction or higher temperature of extraction (Krampitz and Werkman, 1947).

Immediate processing of the samples proved necessary since delays in reaching the trichloracetic acid hydrolysis were accompanied by considerable loss of nucleic acid. The possibility that ribonuclease of the bacteria acts to destroy RNA is suggested by the work of Krampitz and Werkman (1947) (see above). Possible enzyme action would be stopped by trichloracetic acid.

The determinations were carried out on *Staphylococcus aureus* of the "L" strain from the department of Microbiology of Boston University School of Medicine. All cultures were grown in a medium of casein hydrolysate and yeast extract. Fifteen grams of Bacto-Casaminio Acids and five grams of Bacto-Yeast Extract, both of Difco Co., are made up to one liter with tap water. The reaction is adjusted to pH 8. and the medium auto-
claved at 10 lbs. pressure for twenty minutes and then refrigerated. The
parent stock was grown on a blood agar slant and kept at 5 C. All cul-
tures were checked for contamination by Gram stain of a smear.

In order to have reproducible lag period behavior, it was neces-
sary to have an inoculum in a resting or decay stage but which contained
a minimum of nonviable bacteria, since in the lag phase samplings up to
an hour contain the same per cent of nonviable bacteria as the inoculum.
Similarly the inoculum must be large enough to come within range of the
chemical techniques employed. It was discovered that an inoculum large
enough to allow accurate duplicate determination within the first hour
was too large to permit normal growth in the logarithmic phase.

The inoculum was prepared by incubating for 24 hours 1 ml. of
a previous culture in 100 ml. of medium in 250 ml. Erlenmeyer flasks
at 37 C. in an open water bath. Fifty ml. for lag phase, and 25 ml. for
logarithmic phase, of this was spun down in a refrigerated centrifuge
and inoculated into 500 ml. of medium. The growth curve was followed by
means of turbidity measurements at a wave length of 600 mu. All photo-
metric measurements were made on a Coleman Junior Spectrophotometer.

Samples of 100 to 200 ml. were removed at selected times, spun
down, and washed with distilled water and made up to 20 ml. Aliquots
were removed for total nitrogen determinations. The samples were then
washed with cold 10 per cent trichloracetic acid and with 95 per cent
ethyl alcohol. It was found desirable to modify the washing procedure
by resuspending the centrifuged bacteria in 1 or 2 ml. of water before
adding the trichloracetic acid or alcohol to prevent clumping. The bac-
teria were stirred for 10 minutes with each washing. The samples were
then hydrolyzed with two 5 ml. portions of 5 per cent trichloracetic
acid in a water bath at 90 C. The hydrolysates were combined and centrifuged a second time to remove particulate material and made up to 18 ml. with trichloracetic acid. Duplicate aliquots were now taken for determination of RNA, DNA, nucleic phosphorus and nucleic nitrogen.

The orcinol method (Mejbaum, 1939) was used in measuring RNA. The photometric measurements were made at a wave length of 670 mu. The procedure was tested by measuring known mixtures of RNA and DNA standards with this reagent. (The necessity for a correction factor is treated by Schneider (1945) (See above)). On the basis of an orcinol - DNA curve, a correction was made, in a sample computation, of 9 per cent of the weight of RNA, which then came within one per cent of the known amount of RNA.

DNA was measured with the diphenylamine reaction of Dische (1950). The photometric readings were made at 600 mu. Commercially prepared ribonucleic acid and desoxyribonucleic acid of Nutritional-Biochemical Laboratories were used as standards in these two reactions. The preparations were evaluated by total phosphorus determinations which agreed well with chromatography findings on the material. The preparations were about 90 per cent nucleic acid as calculated from phosphorus determinations. No free phosphate was found when the material was reacted prior to digestion. A single impurity, which was 10 per cent of the preparations, was found by chromatography.

Phosphorus determinations were by the method of Fiske and Subba-Row (1925). The reagent used in Kjeldahl digestion for the total nitrogen was 1 ml. of selenium oxide and 1 ml. of a saturated solution of cupric sulfate made up to 100 ml. with concentrate sulfuric acid. Nitrogen was measured as ammonia by Nesslerization using the formula of Koch-McMeekin.
(Hawk, Oser, and Summerson, 1947). The photometric measurements were carried out at a wave length of 520 μμ.

Nucleic acid values have been related to total nitrogen of whole bacteria washed with distilled water.

