LOCALIZATION OF MACROMOLECULES IN *ESCHERICHIA COLI*

II. RNA and its Site of Synthesis

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ABSTRACT

The distribution of RNA in cells of *E. coli* 15 T⁻U⁻ labeled with uridine-H^3 was studied by methods involving the analysis of radioautographic grain counts over random thin cross-sections and serial sections of the cells. The results were correlated with electron microscope morphological data. Fractionation and enzyme digestion studies showed that a large proportion of the label was found in RNA uracil and cytosine, the rest being incorporated as DNA cytosine. In fully labeled cells the distribution of label was found to be uniform throughout the cell. The situation remained unchanged when labeled cells were subsequently treated with chloramphenicol. When short pulses of label were employed a localization of a large proportion of the radioactivity became apparent. The nuclear region was identified as the site of concentration. Similar results were obtained when cells were exposed to much longer pulses of uridine-H^3 in the presence of chloramphenicol. If cells were subjected to a short pulse of cytidine-H^3, then allowed to grow for a while in unlabeled medium, the label, originally concentrated to some extent in the nuclear region, was found dispersed throughout the cell. The simplest hypothesis which accounts for these results is that a large fraction of the cell RNA is synthesized in a region in or near the nucleus and subsequently transferred to the cytoplasm.

The distribution of RNA in *Escherichia coli* and the localization of its site of synthesis are interesting both from the point of view of bacterial cytology and, more generally, from that of nucleocytoplasmic relationships. RNA, in the form of soluble RNA and in that of ribonucleoprotein particles, appears to be actively involved in the various steps of protein synthesis and is a likely candidate for the role of information carrier between DNA and proteins. In normal *Escherichia coli* 75 to 80 per cent of the cell RNA is in the form of ribonucleoprotein particles, or ribosomes (1), the rest being in the form of soluble RNA. The structural organization of the cell is simple: a central nuclear region containing most or all of the cell DNA (2), in apparently direct contact with a uniformly granular cytoplasm, probably composed mostly of ribosomes. The cell is bounded by a thin membrane and surrounded by a cell wall. In such an organism many of the structural elements interposed in higher cells between nucleoplasm and cytoplasm (nuclear membrane, endoplasmic reticulum etc.) are absent, and hence is might be hoped that nucleocytoplasmic relationships are more direct. We have in two previous papers (2, 3) indicated how the difficulties caused in radioautographic studies by the small size of the organisms could be overcome to some extent by the analysis of grain counts over thin sections of the bacteria. We shall now apply this method to

the study of RNA localization and to an attempt at identifying the site of RNA synthesis in *Escherichia coli*. This will be done by studying the distribution of label in cells fully labeled with uridine-H^3 and in cells labeled with very short pulses of uridine-H^3 or cytidine-H^3. In the latter case the distribution of label will be examined immediately following the pulse and compared to the distribution obtained when the cells are allowed to grow for some time after the pulse. The special case of the RNA synthesized in the presence of chloramphenicol will also be examined.

MATERIAL AND METHODS

The strain of bacteria, the methods of culture, labeling, specimen preparation, radioautography, and analysis of results have been described in the previous paper (2).

Label: The label used was uridine-H^3 with a specific activity of 680 mc./mM, from New England Nuclear Corp., and cytidine-H^3 with a specific activity of 1000 mc./mM, from Schwarz Laboratories, Inc. The purity of the compounds was checked by paper chromatography with a butanol-acetate solvent (4).

Geiger Counting: To measure uptake of uridine-H^3 in cells, 10 μl. aliquots of a culture were taken at various intervals after addition of the label and mixed on a stainless steel planchet with 0.5 ml. of a 10 per cent solution of formalin containing a high concentration of cold uridine to reduce absorption of label to the planchet. After 10 minutes the planchets were dried on a 45°C. hot plate and washed in 4 changes of water. The radio-activity incorporated was measured in a Packard windowless flow counter operating in the Geiger region. By counting pre-washed cells after each of four successive washings it was verified that cells were not lost during this procedure. To measure background, cells pre-killed with formaldehyde were introduced into labeled medium and samples were taken, processed, and counted as described above. The attenuation due to self-absorption within the bacteria was measured and found to be approximately 35 per cent.

When measuring the radioactivity of fractionation products, samples of the various supernates were placed on planchets, 0.5 ml. of distilled water was added to spread the sample, and the planchets were dried and counted. The amount of self-absorption for each specimen was measured by adding a known amount of a standard solution of uridine-H^3 to parallel samples and measuring the attenuation introduced in it by mixing with the sample. All counts were thus corrected for self-absorption.

Fractionation: The method of separation used was the Schmidt-Thannhauser procedure (5) adapted to the use of a micro-centrifuge (Misco) (6), and modified by Barner and Cohen (7). This last modification includes the precipitation by ethanol of the DNA present in the KOH fraction and is made necessary by the unusual solubility of *E. coli* 15 T^-U^- DNA in KOH (8). Samples from each supernate were counted as described above.

