THE METABOLISM OF RIBONUCLEIC ACID IN NORMAL AND BACTERIOPHAGE INFECTED *ESCHERICHIA COLI*¹

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Cohen (1948a) has reported that during the multiplication of the bacteriophages T2r⁺ and T4r⁺ in *Escherichia coli*, strain B, the ribonucleic acid content of the host remains constant. Similar observations were made by Kozloff *et al.* (1951) who investigated the origin of the nitrogen of T6r⁺ multiplying in the same host. Cohen (1948b) also examined the possibility that the ribonucleic acid was undergoing turnover during T2r⁺ multiplication. Using the incorporation of inorganic P³² into the ribonucleic acid as an indicator of such a turnover, he found that almost no P³² was incorporated into the ribonucleic acid. In a recent paper by Koch *et al.* (1952) these authors state in a footnote that "purines, like phosphorus, do not turn over in the RNA of the infected cell".

The apparent inertness of the ribonucleic acid of the infected cell might be explained in several ways: (1) It is possible that in the growing host the enzymatic pathway leading from precursors such as glycine and inorganic phosphate to ribonucleic acid is a reversible one and accordingly in a state of dynamic equilibrium. If this is the case, isotopic precursors could be incorporated into the ribonucleic acid without a net increase of the ribonucleic acid. Incorporation of isotope by such a mechanism could be termed "incorporation by exchange". To explain the lack of incorporation of isotope into the ribonucleic acid during phage infection, one would have to postulate that there is an inhibition during phage infection of one or more of the enzymes in this pathway, preventing incorporation of isotope into the ribonucleic acid. (2) Synthesis and degradation of the ribonucleic acid of the growing cell could be taking place by different enzymatic pathways, both essentially irreversible. In this case, isotope could be incorporated into the

ribonucleic acid only when there was a net synthesis of ribonucleic acid. Therefore, during phage infection there would have to be an inhibition of both pathways, in order to obtain the results that were quoted above. (3) Since the ribonucleic acid increases during growth, there must be a pathway for net synthesis. There may not be, however, an *in vivo* pathway for ribonucleic acid degradation. That is, the ribonucleic acid of the growing culture may *not* be in a dynamic equilibrium with its environment and may not turn over. Under these conditions isotopic precursors could be incorporated into the ribonucleic acid only when there is a net synthesis of ribonucleic acid. Once incorporated, the isotope would remain in the ribonucleic acid until the cell is destroyed by lysis. If this were taking place in the growing cell, then during phage infection of such a cell, when there is no synthesis of ribonucleic acid, there would be no incorporation of isotopic precursors into the ribonucleic acid and also no loss of isotope already incorporated.

This paper presents data obtained from experiments designed to differentiate between these three possibilities. In these experiments, the total ribonucleic acid of a growing culture is considered as consisting of two parts, the original ribonucleic acid that is present at the beginning of the incubation and the newly synthesized ribonucleic acid that is formed during the incubation. Incorporation of an isotopic precursor into the original ribonucleic acid would indicate that there are enzymatic pathways for both the synthesis and degradation of ribonucleic acid. Incorporation of isotope only into the newly synthesized ribonucleic acid would suggest that there is no mechanism for the degradation of ribonucleic acid but only its synthesis.

This study indicates that the renewal of the original ribonucleic acid of a growing culture of *E. coli* is extremely slow, if it occurs at all. Data are presented which indicate that ribonuclease, which can be extracted from the cells,

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is not acting on the endogenous ribonucleic acid of the growing or infected host. Therefore it is suggested that the inertness of the ribonucleic acid of the infected host supporting phage multiplication can be explained by the fact that *de novo* synthesis of ribonucleic acid has been prevented. Thus, during phage multiplication there is no degradation of the endogenous ribonucleic acid, no incorporation of isotopic precursors into the ribonucleic acid, and no loss of incorporated isotope from the ribonucleic acid.

