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**CANAVANINE DEATH AND THE
MECHANISM OF DNA REPLICATION
IN E. COLI AND MAMMALIAN CELLS**

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1. PROJECT ABSTRACT

Our experiments have revealed that the mechanism by which the amino acid analogue, canavanine, causes death of Escherichia coli cells is intimately related to a disruption of the normal organization of the bacterial genome.

The irreversible positioning of 2 to 8 canavanyl-protein bodies on the bacterial membrane appears to jam the DNA replication process causing an abnormal binding of DNA to the membrane, and disrupt the nucleus. Studies employing cell-free extracts and priming DNA synthesis with ϕX single stranded DNA show that extracts from canavanine-killed cells convert ϕX SS to RFI and RFI as do normal cell extracts. However, the canavanine cell extracts from Pol A⁻ and I⁻ strains carry out an rATP dependent formation of short pieces of DNA in the absence of added primer DNA in contrast to extracts made from arginine-starved or normal cells. This short-piece-synthesis is blocked if canavanine cell extracts are complemented by arginine-starved or normal cell extracts.

Incorporation of canavanine into protein also results in complete blockage of net RNA synthesis in about 30 min. However, during this period both rRNA's and mRNA's are synthesized, but are unstable. Using MS2 RNA and Polyuridylic acid as templates we found that extracts from these cells lost the ability to initiate normal protein synthesis but can elongate peptide chains. Thus accumulation of abnormal initiation factors results in faulty translation and abnormal proteins are made.

II. SCOPE AND OBJECTIVES OF RESEARCH UNDER THIS CONTRACT

- A. The scope of work under this contract is unclassified and consisted of studies of canavanine death in E. coli and mammalian cells. The mechanism by which abnormal amounts of canavanyl-proteins are positioned on cell membranes was studied. The resultant abnormal binding of DNA to membranes, inhibition of DNA replication and RNA synthesis was investigated.
- B. The planned objectives for this contract were as follows:
1. To examine in detail the nature of binding of DNA to the canavanyl-protein-membrane complex by development of in vitro conditions for assembly and by studying conditions for disassembly.
 2. To study the effect of formation of canavanyl-protein-DNA-membrane complexes on the enzymic reactions associated with DNA replication.
 3. To investigate the regulatory system that controls the quantity and pattern of membrane proteins that is upset as a result of canavanine incorporation into protein.
 4. To study the mechanism by which canavanine blocks net RNA synthesis and alters the normal pattern of RNA transcription by the genome.
 5. To investigate in detail the molecular events causing mammalian-cell death by canavanine and other amino acid analogues.

III. RESEARCH ACCOMPLISHMENTS AND EVALUATION

A. Summary of Early Experimental Findings.

1. We showed that growth of Escherichia coli with L-canavanine causes exponential death of these bacteria dependent upon incorporation of this amino acid analogue into protein in place of L-arginine (J. Mol. Biol. 14:474-489, 1965). The production of fraudulent canavanyl-protein by E. coli eventually halts all net RNA and protein synthesis although the enzymic machinery and precursors for these processes remain intact (J. Mol. Biol. 33:843-860, 1968). We also showed that the mechanism of canavanine-death involves the formation of large electron dense membrane-bound canavanyl-protein bodies and extensive physical disruption of the genome region (J. Mol. Biol. 33:861-872, 1968).
2. During the course of this research we made two additional observations that had bearing on our later work:
 - a. Canavanine-dead Escherichia coli are unable to support the growth of T-even bacteriophage. Loss of the ability to make virus is directly related to the kinetics of canavanine death and is due to the synthesis of host protein containing canavanine. T4-infected canavanine-dead cells did not form phage-induced messenger RNA and consequently no phage-induced protein and DNA synthesis were observed.
 - b. Canavanine-resistant mutants have been isolated that still heavily incorporate canavanine into protein, form a few canavanyl-protein bodies, but suffer none of the other defects leading to death. Further, these mutants contain an altered arginyl-RNA synthetase (Faanes and Rogers, 1972).