Identification of the products was made by ascending paper chromatography in a butanol-pyridine solvent (9) or an *n*-propanol-ethyl acetate solvent (10). Radioactivity on the chromatograms was measured both by direct counting of paper strips and by elution of the strips in 10 per cent propanol in the planchets.

Concentration of nucleic acid products was calculated from UV absorption at 260 mμ by comparison with standard solutions of DNA and RNA (Worthington) degraded in the same manner. A UV absorption spectrum was taken to insure that absorption was due to nucleic acid derivatives. The identification of RNA and DNA was made on the basis of base composition (presence or absence of uracil compounds).

Fixation Control: The loss of incorporated uridine-H^3 during fixation, embedding, and specimen preparation for radioautography was measured as described previously for thymidine and leucine (2) and was found to be less than 1 per cent of the total label.

RESULTS

Characterization of Label in the Cell

Using a method of isotopic competition, Siminovitch and Graham (11) have shown that uridine can be a precursor to all RNA and DNA pyrimidine bases in *E. coli* B. Lichtenstein, Barner, and Cohen (12) have shown that in *E. coli* B_u- labeled with uracil-C^{14} all pyrimidine bases are equally labeled.

A situation where a large fraction of the label was to have been incorporated into DNA might have proved bothersome in RNA localization studies. It was considered that the use of a thymidine and uracil requiring mutant of *E. coli* 15: *E. coli* 15 T^-U^- (7) might improve the situation. Since no information was available on the uptake of uridine by these cells a study of the distribution of label using fractionation techniques, paper chromatography, and enzyme digestion was performed.

The results of the Schmidt-Thannhauser fractionation procedure are summarized in Table I. The cold TCA and ethanol fractions appear chromatographically almost similar. They contain mostly uridylic and cytidylic acid in a proportion

TABLE I

Fraction	Counts/ml./min. × 10^{-3}	Per cent Total activity	Chromatogram	Amount
Cold TCA	450	1.9	UA, CA	
H_2O	100	0.4	UA, CA	
Ethanol	90	0.4	UA, CA	
KOH	20,800	88.0 } 90.1	UA, CA	} 925 μg.
H_2O	500	2.1 }	UA, CA	}
Hot TCA	1,800	7.6	C, DCA	116 μg.
Residual	214	0.9	U, C	
Total cells	23,620	100.0		

Note: The per cent values are with respect to the total activity measured in intact cells. All values are corrected for self absorption. The amounts of RNA and DNA are calculated from UV absorption data. UA = uridylic acid; CA = cytidylic acid; DCA = deoxycytidylic acid; U = uracil; C = cytosine.

of about 2:1. The possibility that some of the label in the two major peaks was in the form of uridine and cytidine di- or triphosphates was not investigated. These fractions are presumed to represent low molecular weight components, to be similar to that fraction lost in fixation, and are probably not seen in our autograph experiments. The KOH and subsequent H_2O fractions (after ethanol precipitation of DNA) are presumably the RNA fraction. The activity appears as uridylic and cytidylic acids in approximately equal amounts. It, therefore, seems that in *E. coli* 15 T^-U^- cytidine and uridine are labeled equally well during growth in uridine-H^3. The hot TCA fraction is composed almost exclusively of cytosine (~78 per cent) and deoxycytidylic acid (~22 per cent) and represents DNA. The ratio DNA/RNA is approximately 1:8.

Contrary to the situation found in *E. coli* B (11) and in *E. coli* B_u-, (12) no trace of labeled thymidine was found. This indicates that in *E. coli* 15 T^-U^- the metabolic block occurs between the formation of uridine-5-phosphate or 2-deoxyuridine-5-phosphate and that of thymidine-5-phosphate. This was suggested by the finding of Barner and Cohen (8), who demonstrated the release of a uracil compound when *E. coli* 15 T^- was grown in the absence of thymidine. The use of the double mutant in this study is therefore justified.

Remembering that such materials as mononucleotides, precursor pools, etc. will not appear in the fixed cells, the distribution of label in the cells used in our experiments will be approximately as follows: RNA—92 per cent, DNA—8 per cent, others—0 per cent.

As short pulses of label were used it was important to show that the distribution remained similar to that found in fully labeled cells. Since the small amount of label incorporated in times as short as 1 minute did not make a fractionation study practical, ribonuclease was used to demonstrate this point. Fig. 1 shows the uptake at 24°C. of uridine-H^3 by a culture of *E. coli* 15 T^-U^-. Since the time involved represents only a small fraction of the total division time, the uptake appears nearly linear. After counting, the planchets were treated with 100 μg./ml. of RNase in 0.005 M NaCl at 37°C. for 15 minutes, washed four times, and counted again. The proportion of activity removed is constant at about 87 per cent of the label present and equal to that removed by similar treatment from fully labeled cells. Similar treatment of cells fully labeled with thymidine-H^3 failed to remove any appreciable amount of label, showing that cells were not washed out during the procedure. Furthermore, comparable results were obtained by radioautography of individual cells following RNase treatment.

