PHAGE FORMATION IN STAPHYLOCOCCUS MUSCAE CULTURES

XI. The Synthesis of Ribonucleic Acid, Desoxyribonucleic Acid, and Protein in Uninfected Bacteria

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Previous studies from this laboratory have shown that resting cells of *S. muscae* have approximately the same amount of ribonucleic acid (RNA) and desoxyribonucleic acid (DNA). During the early growth phases, when there is little or no cellular multiplication, both the RNA and DNA content of the cells begin to increase. When the RNA content per cell has increased to 2 to 2½ times over the DNA content, the cells begin to divide (1). These results have recently been confirmed by Fish, Asimov, and Walker with *Staphylococcus aureus* (2).

In this paper the synthesis of RNA, DNA, and protein in uninfected *S. muscae* cells has been studied under varying conditions. The main problem investigated was the relationship between protein and ribonucleic acid synthesis since it is widely held, due mainly to the work of Brachet (21) and of Caspersson (3), that ribonucleic acid is involved in protein synthesis. According to this hypothesis, it would be expected that an increase in the rate of protein synthesis would require an increase in the turnover of RNA, or an actual increase in the amount of RNA. With the methods used in this work, it was only possible to study whether an increase in protein synthesis resulted in an increase in the RNA content of the cells.

Since the completion of the experiments to be reported in this paper, the recent work of Caldwell, Mackor, and Hinshelwood was called to my attention (4, *cf.* also 18). These authors (4) made the observation that while the DNA content per cell of *Bacterium lactis aerogenes* remained constant over a wide range of conditions, the ratio of RNA to protein was proportional to the rate at which the cells were growing. Similar results have been obtained with with *S. muscae* and will be reported in this paper.

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Experimental Results

The synthesis[1] of RNA, DNA, and protein was studied in resting cells suspended in fresh medium, in early log phase cells, and in cells forming an adaptive enzyme for lactose utilization.

RNA, DNA, and Protein Synthesis During the Lag Phase.—Fig. 1 shows the increase in RNA N, DNA N, and protein N of resting cells suspended in fresh Fildes medium during the lag phase. Seven experiments were carried out, each experiment giving the same qualitative results and essentially the same quantitative results. The average error of the mean (1) for the different experiments for the same time interval varied from 5 to 15 per cent. The values shown in Fig. 1 for each 15 minute interval represent the average plot for each time interval. It may be seen that the increase in RNA N closely parallels the increase in protein N. DNA N does not begin to be synthesized until an interval of about 50 minutes has elapsed.

Fig. 1 illustrates that there is a 60 per cent increase in protein N and RNA N without any increase in the turbidity of the bacterial suspension. Table I shows that there is also a 50 per cent increase in total nitrogen during this time, and a 50 per cent increase in dry weight. Since the dry weight is 25 per cent of the total weight of staphylococcus (19), it appears that there should be at least an increase of 12 per cent in the turbidity under the above conditions. This is outside of the experimental error of the turbidity measurement, which is ± 2.5 per cent (5). It would seem that during this time in the lag phase the optical properties of the cells remain the same, although the percentage dry weight increases from 25 to 37 per cent. Constant turbidity readings therefore do not necessarily mean that no synthesis of protein occurs. Once the cells have reached the end of the lag phase, the dry weight per cell remains constant during the log phase period (Table II).

The Increase of RNA, DNA, and Protein in Log Phase Cells.—In studying the synthesis of early log phase cells, three strains were used. Strain I, under the experimental conditions, has a divison time of about 140 minutes in Fildes' synthetic medium. Strain II will not multiply in Fildes' synthetic medium unless acid-hydrolyzed casein is present in the medium, the division time being determined by the concentration of hydrolyzed casein added to the medium (6).[2] Strain V grows very poorly in Fildes' synthetic medium and

[1] Synthesis as used in this paper means an actual increase in RNA, DNA, and protein of the cell suspension. While such experiments should ideally be carried out on a suspension of cells all physiologically alike, it is impossible to obtain such a system. The RNA, DNA, and protein values that are obtained represent the *average* values for that particular cell suspension. This fact should be kept in mind in interpreting such results.

[2] Each bottle of acid-hydrolyzed casein must be titrated in order to find the correct amount to add to the medium to get the desired multiplication rate.