MATERIALS AND METHODS

Since several investigators (Badger and Krampitz, 1950; Sutton *et al.*, 1951; Edmonds *et al.*, 1952) have shown that glycine is a precursor of bacterial purines, glycine-2- C^{14} incorporation into the adenylic and guanylic acids of the ribonucleic acid was used as an indicator of ribonucleic acid metabolism. In order to obtain the most efficient utilization of glycine-2- C^{14} , the host organism used was a glycineless mutant of *E. coli*, strain B. This organism, *E. coli*, strain B G1-5, was obtained by the method of Davis (1948). The organism was maintained on nutrient agar slants. Transfers were made biweekly. The basal synthetic medium was the basal synthetic 8 medium described by Spizizen *et al.* (1951) to which was added 0.05 g L-tryptophan per liter. The mutant grew in the basal synthetic medium plus 25 μ g of glycine per ml as well and as rapidly as *E. coli*, strain B, did in basal synthetic medium.

Stocks of bacteriophage T4r were prepared by infecting a growing culture of *E. coli*, strain B, in basal synthetic medium under conditions of aeration until complete lysis was observed. Ten L aliquots of the completely lysed culture were passed through a Sharples ultracentrifuge (bowl speed 50,000 rpm). The residue was resuspended in basal synthetic medium (lacking glucose), centrifuged at 5,000 rpm for 10 minutes to remove debris, yielding 25 ml of phage suspension assaying 2 to 5×10^{12} particles per ml. Such stocks did not decrease in titer appreciably when stored at 3 to 5 C for several weeks.

E. coli, strain B G1-5, was grown in basal synthetic medium plus 25 μ g of glycine per ml with aeration at 37 C. Cells to be infected with bacteriophage were harvested from a 9 hour culture, grown from a one per cent inoculum of an overnight culture. Such cells, still in the logarithmic

phase of growth, were suspended in one liter quantities of basal synthetic medium plus 25 μ g of glycine per ml at a concentration of 3×10^9 organisms per ml. Infected and control cells were incubated at 27 C with aeration for the indicated times. To stop the reaction, the cell suspension was poured with stirring into two volumes of acetone (precooled to -20 C) and refrigerated overnight. The precipitate was filtered through Whatman no. 54 paper and washed with acetone. The acetone powder then was dried *in vacuo*.

The acetone powders were suspended in 25 ml water and dialyzed as indicated in subsequent sections. The nondialyzable portion was adjusted to pH 13 and incubated overnight at 37 C. After incubation the pH was adjusted to 2 with HCl. The precipitate, containing desoxyribonucleic acid, was removed by centrifugation. The supernatant was adjusted to pH 3.5 to 4.0, and the precipitate which occasionally formed was removed by centrifugation. The pH was raised then to 8.2. A Ba fractionation was carried out according to Umbreit *et al.* (1945). The ribonucleotides were found in the water-soluble, alcohol-insoluble fraction. The Ba salts were extracted with water several times. A 75 per cent yield (calculated by comparing the amount of ultraviolet absorbing material present in the extract to the amounts of ultraviolet absorbing material in solution before Ba precipitation) of ultraviolet absorbing materials was obtained in this step. Ba ions were removed as $BaSO_4$. The solution of ribonucleotides, adjusted to pH 9 to 10, was adsorbed onto an anion exchange resin column, "dowex-1", 200 to 500 mesh in the SO_4^{--} form, 10 cm deep by 2.0 cm² in cross section. The individual nucleotides were eluted according to Cohn (1950), using 0.002 N H_2SO_4 , 0.003 N H_2SO_4 , and 0.005 N H_2SO_4 . Each nucleotide was individually recycled on a second column. The eluates were collected in 10 ml fractions by means of an automatic fraction collector.

The ultraviolet absorption spectra of the nucleotides obtained in this manner agreed well with authentic spectra. All spectra were determined in a Beckman spectrophotometer, model DU. The concentration of the nucleotides in the eluates was determined spectrophotometrically. For specific activity determinations, a neutralized aliquot of an eluate was dried on a

steel planchet under an infrared lamp. No more than 500 μ g total solids were placed on each planchet, thus no corrections were made for self-absorption. All specific activity determinations reported in the tables are averages of at least 4 independent determinations.