Cells labeled with cytidine-H^3 yielded results in all points similar to those obtained with uridine-H^3, as could be expected from the results of Bolton (13) and Siminovitch and Graham (11), who concluded that cytidine and uridine were interconverted freely in *E. coli*.

In general, thus, when *E. coli* 15 T^-U^- is labeled with uridine-H^3 or cytidine-H^3 a small amount of label appears in DNA while the rest appears in RNA. Because of the relative insensitivity (which shall be discussed later) of grain count distribution to small fluctuations in label

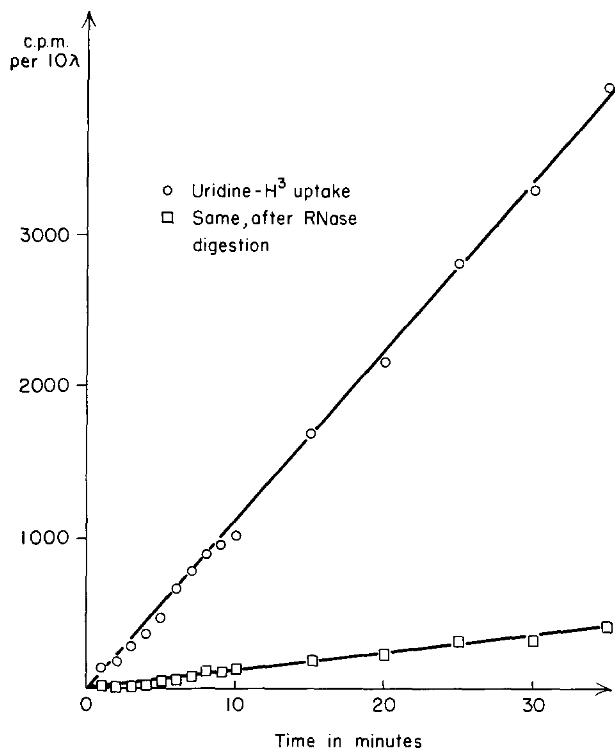

FIGURE 1

Short-time uptake of uridine-H^3 by *E. coli* 15 T^-U^-. The lower curve shows the amount of radioactivity remaining in each sample after RNase digestion.

concentration the proportion of DNA label was judged small enough to permit localization studies on RNA.

Radioautography

The methods of analysis of grain counts over random cross sections and over serial sections of labeled cells have been fully described in the preceding paper (2). Additional comments will be made as needed in the course of this discussion.

(*a*) *Fully Labeled Cells:* Random cross-sections and serial sections of cells of *E. coli* 15 T^-U^-, grown for 7 generations in the presence of 25 mc./ml. of uridine-H^3 at a specific activity of 180 mc./mM were examined in radioautographs. The distribution of grain counts among random cross sections (Fig. 2) approximates closely a Poisson distribution ($\chi^2 = 5.03$; for 4 degrees of freedom $P > 0.20$). The average distribution of grain counts in serial sections (Fig. 7-A) is of the type that would be expected for a random distribution of the label in the cell. We conclude, therefore, that RNA is distributed in a fairly uniform manner throughout the cell.

(*b*) *Fully Labeled Cells Treated with Chloramphenicol:* Fig. 3 shows that the distribution of grain counts in sections of cells labeled as in (*a*) and then treated with 50 μg./ml. of chloramphenicol for 90 minutes is close to a Poisson ($\chi^2 = 1.57$; for 4 degrees of freedom $P > 0.70$). Thus it seems that, as for proteins, the morphological changes due to growth in the presence of chloramphenicol do not markedly alter the distribution of the RNA within the cell.

(*c*) *Cells Labeled with a Short Pulse of Uridine-H^3:* Cells growing at a concentration of 4×10^8 cells/ml. were placed in medium containing 100 μc./ml. of uridine-H^3 (specific activity 680 mc./mM) for 1.3 minutes (1/100 division time) and fixed immediately afterwards.

The distribution of grain counts among random sections is shown in Fig. 4. A fairly strong deviation from the Poisson model is evident: $\chi^2 = 43.2$; for 5 degrees of freedom $P < 0.0001$. It seems safe to assume that this dispersion did not arise by chance, and that the Poisson distribution is, therefore, not a good model for this distribution.

Examination of serial sections of 50 cells gave the average longitudinal distribution of grains

shown in Fig. 7*C*. This seems to indicate a higher than usual concentration of label toward the center part of the cell, site of the nuclear region (compare with diagram for fully labeled cells at *A* and with theoretical diagram at *B* (Fig. 7) for cells containing 80 per cent of the label in the nucleus). For each class of sections (first section at the tip, second section, etc.) the distribution of grains approximated closely a Poisson distribution. Furthermore, if we sum up the total grain count for the first four sections of each cell the distribution obtained is close to a Poisson ($\chi^2 = 4.41$; $P > 0.5$). Therefore, the amount of incorporated label is homogeneous for different cells and proportional to cell length. Therefore, the dispersion from a Poisson of the general grain distribution reflects indeed a non-random distribution of the label in each individual cell.