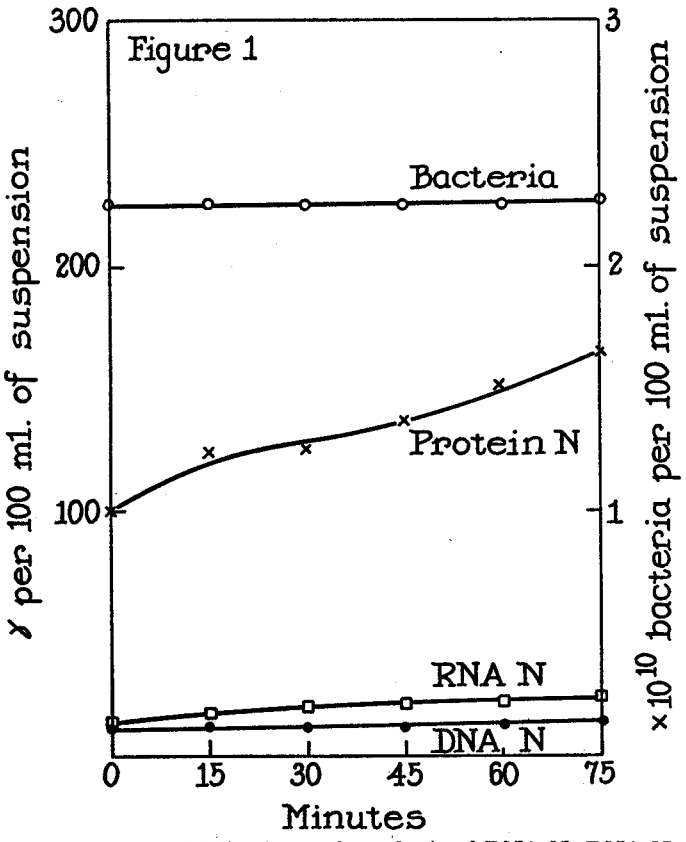


FIG. 1. The cellular multiplication and synthesis of RNA N, DNA N, and protein N of strain I of *S. muscae*. Cells of strain I were grown on veal infusion agar slants containing 0.5 per cent peptone. After growing 20 hours at 34°C., the cells were used to inoculate 500 ml. of veal infusion medium containing 0.2 per cent peptone to give 1.0×10^6 cells per ml. The mixture was then shaken in a 1000 ml. Florence flask for 20 hours at which time the cells had reached 3.1×10^9 cells per ml. and were in the resting state. They were centrifuged out and washed and resuspended in Fildes' synthetic medium as described in *Methods* to give about 1.2×10^8 cells per ml. This mixture was shaken in 1000 ml. Florence flasks at 35°C., and 175 ml. removed at 15 minute intervals and analyzed for RNA, DNA, and protein as described in *Methods*. Higher initial cell concentrations should not be used as they give very irregular results.

its multiplication rate is not influenced by the addition of acid-hydrolyzed casein. Each set of the following figures represents the average plot from four experiments, the average error of the mean varying from 5 to 15 per cent for each time interval. Figs. 2 A and 2 B show the synthesis of RNA N, DNA

N, and protein N in the early log phase of cells of strain I calculated per 100 ml. of suspension and per 3×10^{10} cells respectively. Figs. 3 A, 3 B, 4 A, 4 B, 5 A, and 5 B illustrate the increase of RNA N, DNA N, and protein N of

TABLE I

The Increase in Protein N, RNA N, Total Nitrogen, Dry Weight, and Turbidity at the Beginning and End of the Lag Phase

Cells were prepared as described for Fig. 1. 2450 ml. of synthetic medium was inoculated with 1.0×10^8 cells per ml. 100 cc. of this mixture was poured into an Erlenmeyer flask containing 5.0 ml. of 50 per cent trichloroacetic acid, for protein N and RNA N analysis. Another 100 ml. of the mixture was chilled in a cracked ice–salt bath for 4 minutes and then centrifuged, washed two times with ice cold water, and then analyzed for total nitrogen. 1000 ml. of the mixture was also chilled, centrifuged, washed two times with large volumes of cold distilled water, and used for the determination of the dry weight. The remaining 1250 ml. was shaken in two Florence flasks, 625 ml. in each flask. At the end of 75 minutes, the contents of the two flasks were poured together and samples taken as for the initial flask. Turbidity readings were taken at 15 minute intervals throughout the 75 minute period, ten readings being taken at each interval, the colorimeter being reset to zero after each individual reading. The values were averaged and this figure is shown below. The average error of the mean was ±2 per cent. Microscopic cell counts were carried out in Experiment I as described previously (11).

Time	Total RNA N per 1×10^{11} cells		Total protein N per 1×10^{11} cells		Total nitrogen per 1×10^{11} cells		Dry weight per 1×10^{11} cells		Total cell count		
									Turbidity	Microscopic	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1 and 2	Exp. 1	Exp. 2
min.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*			
0	0.130	0.126	0.83	0.73	1.44	1.5	13.3	14.2	1×10^{11}	1.05×10^{11}	—
75	0.184	0.181	1.25	1.05	2.11	2.2	20.4	20.9	1×10^{11}	9.6×10^{10}	—

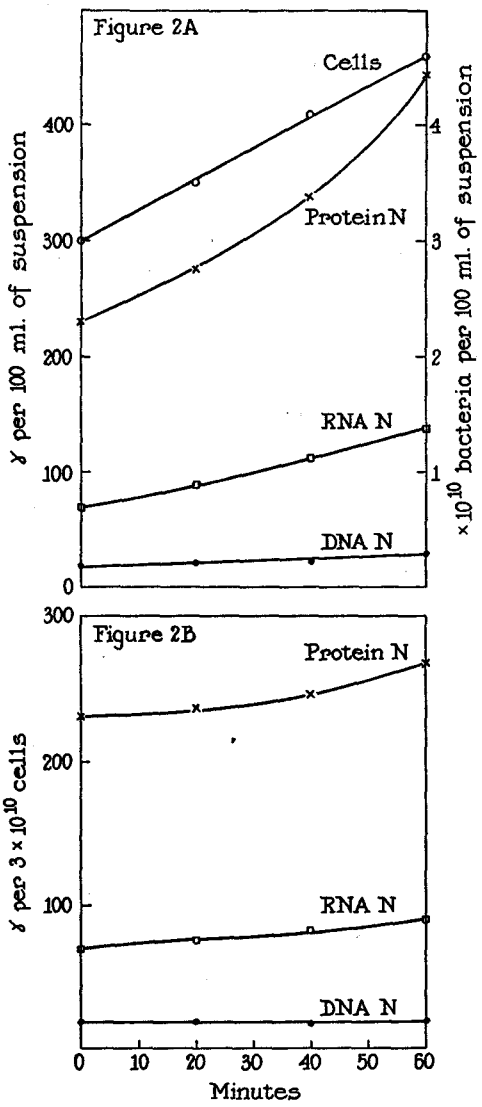
TABLE II

The Dry Weight per 1×10^{11} Resting Cells, Late Lag Phase Cells, and Log Phase Cells