RESULTS

Sensitivity of E. coli, strain B G1-5, to bacteriophage T4r. The characteristics of the one step growth curve of bacteriophage T4r with *E. coli*, strain B, and with *E. coli*, strain B G1-5, were examined in nutrient broth containing 0.5 per cent NaCl and in synthetic media at two temperatures. The methods used in carrying out this study were those described by Adams (1950).

in media which support growth of the host is similar with the two organisms.

The incorporation of glycine-2-C¹⁴ into E. coli during growth. An experiment was performed to determine the incorporation of glycine-2-C¹⁴ into *E. coli*, strain B, and *E. coli*, strain B G1-5, during growth. The flasks containing the growing cultures were placed in aeration trains which permitted the collection of the respiratory CO₂ from each culture during growth. Flask 1 contained 200 ml basal synthetic medium. Flask 2 contained 200 ml basal synthetic medium plus 25 μ g of glycine per ml. To each flask were added 200 μ g glycine-2-C¹⁴ containing 200,000 cts per min. Flask 1 was inoculated with a one per cent inoculum of a 24 hour culture of strain

TABLE 1

Sensitivity of Escherichia coli, strains B and B G1-5, to bacteriophage T4r

ORGANISM	MEDIUM	TEMPERATURE OF INCUBATION	ADSORPTION*	LATENT PERIOD	BURST SIZE
			per cent	min	
<i>E. coli</i> , strain B	Nutrient broth	37	80	23	121
	Basal synthetic medium	37	88	25	77
	Basal synthetic medium	27	85	60	73
<i>E. coli</i> , strain B G1-5	Nutrient broth	37	85	25	153
	Basal synthetic medium	37	67	40-60	39
	Basal synthetic medium + glycine†	37	69	25	144
	Basal synthetic medium + glycine†	27	65	50	162

* Adsorption was for 5 minutes at 37 C and 10 minutes at 27 C.

† The glycine concentration was 25 μ g per ml.

Rabbit antisera to T4r were used in determining the per cent adsorption in a given time. The data obtained are shown in table 1. These studies were carried out under conditions of single infection. The r strain of phage was used to avoid lysis inhibition when experiments were carried out under conditions of multiple infection.

The data show that the multiplication of bacteriophage T4r in *E. coli*, strain B G1-5, is very similar to that in *E. coli*, strain B, in nutrient broth at 37 C. In basal synthetic medium, the mutant organism cannot grow, yet it did give rise to a small burst with a delayed latent period. Whether this indicates that the mutant can support phage multiplication in the absence of glycine, or whether this indicates an intracellular pool of glycine is not known at the present time. It is clear that T4r multiplication

B; flask 2, similarly with strain B G1-5. The cultures were grown for 9 hours at 37 C. The cells then were harvested by centrifugation, washed 4 times with 10 ml each of 0.001 M glycine, then dried *in vacuo* over phosphorous pentoxide. Samples of the dried cells of each strain were oxidized to CO₂ using the oxidizing reagent of Van Slyke and Folch (1940). The CO₂ was converted to BaCO₃ and the radioactivity determined. The radioactivity of the respiratory CO₂ was determined in a similar manner. The results obtained are reported in table 2. The data show that the mutant incorporated twice as much C¹⁴ per mg dry weight of organisms as the parent strain.

Metabolism of ribonucleic acid in E. coli. In these experiments the metabolism of the ribo-

nucleic acid in uninfected and T4r infected cells was compared.

In the first set of experiments, the nucleic acids of the host were labeled by growing the organism in the presence of glycine-2- C^{14} . Three liters of medium containing 27.7 μ g glycine per ml with a specific activity of 1,600 cts per min per μ M were inoculated with strain B G1-5 and grown as already indicated. The cells were harvested by centrifugation and washed 4 times with 0.001 M glycine. The cells were suspended in 18 ml basal synthetic medium. Five ml of the radioactive cell suspension were added to 50 ml acetone at -20°C and allowed to stand overnight in the cold (I). Six and one-half ml of the radioactive cell suspension were added to each of

and the specific activity of each was determined. One sample of guanylic acid and two of adenylic acid were hydrolyzed by refluxing in 1 N H_2SO_4 for two hours. The adenine and guanine were isolated on "dowex-1" anion exchange resin according to Cohn (1950). The specific activities of the various fractions are presented in table 3.