Using the hint given by the study of serial sections, the hypothesis can be made that the non-randomness arises from a concentration of label in the nuclear region. It is obvious, however, that the distribution is not of the type obtained for DNA, and that the label is not exclusively located in the nucleus. It was assumed, therefore, that label was only partially located in the nucleus, the rest being distributed uniformly in the cytoplasm. Various distributions were tried and it was found that a distribution based on the localization of 70 per cent of the total label in the nucleus fitted the data satisfactorily ($\chi^2 = 9.39$; for 6 degrees of freedom $P > 0.10$).

In order to estimate the possibilities of this method of analysis in cases of partial concentration of label in a given region, theoretical distributions of grain counts were calculated in the following manner: a block diagram of the longitudinal distribution of label in an average cell was drawn for each postulated case, the probability for various classes of sections, characterized by a given label content, was estimated, and a grain count distribution was

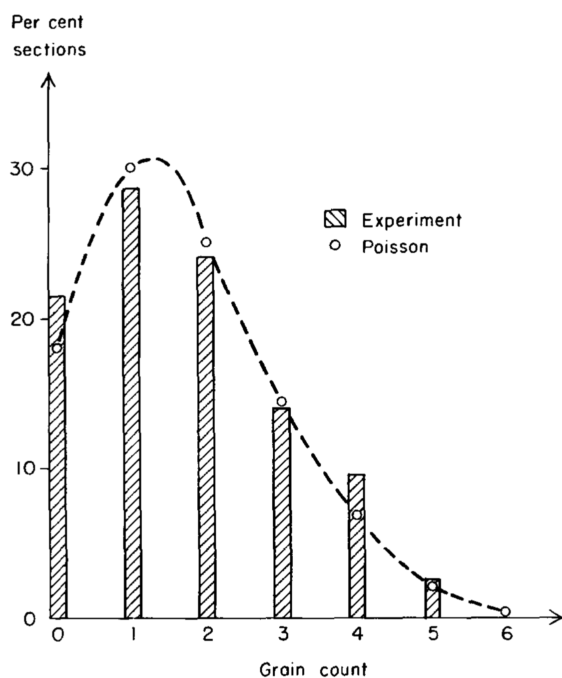

FIGURE 2

Distribution of autographic grain counts above 200 random cross-sections of *E. coli* 15 T⁻U⁻ cells fully labeled with uridine-H^3. A Poisson distribution having same average count is also indicated.

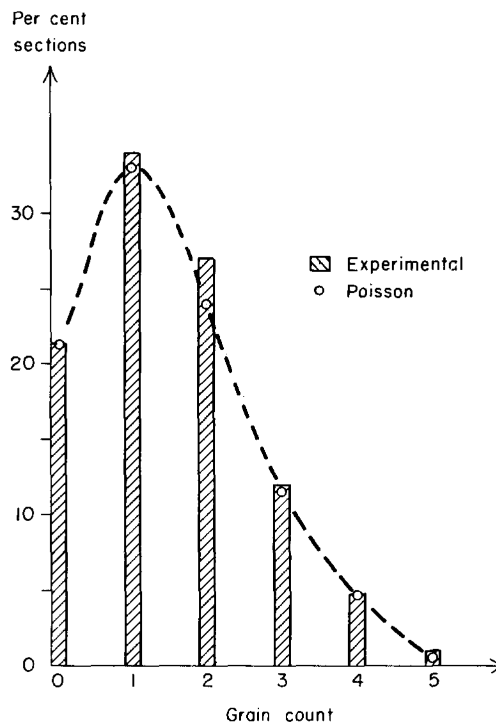

FIGURE 3

Distribution of autographic grain counts above 206 random cross-sections of *E. Coli* 15 T⁻U⁻ cells fully labeled with uridine-H^3 and subsequently placed in presence of chloramphenicol for 90 minutes. A Poisson distribution having same average count is also included.

plotted, using the formula given in the previous paper (2) and an arbitrarily fixed average grain count. It was thus found that a label uniformly distributed in the cell would give a Poisson distribution. If 20 per cent of the label is in the nucleus the situation remains unchanged. If 50 per cent of the label is in the nucleus a deviation from a Poisson occurs, but it is quite small and would be almost undetectable experimentally. When 75 per cent of the label is in the nucleus the skewness of the expected grain count distribution with respect to a Poisson becomes unmistakable. It was concluded, therefore, that a consistent deviation from a Poisson must reflect a significant concentration of label in a limited region of the cell.