B. Relationship Between the Bacterial Genome and Membrane Attachment Sites.

1. One of the objectives of this contract was to study in detail the altered relationship between the bacterial genome and its membrane attachment sites caused by the canavanine-induced membrane bound bodies. Our discovery of an abnormal canavanyl-protein-DNA-membrane complex made by E. coli grown with canavanine is presented in the enclosed report (Schachtele, Anderson and Rogers, J. Mol. Biol. 49:255-261, 1970). The major findings were as follows:
2. Arginine-requiring strains of E. coli grown with canavanine were converted to spheroplasts in the presence of lysozyme and gently lysed either by staged dialysis or with Brij 58 (polyoxyethylene cetyl ether). Zonal sedimentation through sucrose EDTA gradients revealed that 60% of the cell DNA formed a heavy complex with canavanyl-protein-membrane pieces while only 1-2% of the cell DNA sedimented with membrane pieces of untreated cells.
3. Electron microscopy of the heavy fraction employing the Kleinschmidt protein film technique revealed that virtually all of the DNA was complexed with canavanine-induced bodies and their membrane attachment sites. (See electron micrographs in Schachtele, Anderson, Rogers, 1970).
4. The significance of this discovery is that the membrane-bound canavanine bodies were directly shown to contain either an abnormal amount or type of membrane-protein involved in DNA-attachment. More specifically the result suggests that, in the presence of canavanine, E. coli may manufacture an excess of DNA attachment proteins. These proteins or DNA-organizers apparently tend to accumulate in specific clumps at specific regions on the membrane. Thus canavanine-death probably results from jamming of the genome at these abnormally large organizer sites on the cell membrane.

C. The DNA Replication Cycle and Formation of the DNA Canavanyl-Protein-Membrane Complex.

We investigated the relationship between the formation of the abnormal membrane bound DNA forced by growth of E. coli in canavanine and the DNA replication cycle. The view was that if a specific set of organizer proteins are responsible for DNA attachment then they might be produced at a particular time during replication of the genome. The results are have been published (Rogers, Kaden and Kelly. Arch. Biochem. Biophys. 144:636-649, 1971). The major findings were:

The complex forms with DNA made either before or during growth with canavanine and the time course of complex formation follows the incorporation of canavanyl-protein into membrane.

No DNA is degraded during a two hr growth period with canavanine, while extensive binding of DNA to the membrane is found.

Both canavanine-death and formation of the DNA-membrane complex occur at the same rate even if cells are previously brought to the completion of their replication cycles before canavanine is added.

Since canavanine does not allow new DNA synthesis after a cycle is completed, this result clearly indicates that the formation of the canavanyl-protein-membrane DNA complex occurs independently of DNA synthesis.

E. coli chromosomes labeled near their origins were not selectively bound to or excluded from the complex suggesting that a particular portion of the genome is not preferentially bound first.

The fact that DNA synthesis is blocked by canavanine after replication rounds are completed led us to examine DNA nucleotidyl transferase activity in canavanine-dead cells. Control cell extracts contained only slightly higher activities than did canavanine dead-cell extracts.

The results indicate that the canavanyl-protein-DNA complex can be formed independent of the DNA replication cycle and that the role of canavanine in blocking new DNA cycles is not responsible for canavanine death but rather complex formation probably is. These binding proteins are possibly related to processes dependent on protein synthesis identified by Lark and Renger as being essential for later successful DNA replication.

D. Properties of the DNA-Membrane Complex.

One of the objectives of the contract was to analyze the canavanine-induced bodies relative to their role in the DNA replication process. The binding or attachment of DNA was the most evident function involved so this aspect was analyzed (Rogers, Kaden and Kelly, Arch. Biochem. Biophys. 144:636-649, 1971). The major findings are as follows:

Pronase, detergents and urea dispersed or hydrolyzed the fast-sedimenting membrane releasing DNA. DNA was removed by shearing or deoxyribonuclease treatment without disturbing the fast-sedimenting canavanyl protein.

Sucrose gradients containing EDTA showed that strong binding of canavanyl-protein to DNA did not require divalent metals. Sedimentation with Mg^{++} or with magnesium lauryl sarcosinate by the method of Erhart et al. yielded heavy DNA membrane complexes for both control cells and canavanine grown cells.

^{32}P -T4 DNA bound strongly to a heavy membrane fraction of normal cells, but not to arginine-starved or chloramphenicol-treated cells where injected ^{32}P -T4 DNA remained at the top of sucrose gradients. This requirement for protein synthesis after phage infection for phage DNA binding to the membrane was not seen when cells were pre-grown in canavanine. In canavanine-grown cells all injected T4 DNA attached to the membrane with chloramphenicol added. These data show that the canavanyl-protein-membranes entrap T4 DNA very much like host DNA, and in both cases neither new replication nor transcription can occur.