No significant amounts of radioactivity were found in the pyrimidine nucleotides; thus, glycine does not seem to be a precursor of the pyrimidine ring or of the ribose. Glycine recently has been reported not to be a precursor of uracil in yeast (Edmonds *et al.*, 1952). The specific activity of the adenine and guanine was the same as that of the nucleotide from which it

TABLE 2

The uptake of glycine-2- C^{14} by growing cells of Escherichia coli, strains B and B G1-5

ORGANISM	CELLS*	RESPIRATORY CO_2 †
<i>E. coli</i> , strain B	1,170	2,700
<i>E. coli</i> , strain B G1-5	2,296	430

The total activity added to each flask was 200,000 cts/min.

* Activity expressed as cts/min/mg dry cells.

† Total activity as cts/min.

two flasks (II and III) containing 900 ml basal synthetic medium plus 25 μ g glycine per ml. To flask II were added 100 ml basal synthetic medium, and to flask III were added 100 ml of a T4r preparation suspended in basal synthetic medium. These two flasks were aerated at room temperature (27 to 28°C) for 60 minutes. At this time acetone powders were made as indicated. The initial cell concentration in flasks II and III was 3×10^9 bacteria per ml, and the phage concentration in flask III was such that the ratio of phage/bacteria was 4. The number of viable bacteria in each flask during the 60 minute interval was determined by taking samples every 5 minutes and plating appropriate dilutions on nutrient agar plates.

Since the acetone powders of II and III contained most of the salts that had been in the growth medium, they were suspended in 25 ml water and dialyzed against a total of 2,200 ml of water each at 3 to 5°C for 36 hours. The ribonucleic acid was degraded to nucleotides,

TABLE 3

The specific activities of the nucleotides obtained from uninfected and phage infected radioactive cells incubated in a nonradioactive medium

	ZERO TIME CONTROL I	INCUBATED CONTROL II	T4r IN- FECTED III
Cytidylic acid.....	9	-0.4	-0.4
Uridylic acid.....	1	0.6	0.6
Adenylic acid.....	1,500	800	1,340
Guanylic acid.....	1,590	796	1,270
Adenine.....	—	828	1,297
Guanine.....	—	811	—

Values in the table are cts/min/ μ M. Standard error = ± 5 per cent.

was derived, indicating that glycine is also not a significant precursor of purine bound ribose.

From the viable counts that were obtained in experiments II and III, survival curves were drawn (figure 1). Since a cell infected by a phage particle is no longer viable, the curve from experiment III indicated the number of uninfected bacteria throughout the incubation. By integrating the two curves, the number of uninfected bacterial minutes in each experiment was estimated. Since there was no phage added to II, the observed decrease in specific activity of the adenylic and guanylic acids was characteristic of the number of uninfected bacterial minutes that was found from the survival curve. Since the number of uninfected bacterial minutes in III was a fraction of that in II, the specific activity of the adenylic and guanylic acids of III should decrease by this same fraction, i.e., the specific activity of the adenylic and

guanylic acids could decrease to 1,238 and 1,294 cts per min per μM , if the only change occurring in the ribonucleic acid of the whole culture was the *de novo* synthesis of ribonucleic acid in uninfected cells. Since the observed specific activities (table 3) approached these values closely, it was concluded that the adenylic and guanylic acids, and consequently the

Figure 1. Survival curves obtained from the viable cell determinations.

ribonucleic acid of infected cells, were not being renewed during phage multiplication. This confirms the observations made by Koch *et al.* (1952) who used labeled adenine as a tracer, and Cohen (1948b) who used P^{32} .