Thus we are led to the conclusion that in the case of a short pulse of uridine-H^3 an imbalance exists in the distribution of radioactivity which indicates a concentration in or near the nuclear region. For a pulse of $1/100$ division time examined above the concentration is of the order of 70 per cent of the total label. Taking into account the presence of some labeled DNA, this indicates that at least 65 per cent of the newly synthesized RNA is present in or near the nuclear region while the rest is dispersed in the cytoplasm. Taking relative volumes into consideration, this means that at this time the concentration of labeled RNA is roughly 4.5 times higher in the nuclear region than in the cytoplasm. An even higher concentration is suggested when shorter pulses (50 and 40 seconds) are used but because of the low level of radioactivity incorporated, the data are not good enough to make more precise statements.

(d) Cells Labeled with a Short Pulse of Cytidine-H^3, followed by Growth in Unlabeled Medium: In this experiment cells of *E. coli* 15 T^-U^- were growing at 37°C. with a division time of 25 minutes at a concentration of 2×10^8 cells/ml. Cytidine-H^3 at a concentration of 17.5 μc./ml., specific activity 1 c/mM, was added to the medium. After one minute the cells were chilled suddenly. Half the culture was fixed immediately, the other half was washed and transferred to nutrient broth at 37°C. where it was allowed to grow for 20 minutes and then fixed. Exponential growth had been resumed at that time.

Fig. 5 shows the distribution of grain counts among random cross-sections for the cells fixed immediately after the pulse of label. It shows a deviation from a Poisson distribution characteristic of such short pulses. As indicated in the previous section, this is taken to be due to a high concentration of newly synthesized RNA in or near the nuclear region. A comparison of the data with the Poisson model gives $\chi^2 = 77.8$; for 5 degrees of freedom $P \ll 0.0001$, an obviously bad fit.

In contrast, those cells which had been allowed

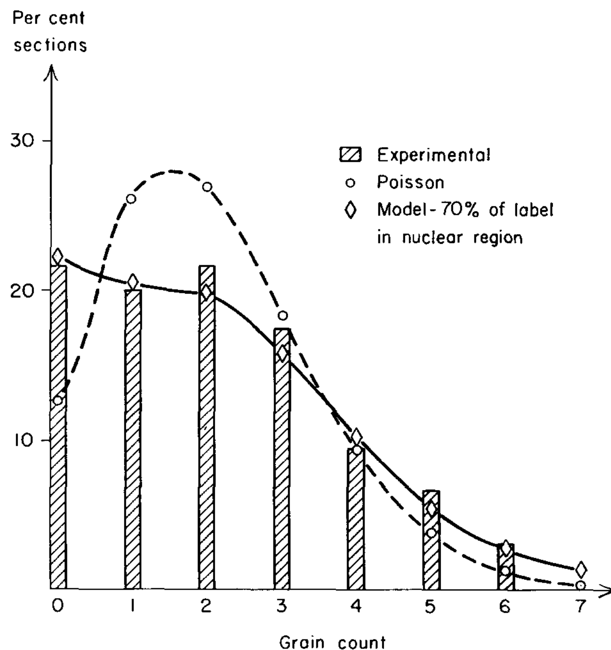

FIGURE 4

Distribution of autographic grain counts above 331 random cross-sections of *E. coli* 15 T^-U^- cells labeled with a 1.3 minute pulse of uridine-H^3 (1/100 division time). Two models are included: (○) Poisson, (◇) Model distribution based on the assumption that 70 per cent of the label is in the nuclear region.

to grow for some time after the pulse in the presence of unlabeled cytidine give a distribution of grain counts (Fig. 6) which approximates a Poisson (χ^2 = 9.6; for 5 degrees of freedom $P \sim 0.1$). This is taken to indicate a random distribution of the label.

The average grain count per section was the same in both preparations, indicating that no label was lost and that few cell divisions occurred during the period of growth following labeling. The fact that a Poisson distribution was obtained serves as an internal check on the homogeneity

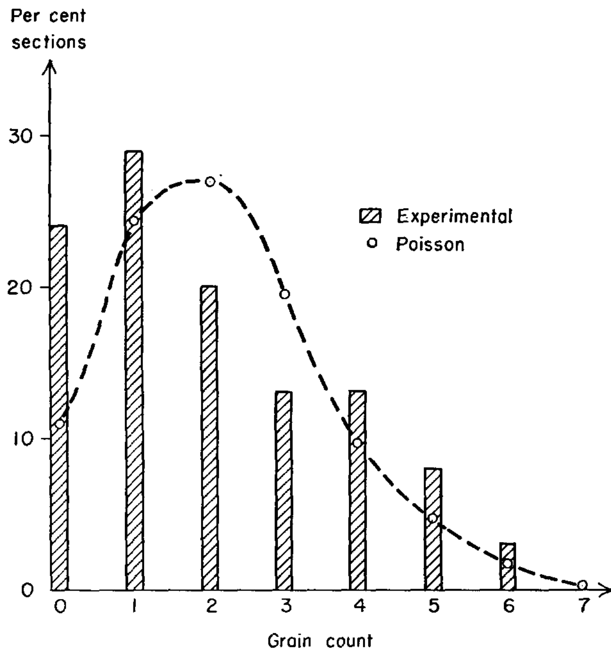

FIGURE 5

Distribution of grain counts above 300 random cross-sections of *E. coli* 15 T^-U^- cells labeled with a 1-minute pulse of cytidine-H^3 (1/25 division time). A Poisson distribution is also shown.