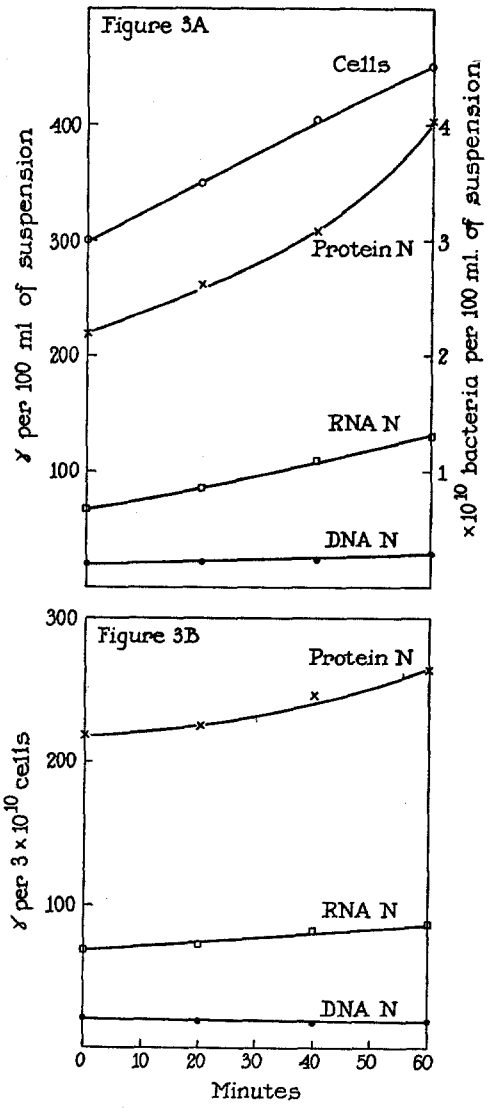
The figures taken for resting and late lag phase cells are from Table I. Log phase cells were prepared as described for Fig. 2.

Type of cells	Dry weight per 1×10^{11} cells	
	Exp. 1	Exp. 2
	mg.	*mg.*
Resting	13.3	14.2
Late lag phase	20.4	20.9
Log phase	21.0	21.2

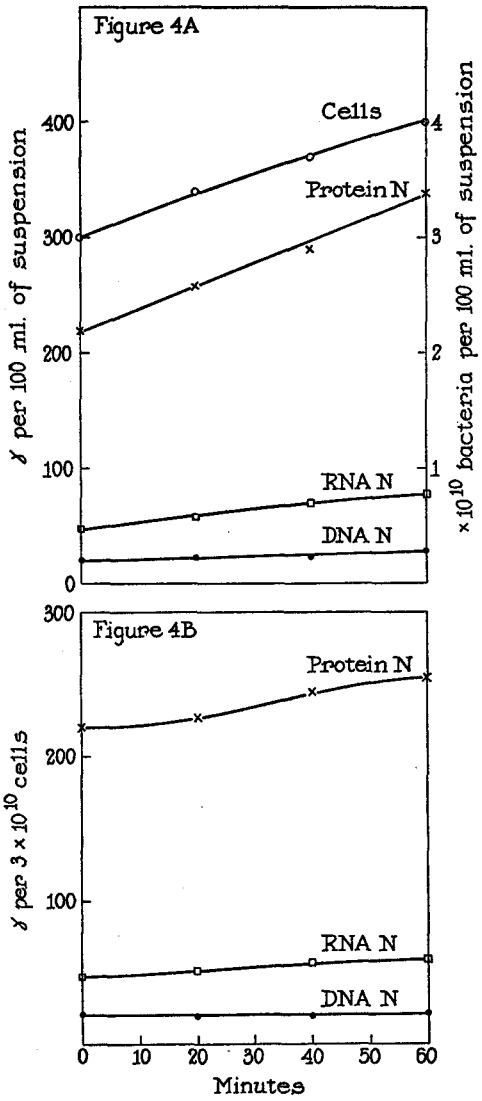
strain II, calculated per 100 ml. of suspension and per 3×10^{10} cells, with varying amounts of acid-hydrolyzed casein added to the medium. Figs. 6 A and 6 B show the synthesis of RNA N, DNA N, and protein N for strain V per 100 ml. of suspension and per 3×10^{10} cells during the early log phase.



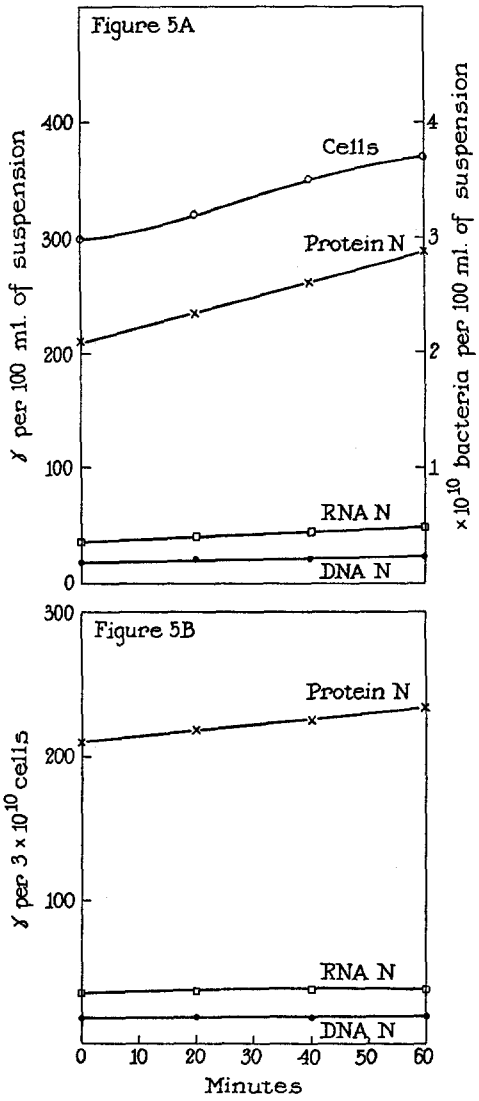
FIGS. 2 A and 2 B. The synthesis of RNA N, DNA N, and protein N of strain I during the early log phase. Strain I was prepared as described in *Methods*. A 1000 ml. Florence flask containing 700 ml. of Fildes' synthetic medium containing 0.03 ml. of 0.1 M $CaCl_2$ and 10 mg. of acid-hydrolyzed casein per 10.0 ml. of medium was inoculated with 1.3×10^8 cells per ml. When the cell count had reached 2.0×10^8 cells per ml., 150 ml. samples were removed and treated as described in *Methods*. Fig. 2 A shows the increase in RNA N, DNA N, and protein N per 100 ml. of suspension. Fig. 2 B shows the same result calculated per 3×10^{10} cells.