The significance of this unusual property of DNA binding developed by membranes of canavanine-grown E. coli is not yet clear. However,

it is evident that the positioning of canavanine-proteins on the membrane somehow irreversibly blocks all normal metabolic events associated with genome function. Also this system provides a unique opportunity for study of DNA-membrane complexes, since apparently an excess of DNA-binding sites are produced during canavanine-death.

E. The Envelopes of Canavanine Grown Cells.

1. The substitution of canavanine for arginine was shown earlier to cause an unusual relative commitment of total protein synthesis into envelope or membrane protein (Schachtele and Rogers, 1968; Rogers, Kaden and Kelly, 1971). Further isolated canavanine-induced protein bodies together with pieces of membrane showed a normal composition of amino acids relative to whole E. coli protein (Schachtele and Rogers, 1968). Recently we have studied further the kinetics of synthesis and the structural components found in envelopes from canavanine-grown E. coli. This work (T.D. Allen, Ph.D. Thesis, University of Minnesota) is not yet published. The major findings are as follows:
2. An abnormally large commitment of protein synthesis to the envelope fraction in canavanine-grown cells was shown by first growing cultures in ^{14}C -canavanine or ^{14}C -arginine and then isolating envelopes by sonic disruption and centrifugal fractionation.
3. A large amount of envelopes from canavanine-grown cells was compared with those from an equal quantity of cells taken just before canavanine-growth. Chemical analysis revealed a 6.3 fold increase in envelope protein and only a 10% increase in cytoplasmic protein. Lipids and carbohydrate in the envelopes of canavanine-grown cells only doubled at the same time. Further analysis of the lipid fractions by thin-layer chromatography showed the same phospholipid composition for both canavanine-grown and normal cell membranes.
4. The proteins of membranes prepared by lysozyme-EDTA treatment followed by DNase digestion, centrifugal fractionation, and lipid extraction, were separated by acidic acrylamide gel electrophoresis. About 11 or 12 protein peaks are developed. The most important observation is that canavanine-membrane proteins appear qualitatively the same as normal membrane proteins. However, the relative amount of at least one protein is radically reduced (Peak V) while others are greatly increased (Peaks VII, IX, and X) in canavanine-grown cells. Hence, membrane protein content is definitely distorted suggesting that canavanine-incorporation caused a disruption in the regulation of membrane protein synthesis and/or assembly.
5. Which of these proteins is responsible for the abnormal DNA binding and stoppage of normal genome function? The answer to this is not yet known. The fact that some of these proteins accumulate as membrane attached amorphous protein bodies (Schachtele, Anderson and

Rogers, 1968) indicates that assembly of membrane is abnormal during growth with canavanine.

6. The kinetics of assembly of the canavanine-induced protein into membranes was studied in detail. During the course of growth in canavanine the fate of pulse-labeled protein was tracked. It was found that supernatant protein migrated to envelope sites requiring about 5 min for assembly. This assembly process was inhibited by chloramphenicol added after the pulse of radioactive amino acids. The chloramphenicol experiment further indicates that supernatant proteins destined for membrane are unstable if not assembled and also assembly requires continuous protein synthesis. Pulse-labeling of normal cells for 0.5 or 2.0 min did not reveal a similar slow assembly process for envelope proteins.
7. These results permit the hypothesis that canavanine incorporation promotes the synthesis of an excess of one set of membrane proteins and limits synthesis of another set of proteins. If both sets are required for membrane assembly, a lag in pulse-labeled protein inclusion into membrane would be seen and would be prevented by blocking protein synthesis.