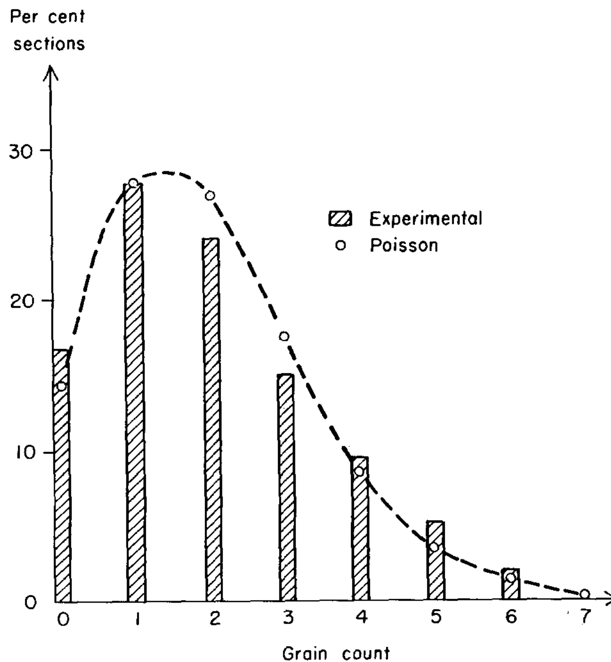

FIGURE 6

Distribution of grain counts above 300 random cross-sections of cells from the same culture as in Fig. 5 after 20 minutes in unlabeled medium, following the pulse of cytidine-H^3. A Poisson distribution is also shown.

of labeling and shows that the initial deviation from a Poisson was indeed significant.

(*e*) *Cells Labeled with a Long Pulse of Uridine-H^3 in Presence of Chloramphenicol:* When normal cells are subjected to a long (20 minutes) pulse of uridine-H^3 a distribution of grain counts fairly close to a Poisson is obtained, indicating that RNA is present in all parts of the cell. In view of the results described above it was interesting to see if the same situation prevailed when the cells were labeled in the presence of chloramphenicol. Under such conditions the rate of uridine uptake per cell remains constant for at least 90 minutes. This was established by subjecting the cells to short pulses of uridine-H^3 at various times after the addition of chloramphenicol to the culture. On the other hand the rate of protein synthesis decreases enormously (14, 15, 16). Bolton has shown that both soluble RNA and ribosomal RNA are made in the presence of chloramphenicol (17) but there is evidence that the ribosomes formed are incomplete or abnormal, as they show different column behavior and have a low sedimentation constant. Aronson and Spiegelman (18) have also found that a large fraction of the RNA synthesized in the presence of chloramphenicol was bound to a particulate component. The localization of such a component is of some interest.

A concentration of 25 μg./ml. of chloramphenicol was added to an exponentially growing culture at 24°C., containing 9.2×10^7 cells/ml. After 60 minutes 200 μc./ml. of uridine-H^3 with a specific activity of 680 mc./mM were inoculated into the culture. The specimen was fixed 20 minutes later. The distribution of grain counts among random sections is shown in Fig. 8. It does not fit the Poisson model: $\chi^2 = 141$; $P < 0.00001$ for 7 degrees of freedom. The average distribution of grain counts along serial sections (Fig. 7*D*) suggests a concentration of label in the nuclear region (compare with the distribution expected for a concentration of 80 per cent of label in the nucleus in Fig. 7*B*). The theoretical distribution among random sections obtained by making the hypothesis that 80 per cent of the label is in the nucleus fits the data well (Fig. 8): $\chi^2 = 7.0$; $P \sim 0.5$ for 8 degrees of freedom. Data from serial sections indicated that the cells were homogeneously labeled. Taking into consideration the presence of some labeled DNA we conclude, therefore, that at least 75 per cent of the newly synthesized RNA is located in the nuclear region. The concentration of labeled RNA is approximately 7 times higher in the nuclear region than in the cytoplasm.

DISCUSSION

We have reached the conclusion that within the resolution of the methods used, RNA is distributed evenly throughout the entire cell. Since some 80 per cent of the total RNA is in the form of ribonucleoprotein particles there seems no reason to doubt that the cytoplasmic granular material seen in sections of *E. coli* represents tightly packed ribosomes such as have been observed in lysates in the ultracentrifuge (19) or the electron microscope (20). The action of chloramphenicol does not seem to disturb the distribution of previously synthesized RNA. It is impossible to make a statement as to the presence or absence of RNA in the nuclear region. In view of the results obtained with pulses of uridine-H^3 it seems likely that some RNA is present in or in close contact with the nuclear region.

