COO-3225-35

ENZYMATIC STUDIES OF RADIATION DAMAGE

Progress Report

1977 - 1978

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Including the summary for the triennium

1975 - 1978

PREPARED FOR THE UNITED STATES DEPARTMENT OF ENERGY
UNDER CONTRACT NO. AT(11-1)3225

REA

DISCLAIMER

Publications:

COO-3225-36 Removed

Pritchard, A.E., Kowalski, D. and Laskowski, M., Sr., An Endonuclease Activity of Venom Phosphodiesterase Specific for Single-Stranded and Superhelical DNA. (1977) *J. Biol. Chem. 252*, 8652-8659.

COO-3225-37 Removed

Fujimoto, M., Pritchard, A.E., Kowalski, D. and Laskowski, M., Sr., Accessibility of Some Regions of DNA in Chromatin (Chicken Erythrocytes) to Single-Strand Specific Nucleases (1978) *Fed. Proc. 37*, 1738, abst. 2574.

COO-3225-38 Removed

Pritchard, A.E. and Laskowski, M., Sr., Discrete Fragments Produced by Limited Digestion of Superhelical PM2 DNA with Venom Phosphodiesterase: Cleavage Site and Mode of Action (1978) *J. Biol. Chem.* accepted, preprint enclosed.

Activities other than research:

1. I was invited to attend the International Symposium, entitled "Nucleases, Structure, Function and Application," September 6-8, 1977, Szeged, Hungary. I chaired the afternoon session of September 6, and presented a 45-minute lecture "Single-Strand Specific Nucleases" on September 7th. The trip was paid by UNESCO.

2. Since 1977, the American Cancer Society reestablished the title of American Cancer Society Professor, as well as some functions connected with it. I participated in the meeting November 28 - December 2, 1977, in Phoenix, Arizona and spoke on "Novel Properties of Venom Phosphodiesterase."

3. I was invited to contribute to the Methods in Enzymology, Nucleic Acids, Part H, on both enzymes that are actively worked in our laboratory, mung bean nuclease and phosphodiesterase. The two articles, hopefully, will be reported next year as published.

4. In January 1978, I was a corecipient of the Jurzykowski Award that was split between my son and myself.

Summary

This year's list of publications is short: one full paper and one abstract have been published; the second paper was accepted by the Journal of Biological Chemistry but is listed now, because its contents have never been presented, and because the two papers are closely connected and represent our major accomplishment during this year. The work on venom phosphodiesterase has been going on in our laboratory for about 25 years and was supported first by AEC, then by ERDA. Until last year the enzyme was considered a typical exonuclease. It was recently found that venom phosphodiesterase also has properties of single-strand specific enzymes; it cleaves circular superhelical DNA and produces a full-length linear DNA (form III) plus a number of discrete bands of fragmented double-stranded DNA. The cleavages that lead to these fragments occur in pairs. Each pair accounts for the total length of the genome. Mapping of fragments showed that 4 our of 5 susceptible sites corresponded to previously established (Brack, C., Bickle, T.A. and Yuan, R. (1975) *J. Mol. Biol. 26,* 693-702) early denaturation sites.

Single-strand specific enzymes were studied with chromatin (chicken erythrocytes) as substrate. So far only an abstract has been published. Chromatin is reluctantly digested to products of very high molecular weight ranging from 30 to 100 S with an average of about 60 S. Further digestion of these products with micrococcal nuclease leads to nucleosomes suggesting that nucleosomes remain preserved.

Research:

Documents COO-3225-36 and COO-3225-38 are closely connected and I would like to discuss them together even though the latter has just been accepted for publication by the J. Biol. Chem. and has not been presented anywhere except Phoenix, Arizona and a brief reference during the Symposium in Szeged, Hungary. Document COO-3225-36 is a full paper of the abstract COO-3225-34 discussed in detail last year. The paper also incorporates an improvement of the method of preparation of venom phosphodiesterase described in the document COO-3225-28. This improvement not only makes the preparation of phosphodiesterase more reproducible but also removes the last traces of endonuclease. However, the incorporation of the methodological part into the document COO-3225-36 is only a peripheral issue. The central issue of this paper is the mechanism of action of phosphodiesterase on circular supercoiled DNA. Under specific ionic conditions the circles are first nicked (form II); then linear DNA (form III) is produced, but besides the full length DNA a number of discrete double-stranded fragments is also produced. The linear double-stranded fragments is also produced. The linear double-stranded DNA and the fragments are then shortened by exonucleolytic action of phosphodiesterase. Contrary to the previous belief of unidirectional 3' → 5' action, the linear fragments are shortened from both ends because no cohesive (single-stranded) ends remain. Presumably, as soon as the degradation from the 3'-OH terminus denudes a short fragment of a complementary strand (probably no longer than a tetranucleotide), and endonucleolytic cleavage occurs, and is followed by a rapid production of 5'-mononucleotides. The

discrete bands representing fragments shorter than the whole length (form III) are quite sharp and resemble bands seen with restriction enzymes.