F. Block of DNA Replication in Ether-treated Canavanine Grown Cells.

1. A series of arginine auxotrophs were produced from strains containing combinations of End I⁻, Pol A⁻, and RNase⁻ mutations. All of these strains were sensitive to growth with canavanine and large membrane-bound bodies were observed as reported previously for other E. coli strains (Schachtele, Anderson, and Rogers, J. Mol. Biol. 33:861, 1968).
2. Cells grown with canavanine (+ Can) were compared to cells starved for arginine (-Arg) or grown normally in exponential phase (+ Arg) and cells were rendered permeable to nucleotides by ether treatment. (Vosberg and Hoffmann-Berling, J. Mol. Biol. 58:739, 1971). When supplemented with a complete set of dNTPs together with rATP and Mg⁺⁺ the +Arg cells carry out synthesis of DNA, while, in contrast, both -Arg and +Can cells produce less than 10% of the normal activity. In Pol A⁻ strains or End I⁻ strains the synthesis is entirely PCMB sensitive.
3. Following pretreatment with pancreatic DNase, ether-treated Pol A⁺ End I⁻ cells grown + Arg, - Arg or + Can all carry out a PCMB insensitive repair synthesis of DNA. In contrast, Pol A⁻ End I⁻ ether treated cells grown + Arg, - Arg or + Can carry out no repair synthesis following DNase pretreatment.
4. The above results with ether-treated cells, first of all, establish that the block in DNA synthesis caused by canavanine is not due to a lack of dNTP's or rATP, since these are supplied during the reaction. Secondly, the block is probably not due to random extensive cutting of DNA since PCMB resistant repair in + Can cells

occurs only after treatment with DNase in Pol A⁺ cells. Also no new repair enzyme is present in + Can cells since in Pol A⁻ cells following DNase treatment no DNA synthesis occurs. The lack of recovery of + Can cell ability to produce an active DNA synthesis in ether-treated cells following growth in arginine, suggests that the block in host genome replication is irreversible as was previously reported using whole cells. (Schachtele and Rogers, 1968).

5. We are presently studying the ability of ether-treated + Can cells to carry out conversion of ϕ X DNA to its replicative form using the methods of Hess, V. et al., J. Mol. Biol. 73:407, 1973.

G. Effect of Growth in Canavanine on Cell Free DNA Synthesis.

1. Cell-free extracts of + Arg, + Can, and - Arg grown cells were prepared from Pol A⁻ and I⁻ and Pol A⁻ and I⁺ cells by two methods. The method of R.D. Wickner, et al., Proc. Natl. Acad. Sci. 69: 3233, 1972 employing lysozyme and Brij-58 was used. The second method developed by Hoffmann-Berling (personal communication) is essentially the production of a concentrated suspension of ether-treated cells, followed by a 45 min incubation with lysozyme in ice and centrifugation at high speed yielding a concentrated protein supernatant with only 1% of the cellular DNA. These two methods of preparation gave strikingly different results with canavanine grown cells (+ Can) but the same results with normal cells. DNA synthesis is primed with added fd or ϕ X single stranded DNA and requires the four dNTPs and rATP and continues for 25 to 30 min at 31°C with almost complete conversion of SS $\rightarrow$ RF. We found a definite inhibition of DNA synthesis in + Can cell extracts prepared by the Brij lysozyme method. Secondly, canavanine grown cells in extract make DNA without added primer (fd or ϕ X).
2. Inhibitor of DNA synthesis in Brij 58-treated canavanine grown cells. Extracts of + Can cells prepared by the Brij-lysozyme technique were combined with extracts from + Arg cells prepared either by Brij-lysozyme or by ether-lysozyme. We discovered a 80 to 90% inhibition of normal DNA synthesis primed by SS ϕ X, dependent upon the concentration of + Can extract added. The inhibitor activity was found to be heat unstable, non-dialyzable and not precipitated with 7% polyethylene glycol. Chromatography of the + Can extract on DNA agarose columns revealed a major peak of inhibitor activity eluting at 0.1 M NaCl.

Our studies indicated that the inhibitor activity was not blocking available replication sites on the ϕ X DNA nor was it reversed by adding excess normal extract. Preincubation of the + Can extract with various combinations of the reaction mixture, DNA, and normal extract, revealed that the inhibitor activity was due to dephosphorylation of deoxyribonucleoside triphosphates. This was confirmed by thin layer chromatography. Although dNMP kinase activity was found in + Can extracts, conversion of NMP to dN + Pi was very

rapid. Phosphatase activity and NMP kinase activities were monitored in ether-treated cells of + Arg, - Arg, and + Can cultures. In contrast to Brij-lysozyme extracts, the ether-treated cells had apparently normal levels of dNMP kinases and the phosphatase activity. These results indicate that Brij-lysozyme-treated + Can cells are peculiar in excessive release of the phosphatase activity, since no extracts of - Arg or + Arg cells prepared by ether-lysozyme or Brij-lysozyme yielded inhibitor activity. Further, adding Brij-58 to ether-treated + Can cell extracts did not unmask an inhibitor activity. The significance of this Brij-releasable inhibitor activity is not yet clear. It may indicate an instability of the membrane of E. coli caused by incorporation of canavanine-proteins, since 70% of these abnormal proteins accumulate on the membrane.