In the case of uridine pulses in normal cells, the distribution of grains among random cross-sections deviates from a Poisson. Since in most cases we can eliminate the possibility that the observed deviation is due to an uneven labeling of the cells, the only explanation is that for short periods of uridine incorporation regions of high label concentrations exist in the cell. The results obtained in serial sections indicate that the region of high incorporation is correlated with the position of the nuclear region. From the data obtained it is impossible to decide whether the region of high incorporation is the nuclear region itself, or a narrow band of cytoplasm surrounding it. This alternative is implied whenever we speak of nuclear region.

It will be noted that Figs. 4 and 5 show slightly different distributions of grain counts. This reflects the fact that experimental conditions were slightly different in the two cases. The actual distribution obtained depends on the length of the label pulse, divison time of the bacteria, temperature and composition of the medium, thickness of the sections, and morphology of the fixed cells. Because of the large number of influential variables, reproducible distributions of grain counts are almost impossible to achieve in the case of short pulses of uridine-H^3. A constant factor, however, is the

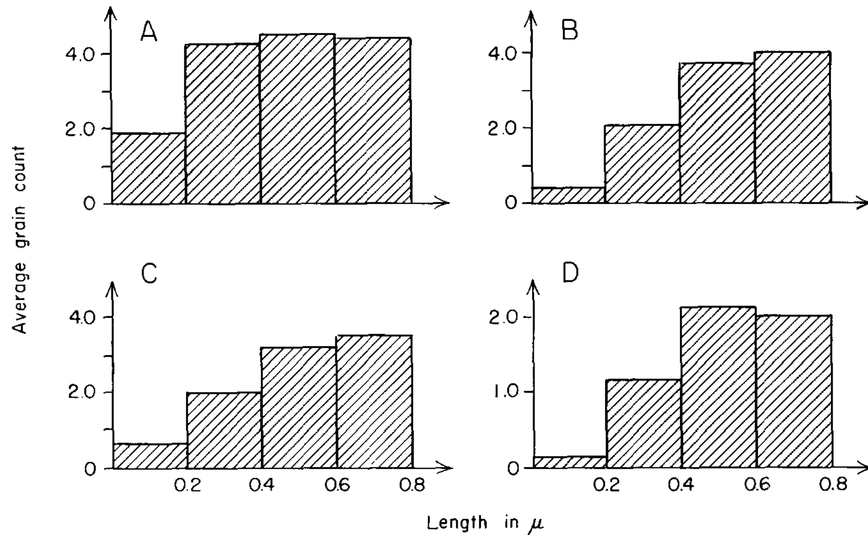

FIGURE 7

Average grain counts over the first four serial sections, 0.2 μ thick, starting from the tip of the cell. The average length of the cells is 1.5 μ, that of the nuclear region about 1.0 μ for normal cells and 0.75 μ for chloramphenicol-treated cells.

A. Cells fully labeled with uridine-H^3 (50 cells).

B. A theoretical diagram showing the distribution expected if 80 per cent of the label were concentrated in the nuclear region.

C. Cells labeled with a 1.3-minute pulse of uridine-H^3 (50 cells).

D. Cells labeled with a 20-minute pulse of uridine-H^3 in presence of chloramphenicol (50 cells).

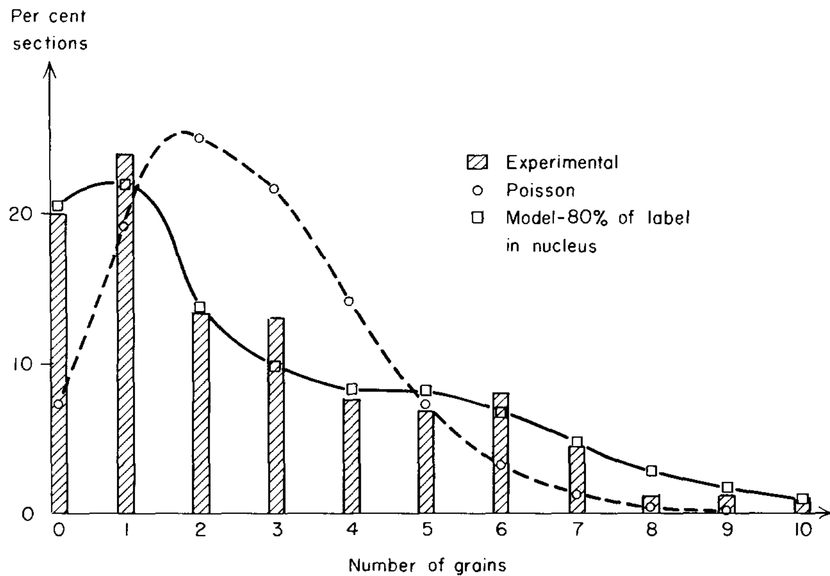

FIGURE 8

Distribution of grain counts over 250 random cross-sections of cells of *E. coli* 15 T⁻U⁻ labeled with a 20-minute pulse of uridine-H^3 in presence of chloramphenicol. Two models are included: (○) Poisson, (□) Model distribution based on the assumption that 80 per cent of the label is in the nucleus.

fact that a significant deviation from a Poisson distribution is always obtained in the case of short pulses of uridine or cytidine-H^3, but not in the case of fully labeled cells.