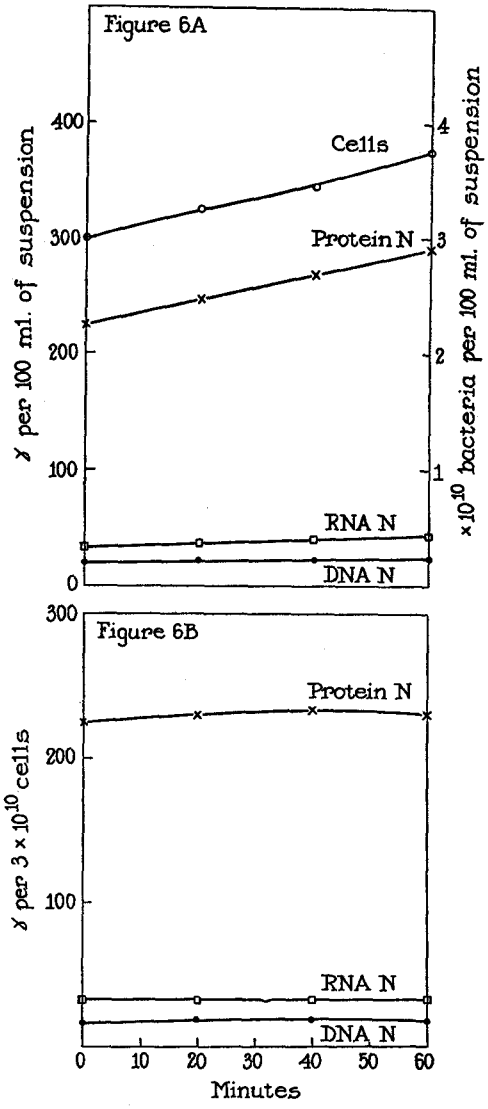
FIGS. 3 A and 3 B. The synthesis of RNA N, DNA N, and protein N of strain II during the early log phase with a high concentration of hydrolyzed casein added to the medium. This experiment was carried out exactly like that described in Fig. 2 except that strain II was used. Fig. 3 A shows the increase in RNA N, DNA N, and protein N per 100.0 ml. of suspension, while Fig. 3 B gives the same result calculated per 3×10^{10} cells.



FIGS. 4 A and 4 B. The synthesis of RNA N, DNA N, and protein N of strain II during the early log phase with a medium concentration of hydrolyzed casein added to the medium. This experiment was carried out exactly like that described in Fig. 2 except that strain II was used and only 20 γ of hydrolyzed casein was added to the medium per 10.0 ml. of solution. Fig. 4 A shows the increase in RNA N, DNA N, and protein N per 100.0 ml. of suspension, while Fig. 4 B gives the same result calculated per 3×10^{10} cells.



FIGS. 5 A and 5 B. The synthesis of RNA N, DNA N, and protein of strain II during the early log phase with a low concentration of hydrolyzed casein added to the medium. This experiment was carried out exactly like that described in Fig. 2 except that strain II was used and only 10 γ of hydrolyzed casein was added per 10.0 ml. of solution. Fig. 5 A shows the increase in RNA N, DNA N, and protein N per 100.0 ml. of suspension, while Fig. 5 B gives the same result calculated per 3×10^{10} cells.



FIGS. 6 A and 6 B. The synthesis of RNA N, DNA N, and protein N of strain V during the early log phase. This experiment was carried out exactly like the experiment described in Fig. 2 except that strain V was used. Fig. 6 A gives the increase in RNA N, DNA N, and protein N per 100.0 ml. of suspension, while Fig. 6 B shows the same result calculated per 3×10^{10} cells.

It may be seen that during this period of multiplication the RNA N, DNA N, and protein N content per cell are fairly constant for all strains studied, there being slight increases in the protein and RNA content per cell in Figs. 2 B, 3 B, and 4 B.

Table III illustrates that the RNA N/protein N ratio per cell is much higher for fast growing cells than for the slower growing cells. When

TABLE III

The Relationship between the RNA N/Protein N Ratio and the Division Time

The strain I values are shown in Fig. 2, the strain II values are shown in Figs. 3, 4, and 5, and the strain V values are given in Fig. 6.