A similar conclusion was drawn from experiments done in the opposite way. The strain B G1-5 cells were grown in a nonradioactive medium, harvested, and suspended in one liter aliquots of basal synthetic medium to a concentration of 3×10^9 bacteria per ml. Glycine-2- C^{14} was added at zero time to a final concentration of 26.9 μg per ml (specific activity, 2,710 cts per min per μM). Experiment IV was the uninfected control; to experiment V phage

was added to give a multiplicity of 4, and to experiment VI T4r was added to give a multiplicity of 15. The flasks were incubated for 50 minutes at room temperature with aeration. Acetone powders were made. Each acetone powder was suspended in 25 ml H_2O and dialyzed for 24 hours against one liter of 0.001 M glycine, then against two changes of one liter each of water for 24 hours. The specific activities of the nucleotides are reported in table 4. The specific activity that should have been found if glycine-2- C^{14} was incorporated only into the ribonucleic acid of the uninfected bacteria in V and VI also is included. These data again suggest that there was only a slight renewal of the

TABLE 4

The specific activities of the purine nucleotides obtained from uninfected and phage infected cells incubated in a radioactive medium

EXPERIMENT NO.	ADENYLIC ACID		GUANYLIC ACID	
	Calculated	Found	Calculated	Found
IV	—	1,255	—	1,279
V	470	575	476	538
VI	122	213	123	133

Values in the table are in cts/min/ μM .

ribonucleic acid in infected cells. There seem to be none in the guanylic acid fraction of VI and a little in the adenylic acid. The significance of these differences is somewhat in doubt due to the small number of uninfected cells present during the incubation (figure 1) and consequently to the small amount of radioactivity found in these fractions. It can be safely concluded that either there is very slight renewal of the ribonucleic acid or none at all in the infected organism.

It was possible to estimate the turnover of ribonucleic acid in uninfected growing cells from the data available in experiments I, II, and IV (tables 3 and 4). In experiment I the specific activity of the glycine in the growth medium was 1,600 cts per min per μM . The specific activities of the isolated adenylic and guanylic acids in experiment I approach this value closely. If this is true for experiment I, it should be true also for experiments II and IV since in each case cells in the exponential phase of growth are being examined. Therefore, the

adenylic and guanylic acids of any newly synthesized ribonucleic acid should have the specific activity of the glycine in the medium. Assuming that the change in the specific activity of the adenylic and guanylic acids is due only to newly synthesized ribonucleic acid having the specific activity of the glycine in the medium, it is possible to calculate a theoretical specific activity for the nucleotides of the total ribonucleic acid. The difference between this and the observed value should be a measure of the rate of renewal of the ribonucleic acid. These data, presented in table 5, suggest that there could be only a slight renewal of the ribonucleic acid of unin-

TABLE 5

The changes observed in the specific activities of the purine nucleotides obtained from growing Escherichia coli, strain B G1-5

EXP NO.	TOTAL RNA*	MEDIUM GLYCINE	ADENYLIC ACID		GUANYLIC ACID	
			Calculated	Observed	Calculated	Observed
I	46	1,600		1,500		1,590
II	82	0	840	800	890	796
IV	79	2,710	1,135	1,255	1,135	1,279

Values in this table are in cts/min/ μ M.

* This was determined from the ultraviolet absorption of the alkaline digest of the acetone powder. D_{260} for 1 μ g ribonucleic acid per ml = 0.0354 at pH 7.

fected cells if any at all. The greatest part of the change in specific activity of the nucleotides of ribonucleic acid found after incubation is due to the *de novo* synthesis of ribonucleic acid. It should be pointed out that the absence of renewal of ribonucleic acid in uninfected cells is as complete as was found with infected cells (tables 3 and 4).