Document COO-3225-38 is a continuation of this work. Using PM2 superhelical DNA, the discrete bands were particularly pronounced when an appropriate concentration of Mg^{2+} was present. By the appearance alone (intensity) it looked as if bands are produced in pairs. After the molecular weights of the suspected pairs were measured they added to a full-length form III DNA. An additional criterion was used. Restriction enzyme Hpa II cleaves PM2 DNA at only one site (Brack, C., Eberle, H., Bickle, T.A. and Yuan, R. (1976) *J. Mol. Biol. 104,* 305-309). If the pair of fragments was properly selected, only one member of the pair should be susceptible to Hpa II. This indeed has been the case. With the use of Hind III restriction enzyme and the data of Brack et al. it was possible to map the fragments, and to compare phosphodiesterase cleavage sites with the previously mapped easily denaturable regions (Brack, C., Bickle, T.A. and Yuan, R. (1975) *J. Mol. Biol. 96,* 693-702). Four out of five cleavages by phosphodiesterase corresponded to the early denaturation regions.

Since only superhelical DNA gives raise to discrete fragments, and since it is known that a single cleavage relaxes the supercoiled form within milliseconds, the two cleavages leading to a pair of fragments must be almost simultaneous. On the genome map, however, the cleavages leading to a pair are far apart suggesting that they might have occurred at the base of a superhelical branch. The fact that one (out of 5) cleavage site did not correspond to an easily denaturable region, suggestes that besides being rich in A+T,

additional factors (presumably tertiary structure) decide on susceptibility of the locus to phosphodiesterase. Another possible interpretation is that the fifth susceptible site is equally rich in A+T, as are the others, but the easy denaturation of this site has been prevented either because it is too short, or because the neighboring regions are unusually rich in C+G. (Chan, H.W., Dodgson, J.B. and Wells, R.D. (1977) *Biochemistry 16*, 2356-2366).

Document COO-3225-37 is an abstract describing the action of single-strand specific nucleases on chromatin. This is the first attempt on our part to compare mung bean nuclease, venom phosphodiesterase and P_1 nuclease using this substrate. All three enzymes have similar catalytic properties and attack chromatin (chicken erythrocytes) rather reluctantly. The exhaustive digestion leads to "superbeads" that correspond in size to hexanucleosomes and larger. These large bodies were isolated by filtration through Bio-Gel A-15 m and digested with micrococcal nuclease to yield predominantly mononucleosomes. The results were interpreted as indicative of different susceptibility of different spacers (linkers) to single-strand specific nucleases. An alternative is to assume that the space arrangements prohibit the approach of single-strand specific nucleases to make contact with every spacer.

The work on chromatin was conceived because we considered it a good substrate for comparing individual enzymes belonging to the same group of single-strand specific nucleases. The preliminary results, however, encourage further work on the system, even though the difference among the individual enzymes do not seem to be great. We do not expect equally clear-cut results as those obtained with another of our enzymes — micrococcal nuclease — which produces rather uniform

mononucleosomes. We look forward, however, to finding conditions of stopping the reaction on the level of predominantly "superbeads".

Additional experiments with mung bean nuclease have been performed that deserve mentioning. In the first category are experiments done outside of our laboratory using our preparations. The second category comprises the work that is not yet accepted for publication but, hopefully, will reach this stage within a few months.

To the first category belongs the paper of Timble, R.B. and Maley, F. (1977) "The Use of Endo-β-N-Acetylglucosaminidase H in Characterizing the Structure and Function of Glycoproteins," *Biochem. Biophys. Res. Commun. 78,* 935-944. On treatment of mung bean nuclease with the endoglucosidase H, the molecular weight decreased from 39,000 to 31,000 daltons. The peptides produced on reduction of this enzyme with thiol were 18,700 and 12,500 daltons, confirming our previous finding (COO-3225-31) that carbohydrate had been present on both peptides. Penicillium nuclease P_1 was decreased in size from 40,000 to 30,000 daltons. Enzymatic activity was slightly affected, and both activities: nuclease (measured on heat denatured DNA) and ω-monophosphatase (measured on 3'-AMP) were affected to a similar degree.

