in the inhibited enzyme from the imidazole-N of a histidine residue to the OH of a serine residue.

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An extreme example of the coding problem, Avian PPLO 5969*

A problem in the relation of protein to DNA is whether or not a given nucleotide sequence uniquely corresponds to a given protein molecule. This equivalence relation may be termed one to one coding. Among the alternative possibilities would be overlap coding where a segment of DNA may encode portions of the peptide sequence of several different proteins. An approach to this problem is to find an organism where the DNA code is short enough to begin to distinguish between some of the possible hypotheses.

In an attempt to find an organism where the amount of DNA presents a coding limitation to the number of possible proteins, we have directed our attention to small cells capable of reproduction on a non-living medium. While a number of small bacteria have been looked at, the most extreme case we have encountered is the pleuropneumonia-like organism Avian 5969[1].

The individual cells are spherical and in the electron microscope show a diameter of 0.25 μ. This extremely small size has the following consequences. The cell has a volume of $8.2 \cdot 10^{-5}$ ml, and assuming a 75% water content has a dry weight of $2.0 \cdot 10^{-15}$ g, which corresponds to a molecular weight of $1.2 \cdot 10^{9}$. Or from a somewhat different point of view we see that we have a cell which contains in the non-aqueous portion the order of 200,000,000 atoms.

We have recently carried out DNA measurements on this type of cell in the following manner. A large culture grown on a medium of Difco Tryptose Broth plus 1% Difco PPLO Serum Fraction is centrifuged and washed. Portions are taken for dry-weight determination, while other portions are first extracted in 75% ethanol and then in 5% trichloroacetic acid (100° for 45 min). The precipitate is centrifuged out and DNA determinations are carried out on the trichloroacetic supernatant by

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

* PPLO indicates pleuropneumonia-like organism. This strain is also known as *Mycoplasma gallisepticum*.

the method of KECK[2]. The DNA content is between 5 and 7.5 % of the dry weight of the cells.

In order to apply the preceding results to the problem of determining the code length it is necessary to demonstrate that the morphological units seen in the electron microscope are indeed the clone-forming units. To determine this we have determined the number of clone-forming units by getting the most probable number from serial dilution experiments. When this is done we find a DNA content per clone-forming unit, some 3 to 4 times the DNA content per morphological unit. Since, however, considerable clumping is observed in all our cultures this result is consistent with the two units being the same. This view is reinforced by the fact that we observe no morphological entities other than the small spheres and clumps of small spheres.

If the morphological and clone-forming units are identical then the average DNA content of a cell has a molecular weight of 70 million. If we assume that the cell uses one to one coding with no overlap in going from DNA to protein, then 70 million molecular weight of DNA can specify about 7 million molecular weight of protein sequence. (Based on the information-theory restriction that three nucleotides are needed to specify an amino acid and three nucleotides weigh approximately ten times as much as an amino acid.)

If we assume an average molecular weight for proteins of 50,000, then a 7 million molecular weight sequence can include 140 different proteins. If the DNA is double stranded the information is redundant and only 70 proteins can be specified. Thus we can make the following strong statement about Avian PPLO 5969; either the organism is able to operate on a very small number of proteins or there must be overlap in the coding.

To evaluate the previous statement the following facts about the organism may be considered. The organism is mostly DNA, RNA, lipid and protein. There are about a total of 10,000 protein molecules in each cell. The cells have ribosomes (at least three types). There are probably less than 300 ribosomes per cell.

This organism thus presents us with a situation where small size, small total number of macromolecules and a relatively short code enable us to study certain overall features of the coding problem.