3. Degradation of DNA by canavanine grown cell extracts. We prepared double stranded ^{32}P -E. coli DNA and single stranded ^{32}P - ϕX circles in order to test the possibility that exo and endo nuclease activities were enhanced in canavanine-grown cell extracts (+ Can). We found that extracts of + Arg, + Can and - Arg rendered only 16% of the ^{32}P -E. coli DNA acid soluble after 30 min. Using end I⁻ cell extracts we found very little breakdown of ϕX SS DNA in any extracts. Thus growth with canavanine does not cause a significant increase in DNA cutting enzymes.
4. Abnormal synthesis of small DNA pieces by extracts from canavanine grown cells. Extracts prepared from arginine starved cells (- Arg) or normal cells (+ Arg) produce less than 10% and usually about 2% of the DNA synthesis activity found when ϕX or fd SS DNA is added. In contrast, we observed a high rate of DNA synthesis in extracts from + Can cells independent of added DNA. This phenomenon was found in Pol A⁻ end I⁻ strains as well as in end I⁺ strains. We determined that only 1 to 2% of the cell DNA was in the + Can extracts, similar to normal cell extracts. The small amount of DNA could, of course, account for the priming activity but there was no excess DNA present. Neutral sucrose and alkaline sucrose gradient sedimentation revealed that the DNA made by + Can extracts in the absence of added DNA was exclusively in small pieces 10 to 7S. When ϕX DNA was added about one-half of the synthetic activity was shifted to ϕX conversion to RF. Normal cell extracts make only RF II and RF I but do not accumulate small DNA pieces.

Complimentation of + Can extracts with either extracts from + Arg or - Arg cells in the absence of added DNA completely blocked formation of the small DNA pieces. Thus normal cells contain some control elements that prevent this abnormal DNA synthesis. We have found that "control" is heat unstable.

The small-piece synthesis is insensitive to preincubation with 200 $\mu\text{g/ml}$ RNase (pancreatic), it requires rATP and all the dNTPs but not the remaining rNTPs.

5. We conclude from the above results that canavanine grown cells contain the enzymes and proteins to carry out conversion of ϕ X DNA from SS $\rightarrow$ RF II $\rightarrow$ RF I as well as conversion of fd DNA in extracts. This result is in striking contrast to host-dependent DNA synthesis in ether-treated cells, where, apparently, host DNA is not synthesized. The disorganization of synthesis of the host genome appears to be irreversible and may in some manner be related to the abnormal synthesis of short pieces of DNA found in canavanine extracts. This possibility is under investigation. The control of short piece synthesis by mixing normal extracts with + Can extracts suggests that some protein or other element in normal extracts plays a role in modulating abnormal synthesis.

H. Effect of Canavanine on RNA Synthesis in E. coli.

1. Earlier we found that net RNA synthesis stopped after 40 min of growth of E. coli with L-canavanine in place of arginine. We suspected that since canavanyl-tRNA^{arg} levels are always low (Faanes and Rogers, 1972) that the stringent control of rRNA synthesis was responsible similar to that observed with arginine-starved cells. But experiments with RC⁻ cells showed the same blockage of RNA synthesis after growth in canavanine. RNA synthesis was monitored for 10 min periods from 0 to 40 min after adding canavanine to cell cultures. Extracted ³H-RNA was examined by velocity sedimentation through sucrose gradients and an inversion of 16S and 23S peaks was noted. Relatively less radioactivity was incorporated into 23S rRNA. These results suggested that rRNA synthesized during growth of cells with canavanine is unstable.
2. Using pulse labeled ³H-RNA hybridized with E. coli DNA and competing with various concentrations of unlabeled purified rRNA, we found that the relative proportions of rRNA to mRNA synthesis remained constant during growth with canavanine. Thus a preferential block of rRNA synthesis was not noted but rather the data suggest turnover of unused rRNA.
3. Lac-mRNA levels were examined in cells grown with canavanine by pulse labeling with ³H-uridine (2 min) at times 0 through 40 min after canavanine growth. The Lac-mRNA levels were determined by hybridization with λ plac DNA. The results showed a constant level of message at all times during growth with canavanine.
4. The data above suggested that RNA synthesis was not in fact blocked but only that RNA's made are rendered unstable. Experiments with Ether-treated cells (Vosberg and Hoffman-Berling) were performed. ³H-UTP incorporation into RNA required all rNTPs. The ether treated canavanine-grown cells incorporated ³H-UTP into RNA as well as normal ether-treated cells. Further adding ³H-rU, rC, rA or rG together with rATP, we observed formation of all nucleoside triphosphates by canavanine-grown cells indicating that the nucleoside Kinases are working properly.
5. But what is the explanation for instability of the rRNA in canavanine grown E. coli? First, we found a rapid decay (10 fold in 30 min) of ability of canavanine grown cells to produce β -galactosidase upon adding back