Strain	γ Hydrolyzed casein per ml.	Division time	γ RNA N increase in 60 min. per		γ DNA N increase in 60 min. per		γ Protein N increase in 60 min. per		γ RNA N/γ protein N per 3×10^{10} cells	γ RNA N/γ protein N ratio per 3×10^{10} cells × division time
			100.0 ml. of suspension	3×10^{10} cells	100.0 ml. of suspension	3×10^{10} cells	100.0 ml. of suspension	3×10^{10} cells		
		hrs.								
I	1000	2.0	57	108	10	19	212	392	0.27	0.54
II	1000	2.0	58	116	9	18	193	386	0.30	0.60
II	2	3.0	26	78	7	21	118	354	0.22	0.66
II	1	4.0	12	60	4	20	74	370	0.16	0.64
V	1000	4.0	10	50	4	20	70	350	0.14	0.56

TABLE IV

RNA N Content of Resting Cells of S. muscae

Resting cells of various strains of *S. muscae* were prepared by growing cells as described in Figs. 2, 3, 5, and 6 until they had reached the stationary phase, which was about 2–3 × 10^9 cells per ml. RNA was then determined on aliquots from the four samples.

Strain	Division time	γ RNA N per 4×10^{10} cells
	hrs.	
I	2.0	37.1
II	2.0	35.8
II	4.0	34.3
V	4.0	35.9

this ratio is multiplied by the division time a fairly constant value is obtained. These calculations show that the ratio of RNA N/protein N is proportional to the growth rate. Although the RNA N content per cell depends upon the growth rate, the amount of DNA N per cell is fairly constant regardless of the growth rate (Table III).

Although log phase cells having different multiplication rates show great differences in the RNA N content per cell, resting cells of *S. muscae* have

essentially the same RNA N content irrespective of their prospective multiplication rate (Table IV).

RNA and Protein Synthesis in Cells Forming an Adaptive Enzyme.—Since the RNA and protein content continually increase during the log phase, it is difficult to determine whether there is any relationship between RNA and protein synthesis. A system was desired in which the cells could be taken from the log phase and put into fresh medium and show a lag period before

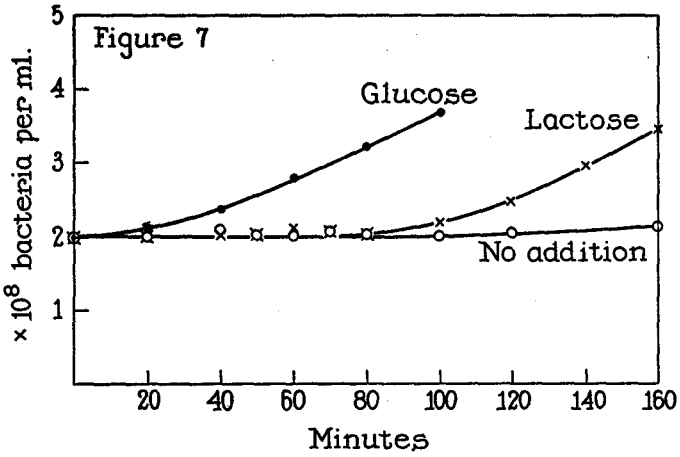


FIG. 7. The multiplication of cells grown in Fildes' synthetic medium containing glucose and resuspended in Fildes' medium containing lactose. 100 ml. of Fildes' synthetic medium was inoculated with enough cells of strain I, prepared as described in *Methods*, to give 7.0×10^7 cells per ml. After the cell count had reached 2.0×10^8 cells per ml., the cells were centrifuged and washed as described in *Methods* and made up to 75 ml. in Fildes' synthetic medium containing no sugar. Three 10.0 ml. portions were then pipetted into three separate test tubes. To tube A was added 20 mg. of glucose in a volume of 0.3 ml., to tube B was added 40.0 mg. of lactose in a volume of 0.3 ml., and tube C received 0.3 ml. of H_2O. All tubes were then shaken at 35°C. and colorimeter readings taken every 20 minutes.

beginning to multiply. The conditions of such a system could be met if the cells could be forced to form an adaptive enzyme before they began to divide. After several attempts, the following method was found satisfactory. The cells were grown in Fildes' synthetic medium containing glucose as its chief energy source until they were in the log phase. They were then centrifuged out, washed quickly with a large volume of warmed Fildes' synthetic medium containing no glucose, and then resuspended in Fildes' synthetic medium containing lactose but no glucose. Under these conditions the cells exhibited a lag period before beginning to multiply (Fig. 7), and manometric measurements confirmed the view that these cells had to adapt to lactose. Fig. 8