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Continuous synthesis of deoxyribonucleic acid in *Escherichia coli*

The synthesis of deoxyribonucleic acid proceeds in a stepwise manner in bacterial cultures whose division has been synchronized by temperature changes or starvation[1]. It has been inferred from these observations that bacteria, like plant or animal cells, reduplicate their nuclear material during only a part of the normal division cycle. More recently it has been found, however, that synthesis of bacterial deoxyribonucleic acid appears to be a continuous process, since autoradiography of freely growing cultures of *Escherichia coli* or *Salmonella typhimurium* after short exposures to tritiated thymidine reveals the labeling of the deoxyribonucleic acid of nearly all of the cells of the population[2]. Thus, the earlier observations of stepwise synthesis probably reflect that disturbance of cell metabolism by which the bacteria were induced to divide in phase rather than the course of events in normal growth. This inference is supported by the finding that deoxyribonucleic acid synthesis is also continuous in cultures of *Bacillus cereus* whose cells divided in synchrony for the first two generations following spore germination[3]. We wish to report an independent observation which likewise indicates that bacterial cells synthesize their deoxyribonucleic acid throughout the normal division cycle.

Bacteria labeled with ^{32}P lose their viability upon decay of the incorporated ^{32}P atoms, the rate of inactivation being proportional to the amount of radioisotope contained in the bacterial deoxyribonucleic acid rather than to the ^{32}P content of other phosphorylated cell constituents[4,5]. Hence after brief incubation in a highly ^{32}P-labeled medium only those cells of a growing non-labeled bacterial population which are actively synthesizing deoxyribonucleic acid should become sensitive to inactivation. Any individuals in the population that do not happen to assimilate any radioisotope into their nuclear structures during that period should remain refractory to decay. Such a short-term labeling experiment has been carried out by inoculating non-labeled cells of *E. coli*, strain B/r into a synthetic glycerol-lactate medium[5,6] labeled with ^{32}P at a specific activity of 120 mC/mg P. The radioactive culture was incubated at 37°, the generation time being about 45 min under these conditions. Samples of the culture were diluted into ice-cold glycerol-casamino acid medium[7] from time to time and aliquots of these dilutions quickly frozen and stored at −196° in liquid nitrogen[4]. From day to day, after various amounts of radioactive decay had occurred, some of the frozen aliquots were thawed and spread on nutrient agar plates for assay of the remaining number of colony formers, *i.e.* viable bacteria. The total amount of ^{32}P incorporated into bacterial nucleic acids was also determined in each sample by the method of SCHMIDT AND THANNHAUSER[8].

The results of this experiment are presented in Fig. 1, where it may be seen that after growth in ^{32}P-labeled medium for only 5 and 10 min, at least 70 % and 95 % respectively, of the bacterial population has already become sensitive to inactivation by decay of incorporated radioactive atoms. In Table I, the average amount of nucleic acid ^{32}P taken up per bacterium after various times of growth is shown and compared with the final slope of the corresponding ^{32}P decay survival curve. It is evident from the last column of Table I that the rate of inactivation per mC of assimilated radioisotope is nearly independent of the total amount of ^{32}P incorporated. Hence it may be concluded that the same mechanism is responsible for inactivation of bacteria labeled by short and by long assimilation periods, *i.e.* that in all the samples of this