Ribonuclease of E. coli, strain B G1-5. It has been reported by Cohen (1948a) and confirmed in this laboratory that the concentration of ribonucleic acid remains constant during phage multiplication. This fact in conjunction with the conclusion noted above, namely that there is almost no renewal of ribonucleic acid in the organism either during growth or during phage multiplication, suggests that *E. coli*, strain B G1-5, does not contain an enzymatic apparatus for degrading ribonucleic acid. This could

explain the apparent constancy of ribonucleic acid during phage multiplication in the absence of synthesis ribonucleic acid. However, this was not found to be the case. This organism contains a ribonuclease which degrades the endogenous ribonucleic acid quite rapidly as soon as the cell is disrupted. The ribonuclease activity was determined by dialyzing the acetone powders obtained in the usual manner and measuring the amount of ultraviolet absorbing material in the dialyzate and comparing this with the amount of nondialyzable ribonucleic acid left inside the dialysis bag. Cells of *E. coli*, strain B G1-5, from a 9 hour culture were harvested and suspended in fresh basal synthetic

TABLE 6

The formation of dialyzable materials from the ribonucleic acid of uninfected and T4r infected Escherichia coli, strain B G1-5

CULTURE	TIME OF INCUBATION	RIBONUCLEIC ACID	
		Dialyzable	Non-dialyzable
	min	mg	mg
Control.....	0	1.7	5.7
Control.....	180	1.6	8.5
T4r infected.....	0	1.7	5.5
T4r infected.....	60	1.5	5.7
T4r infected.....	120	4.6	4.1
T4r infected.....	180	8.1	2.5

medium at a concentration of 3×10^9 bacteria per ml. In the first series of experiments (table 6), 200 ml aliquots of uninfected and T4r infected (ratio of phage to bacteria was 4) were incubated at room temperature for 180 minutes. The nondialyzable ribonucleic acid from the acetone powders was isolated by alkali digestion in the usual manner. The amounts obtained are shown in table 6. The dialyzate gave a strong pentose reaction and no desoxypentose test. It should be noted that the total ribonucleic acid obtained by adding together the values in the two columns increases. There is more ultraviolet absorbing material apparently derived from ribonucleic acid at 180 minutes than at zero time. This perhaps is due to the fact that the extinction coefficient of the pyrimidine ring changes with the degree of polymerization in which it is involved.

The action of the enzyme in phage lysates

was compared with the action of the enzyme liberated from uninfected cells by sonic vibration. Observations made on a typical experiment are reported in table 7. One 50 ml aliquot of cell suspension prepared as in the previous experiments was treated at 1 C in a Raytheon 9 kc oscillator for 10 minutes. This treatment was sufficient to disrupt over 95 per cent of the cells as estimated by turbidity and viable cell count. Another 50 ml aliquot was infected with T4r so that the ratio of phage to bacteria was 10. The flasks were incubated at room temperature for 2 hours, the time interval in the T4r experiment being measured from the onset of lysis. Acetone powders were made before and after incubation and the amounts of dialyzable

released from an infected cell by lysis or from an uninfected cell by sonic treatment, it would seem as if the enzyme does not act intracellularly on the endogenous ribonucleic acid either in the infected cell or in the normal uninfected cell. This suggestion is not unexpected since we have already noted the slow rate of renewal of ribonucleic acid in uninfected as well as in infected cells. The apparent constancy of ribonucleic acid during phage multiplication can now be explained as being due to the fact that in infected cells there is a lack of synthesis of ribonucleic acid. The normal rate of ribonucleic acid breakdown is so slow that no ribonucleic acid is broken down during phage multiplication.

DISCUSSION

The purpose of this investigation was to determine the reason for the apparent inertness of ribonucleic acid during phage multiplication. The data presented indicate that in growing cells of *E. coli* there is very little breakdown of ribonucleic acid. Other investigators have come to the same conclusion using the uptake of inorganic P³² as an indicator of nucleic acid metabolism. Labaw *et al.* (1950) found that phosphorus, once taken up by the bacteria, does not exchange with the nutrient medium during growth. Curves published by Morse and Carter (1949) relating inorganic P³² uptake and growth suggest the same thing. These data taken in conjunction with the observations reported here, namely that the breakdown of ribonucleic acid in growing cells is extremely slow, suggest that the ribonucleic acid is, as it were, a "dead-end street". That is, the materials that are incorporated into the ribonucleic acid are removed from the economy of the cell and are not available for incorporation into any other structures of the cell.