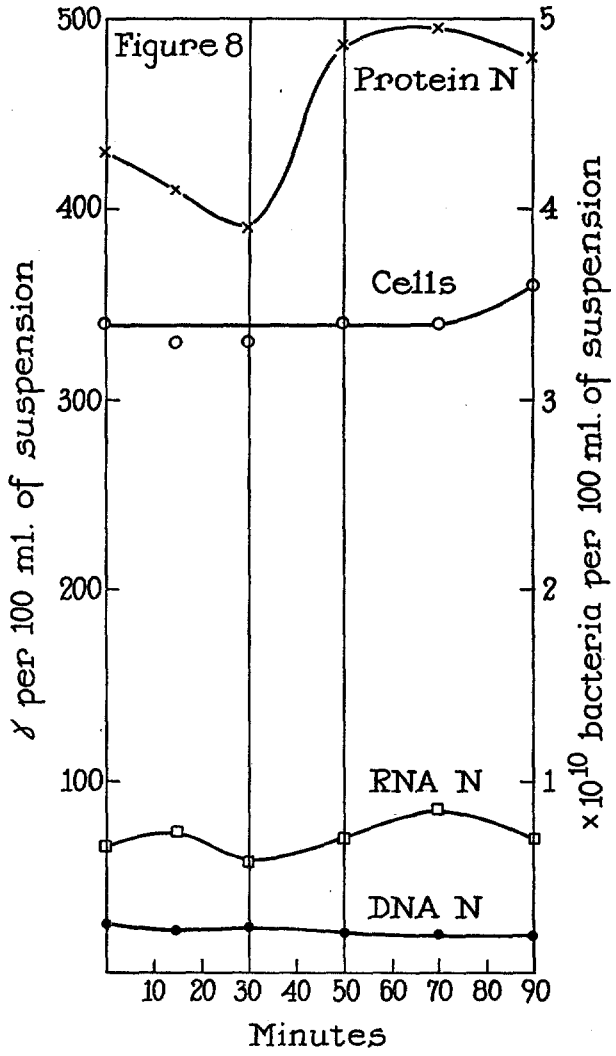


FIG. 8. The synthesis of ribonucleic acid N, protein N, and desoxyribonucleic acid N, in cells adapting to lactose. The cells were grown in Fildes' synthetic medium containing glucose as described in *Methods*. When the cell count had reached about 2.0 × 10^8 cells per ml., the cells were centrifuged and washed as described in *Methods*, and resuspended in Fildes' synthetic medium containing 40 mg. of lactose per 10.0 ml. instead of glucose and the mixture than shaken in Florence flasks. 100 ml. samples were removed, every 10 minutes, and analyzed for ribonucleic acid N, desoxyribonucleic acid N, and protein N as described in *Methods*. Turbidity readings were taken at the intervals shown in the figure.

shows that in such cells there is never an increase in protein unless it is accompanied by a simultaneous increase in ribonucleic acid, although there may be an increase in ribonucleic acid without a corresponding increase in protein. Nine other experiments yielded similar results.

TABLE V

The Synthesis of Protein N and Ribonucleic Acid N in Adaptive Cells

The increase in ribonucleic acid N and protein N shown below was compiled from ten experiments carried out as described in Fig. 8. At the same time the increase in protein N occurred, there was also a simultaneous increase in the ribonucleic acid N. The figures given below show the increase in RNA N which occurred at the same time the increase in protein N was observed. In most of the experiments the ratio of RNA N formed to protein N synthesized was around 0.15.

Experiment No.	γ Protein N	γ RNA N	$\frac{\gamma \text{ RNA N}}{\gamma \text{ Protein N}}$
1	22	9.1	0.27
	63	6.7	0.10
2	48	6.0	0.12
3	96	10.5	0.10
	117	17.7	0.15
4	24	2.7	0.11
	24	6.0	0.25
5	69	12.6	0.18
	33	4.0	0.12
6	71	12.1	0.17
	56	7.5	0.13
7	83	12.1	0.14
8	28	5.4	0.19
	58	7.6	0.13
9	82	14.1	0.17
10	51	7.3	0.14
	61	10.2	0.16

Table V shows the increase in protein N and RNA N found in ten experiments for cells adapting to lactose. All values are for a 10 minute period during the lag period as described in Fig. 8. In all ten experiments, the increase in protein occurred simultaneously with an increase in RNA. The RNA N/

protein N ratio is about 0.15 during these 10 minute intervals. Although the increases on the basis of N do not seem to be very large, they are fairly large in terms of RNA and protein and outside experimental error. It is felt that this fact together with the approximately constant ratio observed makes these values significant.

From Fig. 8, it may be seen that while the cells are adapting to lactose, there may be a loss of RNA during one or more of the 10 minute periods. Further analysis of this reaction shows that there is a loss of RNA as determined by purine-pyrimidine content or ribose estimation (Table VI). Although the purines-pyrimidines are recovered in the cold 5 per cent trichloroacetic acid fraction, there is a total loss of ribose from the system which cannot be

TABLE VI

The Loss of RNA in Cells Adapting to Lactose

The experiment was carried out as described in Fig. 8. However, instead of taking the sample and pouring it into 5 per cent trichloroacetic acid (TCA), it was centrifuged and washed with cold distilled water as described in *Methods*. The 5 per cent trichloroacetic acid fraction was prepared and the determination of purines-pyrimidines, pentose, and RNA was carried out on the various fractions as described in *Methods*. RNA was used as a standard in all cases as described in *Methods*.

Time	γ RNA as determined by		Cold 5 per cent TCA fraction	
	Purines-pyrimidines	Pentose	γ Purines-pyrimidines	γ Pentose
min.				
70	548	561	181	163
80	449	471	240	169

recovered in the cold 5 per cent trichloroacetic acid fraction or in the medium. The loss of pentose from the RNA fraction in *S. aureus* has been reported previously by Krampitz and Werkman who presented evidence that it was oxidized to acetic acid, carbon dioxide, and water (7).

DISCUSSION

Due mainly to the work of Brachet (21), and of Caspersson and his coworkers (3), it has been widely accepted that the synthesis of ribonucleic acid is involved in protein synthesis. All the experiments of Caspersson upon which the above hypothesis is based were carried out by examining different individual cells under different physiological conditions using an ultraviolet microspectroscopic method. The assumption is made in this procedure that the ultraviolet adsorption reading at 2600A represents the ribonucleic content of the cell. However, there is no chemical data to substantiate this assumption, nor is there anything to indicate that he is not simply measuring purine-pyrimidine synthesis. There is also some question from the data Caspersson

has presented whether he is measuring an increase in protein or merely a synthesis of tryptophane, tyrosine, and phenylalanine. In any case, according to Caspersson's interpretation of his data, cells which are actively synthesizing protein show a high ribonucleic acid content; *i.e.*, give a high reading at 2600A in the ultraviolet. Resting cells, however, give a low reading at 2600 A in the ultraviolet. Brachet, on the other hand, used a combination of enzymatic and staining methods to study the relationship between ribonucleic acid and protein synthesis (22). He found that cells producing a large amount of protein also contained high concentrations of ribonucleic acid. A critical evaluation of these results has been given by Brachet (22). It is obvious from the results of both Caspersson and Brachet that, assuming their measurements determine ribonucleic acid and protein synthesis, it is not possible to say whether ribonucleic acid is necessary for protein synthesis or *vice versa* or, indeed, whether one is necessary for the synthesis of the other.