Enzyme		$\frac{\omega\text{-Phosphatase}}{A_{280}}$	$\frac{\text{Nuclease}}{A_{280}}$	$\frac{\text{Phosphatase}}{\text{Nuclease}}$
Mung Bean Nuclease	Before	4,918	2,732	1.80
	After	3,891	2,224	1.75
P_1 Nuclease	Before	2,132	428	5.0
	After	1.906	311	6.1

There are additional experiments worth discussing even though they are not yet accepted for publication. The site specificity of

mung bean nuclease has been investigated on viral linear duplex DNA. It was found that the number of early cleavages decreases with decreasing molecular weight of the substrate, whereas an average size of the final product of this first phase of the reaction is rather similar for all three viral DNA used and accounts for approximately 0.65 million daltons. The stage of the reaction of similar sizes of products is transient, but rather long lasting. It is interesting to speculate that the size and number of fragments produced during the first phase of the reaction of mung bean nuclease on T_7 DNA correspond to those of genes. The agreement may be more than a coincidence. After an exhaustive digestion the final stage of degradation is reached. The products are composed of small oligonucleotides and mononucleotides. The final stage is reached by an exophilic (end-preferring) action of the enzyme that is accelerated by the low ionic strength of the medium.

The next question that was experimentally approached is how much of a gap is required to direct a cleavage to the complementary strand. In T_5st(0) DNA the naturally occurring nicks (known to be located in C+G rich regions) do not direct the cleavage across until the nick is enlarged with the aid of exonuclease III to a gap (5 residues average). In the native T_7 DNA exposed to mung bean nuclease 3 nicks per mole occur before the rate of double-strand cleavages becomes parallel to the rate of nicking. With T_7 DNA and mung bean nuclease the nicks as well as double-strand cleavages occur in A+T rich regions. It is hoped that this part of the work will be reported as published in the next year's report.

The work on the structure of mung bean molecule was also started, but presumably will take a little longer to achieve the publishable

stage. In view of a great similarity of P_1 and mung bean nuclease most of the work was done on P_1 which is relatively easy to prepare, and only critical experiments were checked on mung bean nuclease. So far the interesting results show that presence of Zn^{2+} is necessary not only for activity but also for the integrity of the enzyme molecule as recorded on acrylamide gel. Conditions of inactivation by dialysis at pH 5 and reactivation using thiols and Zn^{2+} were also studied.

Summary for the Triennium 1975 - 1978

Besides the detailed yearly reports for 1975/76, 1976/77, and 1977/78 (attached above) a short summary covering the work of the triennium follows. It is arranged in the same order of 6 subheadings that were used in the report for the previous triennium.

1. Main research accomplishments with special reference to the originally stated objectives.

Our starting point was the assumption that the detection of minor deviations in a regular DNA structure may be accomplished by the single-strand specific enzymes before it can be shown by physical means. Structural changes in DNA are required for the regulation of growth and development of an organism and are also observed with purified DNA *in vitro*. For example, regions of duplex DNA rich in A+T undergo an opening and closing transition termed "breathing". The breathing process is exaggerated when the DNA is supercoiled. Minor structural deviations can also be induced using radiation or mutagenic chemicals. Such alterations in DNA structure are produced in the course of interaction of an organism with its environment. The single-strand specific nucleases provide a sensitive probe for changes in DNA structure that occur in the normal course of development and those that result from the detrimental effects of the environment.

In order to proceed with the use of single-strand specific enzymes as sensitive reagents their purity must first be assured. In the preceding triennium two important enzymes, mung bean nuclease (COO-3225-03) and venom phosphodiesterase (COO-3225-16) were purified to homogeneity as verified by several criteria. In the

presently accountable triennium further improvements were introduced into the method of preparation of each enzyme (COO-3225-28, venom phosphodiesterase and COO-3225-31, mung bean nuclease). Only after highly purified venom phosphodiesterase became available was it possible to classify it as belonging to the group of single-strand specific enzymes. Ironically, this enzyme has been considered as almost private property of our laboratory for the past 25 years. The work was supported first by AEC, then by ERDA. This enzyme gave maximum information concerning the exact locations of susceptible sites in viral DNA (COO-3225-38). As expected single-strand specific enzymes cleaved double-stranded native DNA predominantly at the A+T rich, easily denaturable regions that were previously mapped by Brack, C., Bickle, T.A. and Yuan, R. (1975) *J. Mol. Biol. 26,* 693-702. However, one out of five identified cleavages was found outside of these areas, suggesting that either the enzyme is more sensitive than physical methods, or that irregularity other than the one leading to an easy denaturation is also detected by the enzyme. If in the future it will be found that this fifth region is A+T rich but short, our original supposition that single-strand specific enzymes are more sensitive than any of the physical probes would be confirmed. If, on the other hand, the odd cleavage will be proven to occur in an A+T poor region, it will lead to the conclusion that other aberrations in tertiary structure also affect the vulnerability.