Results

Samples were analyzed from the 24-hour inoculum material to give values of a resting or decay culture. The values of RNA and DNA were about equal and ranged from 0.5 to 0.7 mg. of nucleic acid per mg. of nitrogen.

Samples were also taken at one half hour intervals up to three hours. There is a slight increase of DNA values to a maximum of 0.855 mg. per mg. of nitrogen at one hour and 10 minutes. RNA values increase to about 2½ times the original value, reaching a maximum of 1.54 mg. per mg. of nitrogen at one hour and a half.

The values of Vendrely and Lehoult (1946) related to mgs. of total nitrogen are, for RNA, 0.625 mg., and, for DNA, 0.2 mg. Their very low DNA value suggests that one extraction fails to remove all the DNA.

Our values agree with those of Price (1949) on Staphylococcus muscae in having equal amounts of the two nucleic acids in a resting culture. Price found a larger increase of RNA, which reached 2½ to 5 times the initial amount (see above).

After the modification in the trichloracetic acid wash was adopted, the nucleic acid phosphorus values were in good agreement with the nucleic acid totals determined by colorimetry.

Nucleic nitrogen to phosphorus ratios in eleven out of thirteen
<table>
<thead>
<tr>
<th>Sampling Time</th>
<th>DNA Total N.</th>
<th>RNA Total N.</th>
<th>N/P</th>
<th>Mg P x 10</th>
<th>Mg Total Nucleic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs.</td>
<td>.656</td>
<td>.597</td>
<td>1.68</td>
<td>.22</td>
<td>.151</td>
</tr>
<tr>
<td>24 hrs.</td>
<td>.585</td>
<td>.49</td>
<td>1.61</td>
<td>.28</td>
<td>.151</td>
</tr>
<tr>
<td>24 hrs.</td>
<td>.586</td>
<td>.715</td>
<td>2.5</td>
<td>.16</td>
<td>.155</td>
</tr>
<tr>
<td>37 min.</td>
<td>.51</td>
<td>1.02</td>
<td>2.05</td>
<td>.205</td>
<td>.17</td>
</tr>
<tr>
<td>40 min.</td>
<td>.615</td>
<td>1.47</td>
<td>1.83</td>
<td>.175</td>
<td>.199</td>
</tr>
<tr>
<td>55 min.</td>
<td>.69</td>
<td>1.04</td>
<td>2.45</td>
<td>.145</td>
<td>.159</td>
</tr>
<tr>
<td>55 min.</td>
<td>.665</td>
<td>.95</td>
<td>1.8</td>
<td>.21</td>
<td>.212</td>
</tr>
<tr>
<td>1 hr. 10 min.</td>
<td>.855</td>
<td>1.44</td>
<td>1.61</td>
<td>.186</td>
<td>.195</td>
</tr>
<tr>
<td>1 hr. 30 min.</td>
<td>.764</td>
<td>1.54</td>
<td>1.95</td>
<td>.215</td>
<td>.249</td>
</tr>
<tr>
<td>1 hr. 35 min.</td>
<td>.806</td>
<td>1.54</td>
<td>2.1</td>
<td>.185</td>
<td>.204</td>
</tr>
<tr>
<td>2 hrs. 5 min.</td>
<td>.650</td>
<td>1.41</td>
<td>1.81</td>
<td>.22</td>
<td>.212</td>
</tr>
<tr>
<td>3 hrs. 5 min.</td>
<td>.801</td>
<td>1.34</td>
<td>2.0</td>
<td>.20</td>
<td>.207</td>
</tr>
<tr>
<td>3 hrs. 5 min.</td>
<td>.673</td>
<td>1.15</td>
<td>2.0</td>
<td>.215</td>
<td>.228</td>
</tr>
</tbody>
</table>

Figure 3. Ribonucleic acid and desoxyribonucleic acid values for *Staphylococcus aureus* of the "L" strain over the early stages of the growth cycle.
values reported range from 1.31 to 2.1 as against an ideal of 1.7.

Nucleic phosphorus and total nucleic acids were in good agreement in eight of the thirteen values reported. Price (1949) reports agreement between nucleic acid colorimetry and phosphorus determinations within ± 15 per cent.

Results are recorded in Figure 3, showing DNA and RNA per mg. of total nitrogen, the nucleic nitrogen to phosphorus ratio and the correspondence of nucleic phosphorus and nucleic acids determined. Figure 4 is a graph of the first two columns of Figure 3.
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