Recent experiments in which ribonucleic acid and protein were determined by chemical analysis have not supported Caspersson's hypothesis that ribonucleic acid synthesis is involved in protein synthesis. Thus it has been found that yeast cells will synthesize large amounts of protein from ammonia without any increase in ribonucleic acid, although the ribonucleic acid fraction showed a high turnover rate (8). Abrams has reported that it is possible, using x-rays, to reduce greatly the increase of ribonucleic acid and also the turnover rate of this substance with little effect on the protein synthesis (9). However, this investigator called the incorporation of glycine into protein "protein synthesis" and this may not necessarily be correct. In *E. coli* B phage infected cells, virus protein is synthesized without any increase or turnover in the ribonucleic acid of the infected cells (10).

In *S. muscae* cells the relationship between protein and ribonucleic acid and synthesis described in this paper may be summarized as follows:—

(*a*) When resting cells are resuspended in fresh medium, the increase in ribonucleic acid and protein begins shortly after the cells are resuspended and closely parallels each other, in contrast to desoxyribonucleic acid synthesis which starts about 45 minutes after the former two substances have begun to be synthesized.[3]

(*b*) While the cells are forming an adaptive enzyme for lactose utilization, there is never an increase in protein without a simultaneous increase in ribonucleic acid, the RNA N/protein N ratio during these intervals being about 0.15.

[3] When the first few samples are taken at a shorter time interval than that shown in Fig. 1, the RNA begins to increase shortly before the protein. The sequence, therefore, is RNA synthesis closely followed by an increase in protein which parallels the continuing RNA increase, with DNA synthesis occurring at a later interval. If there is a relationship between RNA and protein synthesis, these experiments would indicate that RNA may be necessary for protein synthesis and not *vice versa*.

(*c*) The RNA N/protein N ratio is proportional to the growth rate of the bacterial cell.

The above three results may indicate that there is a relationship between protein synthesis and ribonucleic acid synthesis under these experimental conditions.

While *S. muscae* cells are adapting to lactose, there is a loss of ribonucleic acid. The purines and pyrimidines can be recovered in the cold 5 per cent trichloroacetic acid fraction, although the pentose component is completely lost from the system. These results would indicate that the ribonucleic acid is eventually split to nucleosides, with the nucleosides being split to free bases and pentose by nucleosidases (20). The pentose may then be oxidized as described by Krampitz and Werkman in *Staphylococcus aureus* (7).

Methods

Determination of Bacteria.—Bacteria were determined turbidimetrically and microscopically as described previously (11).

Preparation of Bacteria.—Strains I and II were grown on agar slants as described previously (6). After growing for 20 hours on the agar slants at 35°C., the cells were washed off with veal infusion. In the case of strain I, 500 ml. of veal infusion, containing 0.2 per cent peptone in a 1000 ml. Florence flask, was inoculated with enough cells to give 1.0×10^6 cells per ml. The Florence flask was then shaken at about 100 oscillations per minute, so as to insure thorough aeration, for 20 hours at 34–35°C. The cells at this time had increased to about 3×10^9 cells per ml. and were in the resting phase. The bacteria were then centrifuged, washed twice with 50.0 ml. of warmed Fildes' synthetic medium (12), and then resuspended in the appropriate amount of Fildes' synthetic medium, containing 10 mg. of hydrolyzed casein (6) per 10.0 ml., to give about $1.0–1.5 \times 10^8$ cells per ml. The bacterial suspension was then shaken in a 1000 ml. Florence flask at 34–35°C. at about 100 oscillations per minute. Strain V was grown in a similar manner.

The Adaptation of Cells to Lactose.—Cells were grown in Fildes' synthetic medium, containing glucose, as described above. However, the initial cell count was about 7×10^7 cells per ml. The mixture was shaken in 1000 ml. Florence flasks until the count had reached about 2×10^8 cells per ml. The cells were then centrifuged out and the tubes carefully drained and excess fluid wiped out with filter paper. The cells were then washed rapidly in 70 ml. of Fildes' synthetic medium (35°C.) which contained no glucose and again centrifuged and the tubes allowed to drain as described above. The cells were then made up quickly in Fildes' synthetic medium (35°C.) containing 40 mg. of purified lactose per 10.0 ml. of medium but no glucose. The mixture was then shaken in 1000 ml. Florence flasks at 35°C. and 100 ml. samples taken for analysis every 10 minutes.

Preparation of Experimental Samples.—Unless otherwise stated, the samples, usually 100 to 150.0 ml., were taken at 10 minute intervals and poured into 500 ml. Erlenmeyer flasks containing 10 to 15 ml. of 50 per cent trichloroacetic acid (TCA). The samples were thoroughly mixed, chilled in an ice bath, and put in the refrigerator overnight. The next day the samples were centrifuged and resuspended in 5.0 ml. of cold 5 per cent TCA and ground with a mechanical grinder as described

previously (1), except that no alundum was added. The cells were centrifuged and again resuspended in 5.0 ml. of cold 5 per cent TCA and ground as above. After centrifuging and washing (1), the residue was extracted two times with 1.5 ml. of 5 per cent TCA at 90°C. for 15 minutes as described previously (1) in order to extract the nucleic acids, allowing the mixture to sit 1 hour in an ice bath after each extraction. The residue from the hot TCA extractions was washed once with 4.0 ml. of cold 5 per cent TCA and then analyzed to determine the protein content of the cell. The amount of nitrogen soluble in a hot alcohol-ether mixture (3:1) was only about 5 per cent of the total nitrogen. As this amount of nitrogen was far too small to be of significance in any of the determinations, the cells were not extracted with alcohol-ether, in the experiments described in this paper.