Similar observations have been made with yeast. Spiegelman and Kamen (1947) observed that the specific activity of the ribonucleic acid of yeast labeled with P³² fermenting glucose in the absence of a nitrogen source remained constant. When a nitrogen source was introduced (thus, there was the possibility of synthesis of ribonucleic acid), only then was there a change in the specific activity of the ribonucleic acid. Abrams *et al.* (1949) report that if yeast grown in a medium containing N¹⁵H₃ were allowed to incubate further in a medium containing ordinary

TABLE 7

The action of the ribonuclease of Escherichia coli, strain B G1-8, on the endogenous ribonucleic acid liberated by phage lysis and sonic treatment

EXPERIMENT	DIALYZABLE RIBONUCLEIC ACID	
	0 min	120 min
	mg	mg
Sonic treated	0.66	1.64
T4r lysate	0.65	1.39

ribonucleic acid determined in each case (table 7). Less ribonucleic acid was converted to dialyzable material in the phage lysate than with the sonic treated cells. Since lysis is not simultaneous in all infected cells of the culture, the lower value in this case only strengthens the contention that ribonucleic acid is not degraded by the ribonuclease until one or both are liberated from the cell. It should be noted also that the same amount of dialyzable material is found at zero time from an acetone powder of a sonic extract as from an acetone powder of whole cells. This indicates that the acetone treatment of whole cells destroys the membrane sufficiently so that all the intracellular ultraviolet absorbing dialyzable material will dialyze out. This is important to note because in other experiments the amount of dialyzable material in acetone powders of infected cells sampled at various times during the latent period was constant. Thus, there is no breakdown of the ribonucleic acid during phage multiplication. Since the enzyme is apparently just as active in degrading the endogenous ribonucleic acid when it is

ammonia, the total amount of N^{15} in the yeast remained constant even though there was a net synthesis of protoplasm. There was no increase in the guanine content during the incubation and also no change in the specific activity of the guanine of the nucleic acids. These data point to the fact that also in yeast the ribonucleic acid is not subject to degradation.

E. coli contains a ribonuclease which will degrade the ribonucleic acid of the organism once the cell has been broken up, either by phage lysis or by sonic treatment. The enzyme does not appear to be acting in growing cells *in vivo*. Whether the enzyme or the substrate is present intracellularly in an unavailable form is not known. The role that this enzyme plays in the life cycle of the organism may be solely that of converting the ribonucleic acid of autolyzing cells to smaller fragments so that they might be available to other growing cells in assimilable form.

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SUMMARY

The metabolism of the ribonucleic acid of a glycineless mutant of *Escherichia coli*, strain B, has been investigated during growth of the organism and during infection with bacteriophage T4r. Glycine-2- C^{14} incorporation into the purines of ribonucleic acid was used as an indicator of the metabolism of ribonucleic acid. The rate of renewal of endogenous ribonucleic acid in growing cells and in cells supporting phage multiplication was found to be very slow and of the same order of magnitude in the two instances.

The organism contains a ribonuclease. This enzyme degrades the endogenous ribonucleic acid to dialyzable materials only if the cell is disrupted either by sonic vibration or phage lysis. Since the rate of renewal of the ribonucleic acid in these cells is very slow, the enzyme must not be acting on the endogenous ribonucleic acid *in vivo*. This argues against the possibility

of ribonucleic acid being converted to desoxyribonucleic acid during growth and also suggests that ribonucleic acid is not in a "dynamic state" in the growing organism.

The apparent constancy of the ribonucleic acid during phage multiplication in *E. coli* therefore is due to the fact that no new ribonucleic acid is being synthesized at this time. Since the normal mechanism for degrading ribonucleic acid *in vivo* is so weak, it is not necessary to postulate that there is a specific inhibition of ribonucleic acid degradation imposed by an infecting phage particle.

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