Some progress was achieved with the further characterization of mung bean nuclease as a protein, even though it did not reach the publishable stage. It was shown that Zn^{2+} is required for the integrity of the enzyme molecule.

Much further advanced is the work on the mechanism of the first

phase of the reaction of mung bean nuclease. It was discussed on page 8. The major finding is that the preexisting nicks in T_5 DNA (known to occur in CG rich region) do not induce cuts across from the nick when exposed to mung bean nuclease. Only after the nick was expanded to a gap (an average of 5 bases) the cut across becomes favored. On the other hand nicks inflicted by mung bean nuclease on T_7 DNA act as inducers for the cuts across, presumably because they are rapidly enlarged to gaps by the exophilic action of the nuclease itself.

The experiments with chromatin as a substrate are too recent to entitle to the far-going speculations. I frankly admit that the success of one of the enzymes discovered in my laboratory — micrococcal nuclease, took me by surprise. The enzyme is specific for the linkers and leaves nucleosomes preserved. It is now a standard enzyme used for the preparation of nucleosomes from various sources. I do not think that we stand a chance of an equally strong specificity of retaining "superbeads" and hydrolyzing linkers between them. I do think, however, that of all so far suggested means of preserving structures more complex than mononucleosomes, some of the enzymes belonging to the group of single-strand specific appear to be the most promising.

2. Plans for the continuation of present objectives and possible new objectives in consideration of past results.

The description of our detailed plans is to be found in the accompanying application for the renewal. Here I am describing only what could be called "speculation on the theme". The observation that an average size of products of the first phase of the reaction of

mung bean nuclease is 0.6 million daltons regardless whether the starting material is a large T_7 DNA or a small PM2 DNA may not be trivial. This average represents a range of variation from 0.2 to 1.4 million daltons and falls in the range of T_7 DNA genes. It is known that A+T rich regions exist between some of the genes of T_7 DNA. Should this speculation that fragments rapidly formed by mung bean nuclease correspond to operational genes be substantiated, the physiological significance of single-strand specific enzymes in preserving the conserved DNA will be strengthened. Single-strand specific nucleases may also allow a closer look on the tertiary structure of viral DNA. The experiments with venom phosphodiesterase are particularly suggestive. The occurrence of two almost simultaneous cleavages places them at the nearest proximity. Yet on a genetic map they are far apart. One may speculate that supercoiling occurs in the non-transcribable areas and thus protects the vital portions.

The use of chromatin as substrate for the single-strand specific enzymes is recent and the facts are meager even for extended speculations. Contrary to micrococcal nuclease only some linkers (spacers) are susbeptible to single-strand specific enzymes which results in a final product that is a multiple of a nucleosome: —a "superbead". The structure of nucleosome is preserved in a superbead, because digestion of the isolated superbead with micrococcal nuclease leads to nucleosomes. So far we found very little variation among different single-strand specific enzymes. Presumably all go through the same stage. The questions we hope to ask: Are the superbeads only a packing arrangements or some functional unit? Are they equally well-defined entities as mononucleosomes?

3. Graduate students trained, degrees granted and postdoctoral tenures completed.

After surgery (November 1974, removal of adenocarcinoma of colon) I stopped accepting graduate students. I have one student, Miss Janice Pruch, who was accepted just before my operation. I still believe that I have a good chance to see her through. Mr. Robert Zellmer was a graduate student for a year in our department. He was assigned to Dr. David Kowalski. However, after a year's stay he decided to switch from biochemistry to physical chemistry and transferred to the Ohio State University.