In some experiments, it was necessary to determine the substances soluble in the cold 5 per cent TCA fraction. In order to do this, the bacterial sample when first taken was chilled in a salt–cracked ice mixture for 2 minutes and then centrifuged for 7 minutes at 2000 × *g* at about 10°C. The supernatant fluid was poured off and the tube carefully drained and the cells washed in 50.0 ml. of ice cold distilled water and again centrifuged as above. The supernatant was poured off, the tube drained carefully, and the cells were then suspended in 4.0 ml. of cold 5 per cent TCA and ground twice as described above. The two supernatants were combined to give the cold 5 per cent TCA fraction, while the residue was treated as above to prepare the nucleic acid and protein fractions.

Determination of Ribonucleic Acid and Desoxyribonucleic Acid.—Ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) were determined as described previously (1). The average error of the mean (1) based on eight analyses was ±2.0 per cent. The amount of DNA found with Dische's reagent agreed to within ±3 per cent of the amount determined by either Cohen's method (13) or Stumpf's procedure (14). In determining DNA, about four times as much of the sample was taken as that used for the determination of RNA and protein nitrogen, as the DNA methods were about four times less sensitive than the two latter methods in our hands. In two experiments, the amount of RNA, DNA, and protein found with Schneider's fractionation procedure (15), which was the method used in this work, agreed to within ±6 per cent of the RNA, DNA, and protein values found when the Schmidt and Tannhauser fractionation procedure (16) was used. The amount of RNA and DNA found by the above two procedures also agreed to within ±9 per cent of the values determined by the ultraviolet adsorption reading at 2600A(1).

Determination of Nucleic Acid by the Ultraviolet Adsorption Measurement at 2600A.—In determining nucleic acid in the Beckman ultraviolet spectrophotometer, the same method was used as described previously (1). As the TCA gives a slight reading at 2600A under the conditions employed, it is essential to run a blank containing the appropriate concentration of TCA alongside the experimental samples throughout the entire procedure. The nucleic acid samples were usually diluted about 1:10 with distilled water before being read as was the 5 per cent cold TCA fraction. Both fractions showed an ultraviolet adsorption peak at 2600A.

Protein Determination.—The nitrogen in the protein fraction was determined by Nessler reagent as described by Umbreit *et al.* (17) after being digested for 17 hours. Instead of carrying out the digestion in a sand bath, however, the samples were put in a rack on a hot plate (Acme Electric Heating Co., 110 volts, 1000 watts, type

1734-9) and digested for 17 hours at medium heat. Three drops of 30 per cent H_2O_2 were then added and the sample heated for 10 minutes as described above. The sample was cooled and 2 drops of 30 per cent H_2O_2 again added and the sample heated for 10 minutes again. This procedure was followed once more except that the heating was carried out for 20 minutes. This procedure insured complete digestion of the sample and longer digestion gave the same nitrogen results. After the complete digestion had been carried out, the sample was made up to 5.0 ml. with water in Klett-Summerson colorimeter tubes and 1.0 ml. taken for the determination. The values determined by this method agreed to within ±3 per cent of those found when similar nitrogen samples were analyzed by the micro-Kjeldahl method. The average error of the mean for the Nessler method, based on eight analyses, was ±2.0 per cent.

It should be mentioned that great care must be taken in preparing the protein sample for analysis, particularly in regard to the centrifuging. The sample must be centrifuged twice, 20 minutes each time, at 2000 × *g* to insure a good recovery during *each* of the various stages used in preparing the protein sample. As the film of cells forms on top of the TCA fluid after the first centrifuging in any single stage of the procedure, the tube should be shaken very gently and then recentrifuged.

SUMMARY

1. The synthesis of ribonucleic acid, desoxyribonucleic acid, and protein in *S. muscae* has been studied: (*a*) during the lag phase, (*b*) during the early log phase, and (*c*) while the cells are forming an adaptive enzyme for lactose utilization.

2. During the lag phase there may be a 60 per cent increase in ribonucleic acid and protein and a 50 per cent increase in dry weight without a change in cell count, as determined microscopically, or an increase in turbidity.

3. During this period, the increase in protein closely parallels the increase in ribonucleic acid, in contrast to desoxyribonucleic acid, which begins to be synthesized about 45 minutes after the protein and ribonucleic acid have begun to increase.

4. The RNA N/protein N ratio is proportional to the growth rate of all *S. muscae* strains studied.

5. While the RNA content per cell during the early log phase depends upon the growth rate, the DNA content per cell is fairly constant irrespective of the growth rate of the cell.

6. Resting cells of *S. muscae* have approximately the same RNA content per cell irrespective of their prospective growth rate.

7. While the cells are adapting to lactose, during which time there is little or no cellular division, there is never an increase of protein without a simultaneous increase in ribonucleic acid, the RNA N/protein N ratio during these intervals being approximately 0.15.

8. Lactose-adapting cells show a loss of ribonucleic acid. The purines-pyrimidines of the ribonucleic acid can be recovered in the cold 5 per cent

trichloroacetic acid fraction, but the ribose component is completely lost from the system.

9. The significance of these results is discussed in relation to the importance of ribonucleic acid for protein synthesis.

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