It is difficult to make precise statements as to the relative content of label in the nuclear region and the cytoplasm. In order to make such statements one must break down the grain distribution curve into its several postulated components. This can be done only if the average grain count is high enough. Unfortunately, the cases in which the deviation from a Poisson is large enough to make such an attempt worthwhile correspond to very short pulses of label. In those cases the radioautographs had to be exposed for 1 to 3 months in order to obtain an average grain count of 1.5. Longer exposures and the recent availability of uracil-H^3 with higher specific activity than the compounds used in this study might help produce additional information in the future.

In the case of uridine-H^3 uptake in the presence of chloramphenicol the situation is improved by the fact that with a pulse as long as 20 minutes there exists a strong deviation from a Poisson distribution. Since the rate of uptake of uridine remains constant this means that high levels of labeling are possible. Fig. 8 illustrates fairly clearly how a high average grain count helps bring about a clearer deviation from the Poisson model.

Thus we have concluded that for short pulses of uridine or cytidine and for longer pulses of uridine in the presence of chloramphenicol, a large fraction of the label appears either in the nuclear region or in a narrow shell surrounding it. Several hypotheses concerning the synthesis of RNA can be formulated.

(*a*) *RNA is synthesized in the same places in which it is incorporated.* In view of our results this would imply that nuclear RNA is being synthesized faster than cytoplasmic RNA. Since we are considering steady state conditions (exogenous uridine was in all cases available to the cells before the addition of label) the relative concentration of RNA in the nucleus would remain the same regardless of the length of the pulse, a fact contradicted by our results with fully labeled cells. This hypothesis is also contradicted by the experiment described in paragraph *d*, in which the label concentrated in a small region of the cell during a short exposure to cytidine-H is found to have diffused throughout the cell after a period of growth in medium containing unlabeled cytidine. Furthermore, in the case of chloramphenicol treated cells, we would have to accept the idea that cytoplasmic synthesis has been depressed and nuclear synthesis increased in such proportions as to keep the rate of uptake constant. This is an unlikely hypothesis.

(*b*) *The hypothesis that RNA is synthesized in the place of final incorporation could be rendered more plausible by making additional assumptions.* One such assumption would be, for example, that nuclear RNA shows a turnover of nucleosides. This is rendered unlikely by the experiments of Hershey (21), van Tubergen (22), and that described in paragraph *d*, showing no exchange with exogenous nucleosides. A more complicated mechanism providing for a total conservation of the label would have to be postulated. Another possible assumption would be that of a longer time lag in the synthesis of cytoplasmic RNA than in that of nuclear RNA. The kinetics of uptake (Fig. 1) do not show any evidence for this.

(*c*) *RNA is synthesized for the most part in a specialized region, and transported subsequently to various parts of the cell.* All of our data favor this hypothesis, the preferred region being the nuclear region or its immediate vicinity. The experiments on short pulses and especially that on a short pulse followed by growth in cold medium agree with this. The uptake in the presence of chloramphenicol can then be explained on a basis of a greatly reduced synthesis of proteins, causing either an accumulation of ribosomal RNA in the nuclear region, or an accumulation of incomplete ribosomes in close contact with this region. Soluble RNA could then account for the activity present in the rest of the cytoplasm. Alternate explanations for any set of data are conceivable. This, however, seems to be the simplest hypothesis that accounts for the known facts without being contradicted by any.

Much recent evidence points to a nuclear origin of cytoplasmic RNA in *Neurospora* (23, 24), in tissue culture cells (25, 26), in root meristem cells of *Vicia faba* (27), in *Amoeba proteus* (28). Our data seem to indicate that a similar situation prevails in bacteria. This idea is supported by a recent report by Ezekiel (29), who found that, in *B. megaterium* protoplasts labeled *in vivo* with pulses of uridine-C^{14}, fractions presumed to represent nuclear material and cell membranes showed the most rapid RNA labeling and showed a turnover of label when the pulses were followed by growth in cold medium.

In all such studies a major question remains to be solved: that is, the question of whether the RNA formed in the nucleus retains its molecular identity at all times during the transfer to the cytoplasm. Such a question does not, however, seem amenable to the type of approach used here.

Within the limits of resolution imposed by the methods the data presented here indicate that in cells fully labeled with RNA precursors the incorporated label is distributed randomly throughout the cell, while in the case of short pulses of label a characteristic non-randomness appears. We have interpreted these results in terms of cytological structures visible in the electron microscope and showed that the data were consistent with the idea that a large part of the label was first incorporated in the nuclear region or in a narrow band of cytoplasm surrounding it. Other interpretations are possible and better methods of localization will have to be developed before the problem of RNA synthesis in bacteria can be solved unambiguously.

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