As has been my custom of many years I am accepting 4 postdoctoral fellows per year. That is my defense against aging. I hope to retain the 4 postdoctoral fellows every year. During the past triennium the funds from ERDA were used to support Dr. Warren D. Kroeker (1974-1976) and Dr. Arthur E. Pritchard, 1976 until present. Dr. Kroeker accepted the position with P.L. Biochemical in Milwaukee and did not break the connection with our laboratory. He still collaborates in preparing block polymers. We keep him as a consultant and he comes occasionally to Buffalo. In addition, five other postdoctorals, whose salaries came from other grants, were profiting from the equipment, supplies, secretarial help, and other services provided partially by ERDA. They were Drs. Siwecka, Oda, Kurecki, Fujimoto and Satoh. The last three are still with us. I did not stop accepting summer students, on the contrary I accept as many as the laboratory space and funds permit. They do not require long-term obligation on my part and bring the enthusiasm which without graduate students would be lacking. I have been accepting summer students for many

years and have found this practice rewarding. Similarly, summer students all supported by different grants, including ERDA, have used facilities and supplies provided in part by ERDA. They were: Lawrence Perry, Paul Paroski, Thomas Foels, Elizabeth Paroski, David Dickman, David Hartman, and Donald Lewicki.

4. Bibliography

During these three years a total of 12 publications supported by ERDA appeared. Of these five were abstracts (COO-3225-27, COO-3225-38, COO-3225-34 and COO-3225-37); five were research papers (COO-3225-31, COO-3225-32, COO-3225-33, COO-3225-36, COO-3225,38) and two were invited reviews (COO-3225-25, COO-3225-30).

COO-3225-25 Kowalski, D. and Laskowski, M., Sr., Functional Characterization of Nucleophosphodiesterases, in *Handbook of Biochemistry*, G.D. Fasman, ed., The Chemical Rubber Co. (1976) 3rd edt., Vol. 2, pp. 491-531.

COO-3225-26 Kowalski, D. and Kroeker, W.D., Physical, Chemical and Catalytic Properties of the Single-Strand Specific Nuclease from Mung Bean Sprouts, *Fed. Proc. 35*, p. 1588, (abst. 1176) (1976).

COO-3225-27 Kroeker, W.D., Kowalski, D. and Laskowski, M., Sr., Action of Single-Strand Specific Mung Bean Nuclease on Native DNA, *Fed. Proc. 35*, p. 1619 (abst. 1324) (1976).

COO-3225-28 Perry, L.E., Pritchard, A.E. and Laskowski, M., Sr., Preparation of Endonuclease, Exonuclease and 5'-Nucleotidase from the Same Sample of Venom, *5th International Symposium on Toxinology*, Costa Rica, P. 29, abst. BC5, August 1976.

COO-3225-30 Laskowski, M., Sr., Sulkowski, E., Duch, D.S., Richards, G.M., Kress, L.F., Dolapchiev, L.B., Sieliwanowicz, B., Yamamoto, M., Tutas, D.J., daRoza, D. and Pruch, J., Nucleolytic Enzymes of the Venom of *Crotalus adamanteus*, in *Animal, Plant and Microbial Toxins*, Vol. 1, eds. A. Ohsaka, K. Hayashi and Yoshino Sawai, Plenum Press, New York, N.Y., pp. 235-248 (1976).

COO-3225-31 Kowalski, D., Kroeker, W.D. and Laskowski, M., Sr., Mung Bean Nuclease I. Physical, Chemical and Catalytic Properties, *Biochemistry 15*, 4457-4463 (1976).

COO-3225-32 Kroeker, W.D., Kowalski, D. and Laskowski, M., Sr., Mung Bean Nuclease I. Terminally-Directed Hydrolysis of Native DNA, *Biochemistry 15*, 4463-4467 (1976).

COO-3225-33 Kroeker, W.D. and Laskowski, M., Sr., Polynucleotide Kinase: Functional Purification and Use in the Direct Kinetic Measurement of Single- and Double-Strand Cleavages of DNA by Restriction and Other Endonucleases of Limited Action, *Anal. Biochem. 79*, 63-72 (1977).

COO-3225-34 Pritchard, A.E., Kowalski, David and Laskowski, M., Sr., A Single-Strand Specific Endonuclease Activity of Venom Phosphodiesterase, *Fed. Proc. 36*, 907 (abst. 3352) (1977).

COO-3225-36 Pritchard, A.E., Kowalski, D. and Laskowski, M., Sr., An Endonuclease Activity of Venom Phosphodiesterase Specific for Single-Stranded and Superhelical DNA. *J. Biol. Chem. 252*, 8652-8659, (1977).

COO-3225-37 Fujimoto, M., Pritchard, A.E., Kowalski, D. and Laskowski, M., Sr., Accessibility of Some Regions of DNA in Chromatin (Chicken Erythrocytes) to Single-Strand Specific Nucleases, *Fed. Proc. 37*, p. 1738, abst. 2574, (1978).