arginine and the inducer, ITPG. This was surprising since lac-mRNA synthesis remained normal. But the result suggested a translational defect produced by incorporation of canavanine into protein.

6. Extracts of canavanine grown and normal cells were prepared and stripped of natural mRNA by the method of Zubay. Formally we had found that Polyuridylic acid translation to polyphenyl alanine was normal in extracts from canavanine grown cells (Schachtele and Rogers, 1968). However, using MS2RNA as messenger we found a rapid decay of ability of these extracts to translate.
7. Ribosomes from both normal and canavanine-grown cells were washed with NH_4Cl and combined with supernatants from the same and opposite extracts. Only the supernatant from normal cells supported translation of MS2RNA using both ribosome fractions. The result indicates that the initiation factors in canavanine grown cells are faulty. This may mean that one or more of these factors are turning over or that canavanyl-initiation factors accumulate and prevent normal initiation of protein synthesis. Thus the change in RNA synthesis observed and instability of rRNA may result from abnormal initiation of protein synthesis.

IV. GRADUATE STUDENTS TRAINED

Charles F. Schachtele, 1965-1968. Ph.D. degree in microbiology with minor in biochemistry. Thesis: "The Mechanism of Canavanine Death in Escherichia coli."

Ronald B. Faanes, 1966-1970. Ph.D. degree in microbiology with minor in biochemistry. Thesis: "The role of arginine and canavanine in regulation of arginine biosynthesis in Escherichia coli."

Theodore D. Allen, 1968-1972. Ph.D. degree in microbiology with minor in biochemistry. Thesis: "The effect of canavanine on protein synthesis."

Matti P. Korhola, 1973-present. Major in Microbiology with minor in biochemistry. Work not completed.

V. PUBLICATIONS ASSOCIATED WITH THIS CONTRACT

1. Schachtele, C.F. and Rogers, P. Canavanine incorporation into Escherichia coli and disruption of protein synthesis. Bact. Proc., 131, 1967.
2. Schachtele, C.F. and Rogers, P. Mechanism of canavanine death in Escherichia coli. I. Effect of canavanine of macro-molecular synthesis. J. Mol. Biol. 33:843, 1968.
3. Schachtele, C.F., Anderson, D.L. and Rogers, P. Mechanism of canavanine death in Escherichia coli. II. Membrane-bound canavanyl-protein and nuclear disruption. J. Mol. Biol. 33:861, 1968.
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6. Rogers, P., Kaden, T. and Kelly, M.V. Abnormal genome attachment to E. coli membrane caused by canavanine. Bact. Proc. 55, 1970.
7. Allen, T.D. and Rogers, P. Membrane-bound canavanine-containing proteins in normal and T4-infected Escherichia coli. Bact. Proc. 150, 1970.
8. Rogers, P., Kaden, T., and Kelly, M.V. Canavanine-induced fast-sedimenting complexes with Escherichia coli DNA and bacteriophage T4 DNA. Arch. Biochem. Biophys. 144:636-649, 1971.
9. Allen, T.D. and Rogers, P. The effect of canavanine on the synthesis of protein in envelope and cytoplasmic fractions of Escherichia coli. In preparation, COO-1562-19.
10. Faanes, R. and Rogers, P. Repression of enzymes of arginine biosynthesis by L-canavanine in arginyl-transfer ribonucleic acid synthetase mutants of Escherichia coli. J. Bacteriol., 112:360, 1972.
11. Rogers, P. and H. Hoffmann-Berling. Effect of growth of Escherichia coli with canavanine on cell-free DNA synthesis. Abstract Ann. Meeting ASM, 161, 1974.

VI. PERCENTAGE EFFORT OF PRINCIPAL INVESTIGATOR

Palmer Rogers, Ph.D. - 20% time during the entire project period.