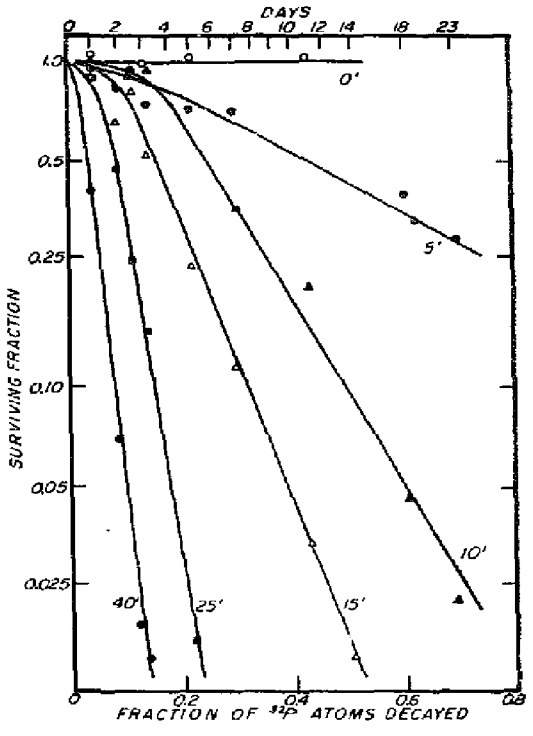

Fig. 1. Colony survival as a function of radioactive decay in samples of a culture of *E. coli* B/r which have been allowed to assimilate ^{32}P of specific activity 120 mC/mg P for the number of minutes indicated on each curve before being frozen and stored at —196°.

TABLE I

Minutes of growth in ^{32}P-labeled medium	*Viable bacteria/ml before decay* $\times 10^5$	*Nucleic acid ^{32}P (mC/cell) (I)* $\times 10^{-10}$	*Final slope of decay survival curve (K)*	*K/I (cell/mC)* $\times 10^{10}$
0	5.8	—	—	—
5	6.1	0.21	— 1.0	—4.8
10	7.8	0.47	— 2.8	—6.0
15	8.0	0.91	— 5.5	—6.0
25	8.1	3.0	—13.8	—4.6
40	11.0	5.2	—18.7	—3.6

experiment the lethal disintegrations represent decay of ^{32}P atoms incorporated into the bacterial deoxyribonucleic acid. The fact, therefore, that after an exposure to ^{32}P amounting to only 11 % of the generation time more than 70 % of the cells have become sensitive to inactivation by decay then indicates that any period during which bacteria of this culture synthesize no deoxyribonucleic acid could occupy at most a very minor fraction of their division cycle, *i.e.* that deoxyribonucleic acid synthesis is *essentially continuous* in *E. coli* cells. This difference in manner of deoxyribonucleic acid synthesis between bacteria and cells of higher forms may reflect the simplicity of nuclear organization of the "lower protista"[9].

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The natural occurrence of β-hydroxyaspartic acid

The formation of β-hydroxyaspartic acid *in vitro* by a transamination reaction between oxaloglycolate and glutamate has been established by studies carried out in this laboratory[1] as well as by the independent work of GARCIA-HERNANDEZ AND KUN[2]. We wish to report the isolation of β-hydroxyaspartic acid from pancreatic digests of casein. The isolation procedure involved the removal of the aromatic amino acids from the hydrolysates by adsorption on charcoal[3] and successive chromatography of the resulting solutions on columns of Dowex-1 formate and Dowex-50, hydrogen form, with [^{14}C]aspartic acid as a column marker.

The isolated compound has been identified by the following enzymic reactions. Incubation of the isolated hydroxyaspartate and α-ketoglutarate with the transaminase preparation from sheep brain[1] resulted in glutamate formation (Table I).

The isolated amino acid (20 μmoles) in the presence of 50 μmoles of [^{14}C]carbamyl phosphate (specific activity, 12,800 counts/min/μmole) and the transcarbamylase preparation from normal rat liver was enzymically converted into a single radioactive compound (total radioactivity, 47,800 counts/min) which on column and paper chromatography was identical with known N-carbamylhydroxyaspartate (ureidomalate)[5].

Further identification of the isolated compound was achieved by chromatographic studies. The isolated amino acid gave the same R_F as authentic hydroxyaspartate in three different solvent systems. The dinitrophenyl derivative of the isolated compound was prepared[6]. Paper chromatography[7] of the derivative gave an R_F (0.24) identical with that of a sample of authentic N-dinitrophenylhydroxyaspartate. Additional studies were carried out on the automatic amino acid analyzer. The isolated compound, synthetic hydroxyaspartate (a mixture of four isomers[8]) and *erythro*-β-hydroxy-L-aspartate[9] were added separately to a synthetic mixture of