COO-3225-38 Pritchard, A.E. and Laskowski, M., Sr., Discrete Fragments Produced by Limited Digestion of Superhelical PM2 DNA with Venom Phosphodiesterase: Cleavage Site and Mode of Action, *J. Biol. Chem.*, accepted (1978) preprint enclosed.

5. Opinion as to the present state of knowledge in this area of research, its significance in the fields of biology, medicine and environment and needed future investigations.

In 1969 the first experiment was performed by Paul Johnson in our laboratory showing that the single-strand specific nuclease from mung bean sprouts cleaved native, double-stranded λ phage DNA into two halves. Since that time general interest in single-strand specific enzymes gradually increased, and in our laboratory during the past 3 years occupied the central position. From the point of view of bio-engineering the single-strand specific enzymes will never compete in significance with the restriction enzymes. I expect, however, that

the significance of single-strand specific enzymes will steadily increase and parallel our understanding of tertiary structure of nucleic acids, particularly, but not only of DNA. Since these enzymes are sugar-blind, the same structural effects of RNA will be decisive as to whether or not a particular region would be susceptible to hydrolytic attack. I still believe that single-strand specific enzymes are the most sensitive probes presently available to detect the minute aberrations of the regular helical structure. In addition, I hope that further studies either on the level of native viral DNA or on the level of chromatin will lead to the understanding of biological function of these enzymes. As yet it is only a speculation that the transcriptionally inactive portions are the most vulnerable to these enzymes. The supporting evidence is limited to the correlation between easily denaturable and easily hydrolyzable regions; and to the coincidence in number and size of genes and fragments of T_7 DNA produced by mung bean nuclease.

The application of single-strand specific enzymes will undoubtedly increase during the next three years. The field of their application will increase even faster. I expect that chromatin will become the major substrate for these enzymes. Additional restraints as well as additional aspects of specificity will make this probe even more valuable.

It also seems to me that the popularity of the individual enzymes in this group is likely to change. Nucleases S_1 (*Aspergillus*) and N_1 (*Neurospora*) will be gradually replaced by P_1 (*Penicillium*) because it is the cheapest representative of the group. I expect mung bean nuclease and venom phosphodiesterase to become leaders when the highest purity will be required. In particular I expect venom

phosphodiesterase to become high in demand for 2 reasons: its unusual mechanism of producing numerous long-lasting double-stranded fragments deprived of the sticky ends, and second, its ability to attack supercoiled DNA much faster (10^4) than the relaxed circles. I foresee the application of phosphodiesterase to such problems as finding the location of a supercoiled branch, or evaluating forking ability of different termini.

6. The present division of federal support for overall research program.

In 1977 the American Cancer Society deleted the word "emeritus" from the title and in agreement with the local administration, Director Dr. Gerald P. Murphy, decided to retain the position of the American Cancer Society Professor until such time that the incumbent felt ready to quit or died. The incumbent participates in the meetings of the American Cancer Society Professor and receives no salary but a $3,500 annual grant to be spent at his discretion, or to be accumulated for equipment. This is only a token amount of money, but it substantially changes the position inside the Institute.

I have three grants in the general area of nucleic acids: ERDA, last year of a triennium; NSF, last year of a triennium; and NIH, first year of a triennium. Each grant deals with a different aspect of the enormous domain of nucleic acids. Only an occasional overlap in substance occurs among the projects, but seldom is the same paper reported to two different agencies. I also have a grant from NIH (last year of a triennium) for the work on proteolytic inhibitors, a topic belonging to a totally different domain excluding the chance of an overlap. The only connection with other topics is that all of

them interest me deeply. For fiscal reasons, however, all grants are acknowledged on all papers (see below).

In order for me to work efficiently the pension from New York State needs a supplement. For the past year I have been paying to myself $8,000 from the NIH Heart and Lung Institute grant and $4,000 per annum from ERDA. With this supplement to pension (somewhat below a salary of a postdoctoral fellow) I can live comfortably and work efficiently.

I have already stated in the previous report that the fiscal difficulties of New York State resulted in freezing many positions at Roswell Park Memorial Institute. I was particularly unlucky and lost a secretary and a laboratory worker (dishwasher). Since all odds and ends were used for substituting, for fiscal reasons I acknowledged (and still do) all grants on all papers. In this report, however, I listed and discussed only such work that has direct bearing on the topics for which the grant was given.