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PROPOSAL #6

RADIATION STUDIES:
PHYSICAL-CHEMICAL STUDIES ON NUCLEIC ACIDS AND DERIVATIVES

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BIOLOGICAL EFFECTS OF RADIATION AND RELATED
BIOCHEMICAL AND PHYSICAL STUDIES

George B. Brown, Ph.D.
Member, Sloan-Kettering Institute for Cancer Research
Chief, Division of Nucleoprotein Chemistry
Principal Investigator

Mary L. Petermann, Ph.D.
Member, Sloan-Kettering Institute for Cancer Research
Head, Physical Chemistry Section
Co-Principal Investigator

Liebe F. Cavalieri, Ph.D.
Member, Sloan-Kettering Institute for Cancer Research
Head, Chemical Physics Section
Co-Principal Investigator

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Sloan Kettering Institute for Cancer Research
410 East 68th Street, New York 21, New York

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Physical Chemistry Section, 5702
Mary L. Petermann, Ph.D., Head

Summary

Studies on the structure of animal ribosomes have been continued. Ribosomes from rat liver and from a rat tumor, the Jensen sarcoma, dissociate into two subunits, two-thirds and one-third of their total mass. Each sarcoma subunit contains a single chain of RNA, while each liver subunit may contain one or more RNA chains. The numerous ultracentrifugal boundaries noted in ribosome solutions can be attributed to changes in conformation of the whole ribosomes and the subunits and to dimerization of the small subunit.

1. The subunits of ribosomes from rat liver and from a rat tumor, the Jensen sarcoma

When the magnesium concentration of the solvent is reduced, the ultracentrifugal patterns of animal ribosomes show, in addition to the whole ribosomes (80 S), a number of slower components (62 S to 28 S). These have been assumed to be subunits, but their sizes and the number per ribosome have been uncertain. We are trying to correlate the molecular weights of these subunits with the sizes of the ribosomal RNA chains.

Ribosomes have been isolated in the presence of a RNase inhibitor, bentonite. The whole ribosomes from sarcoma and the larger subunits (50 S) from both tissues have been separated by sucrose gradient centrifugation and their diffusion coefficients measured. The molecular weights (M) calculated from the sedimentation (s) and diffusion (D) coefficients at a concentration of 1 mg/ml are:

	s	D	M
Sarcoma	78.0 S	1.3×10^{-7}	4.3×10^{6}
	52.0 S	1.3×10^{-7}	2.9×10^{6}
Liver	49.9 S	1.36×10^{-7}	2.6×10^{6}

Since M for liver 80-S ribosomes is also close to 4×10^{6}, both larger subunits are about two-thirds the size of the whole ribosomes. The smaller subunits, 33 S, which have not yet been characterized, should be about half the size of the larger ones.

2. Ribosomal RNA

RNA extracted from sarcoma ribosomes by sodium dodecyl sulfate and phenol in the presence of bentonite has a partial specific volume of 0.53 ml/gm. It contains two heat-stable components, which have been

separated by sucrose gradient centrifugation. Their molecular weights, calculated from sedimentation coefficients and intrinsic viscosities ($[\eta]$) are:

	s	$[\eta]$	M
Sarcoma RNA	29.7	0.51	1.60×10^6
	17.0	0.43	0.65×10^6

Since the ribosomes are about 50 per cent RNA, the larger subunit can accommodate one chain of 30-S RNA. The smaller subunit probably contains one chain of 17-S RNA.

With liver ribosomes the picture is more complicated. As previously reported, they appear to contain mainly 16-S RNA of molecular weight 0.6 x 10^6, with some 28-S, 22-S, and 8-S chains. Either some subunits contain more than one RNA chain, or some of the chains have been broken during isolation.

3. Changes in conformation of ribosomes and subunits

Both the whole ribosomes and their dissociation products have wide ranges of sedimentation coefficients. If there are only two types of subunits, these variations must be attributed to changes in conformation. For sarcoma, components of 83 S to 78 S, and even a 62-S boundary obtained under special conditions, appear to be whole ribosomes of different degrees of compactness. The larger subunit varies from 55 S to 50 S, and in the absence of counterions changes to a component of less than 20 S. The smaller subunit is 33 S until the magnesium is all removed, when it changes to 29 S; it can also dimerize to give a boundary of about 60 S.

The larger subunit of liver ribosomes ranges from 55 S to 46 S, and the smaller subunit, like that of sarcoma, can be 36 S, 28 S, or 60 S.

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Chemical Physics Section, 5704
Dr. Liebe F. Cavalieri

Scientific Background and Scope

Study of the replication of DNA continues to be one of the major efforts of this group. As the work progresses, the relationship between *in vivo* and *in vitro* replication becomes more meaningful. Both phases are still being aided and supplemented by various physical studies with DNA.

All phases of the present work will be continued and, wherever indicated, expanded. The polymerase work (number 1 outlined below) will naturally evolve into one of physical studies of these synthetic polymers, including x-ray and electron microscope studies.

The work outlined under number 2 of this report will expand by the use of other type chemicals and drugs (such as actinomycin F) whose actions have been studied in other connections. These interactions will also be extended to DNA's from other sources.

The work given in number 3 of this report will expand as soon as possible to a characterization of metastable DNA.

1. The In Vitro Synthesis of DNA-RNA Hybrids

A partially purified enzyme from *Escherichia coli* has been shown to yield hybrid molecules in which one strand is a homopolyribonucleotide and the other is a homopolydeoxyribonucleotide. The substrates are the corresponding ribonucleoside-5'-triphosphates and deoxyribonucleoside-5'-triphosphates. The templates used have been poly (A+U), poly (dA+T), or poly (C+I). Thus either a DNA or RNA-like polymer serves as template. Poly (C+I) can produce either poly (dC+G) or poly (dG+C). The same holds for the other templates. This wide variety of hybrids will be useful in the study of the mechanism of DNA synthesis.

2. The Interaction of Actinomycin D with DNA

The interaction of actinomycin D with DNA from calf thymus and *E. coli* has been examined by equilibrium dialysis and light scattering. There are two types of binding sites, one of which has a binding constant about 50 times greater than the other. The binding of actinomycin to the strong sites causes a "dimerization" of the DNA. Upon removal of the drug, the molecular weight reverts to its original value. The radius of gyration of the dimerized complex is not sensibly greater than that of the original DNA; therefore, the doubling of the molecular weight is due to a lateral rather than a head-to-tail aggregation. Subsequent binding of actinomycin has so little effect on the radius of gyration that intercalation can be ruled out. Denaturation of the DNA results in a single type of binding site, with a binding constant of intermediate value, but the total number of sites remains constant. These observations shed light on the nature of the actinomycin

binding sites and their relation to the interaction of RNA polymerase with DNA. It is deduced that actinomycin inhibits RNA polymerase by competing with it for the strong sites on the DNA.

3. The Template for In Vivo DNA Synthesis

When bacterial cells are lysed at 60°, while stirring, about 15 per cent of the DNA appears in a denatured form; no denatured DNA appears if stirring is omitted. The denatured DNA does not exist as such in the living cell but is elicited by this treatment. It exists in a metastable state, therefore, which can appear as "native" or denatured DNA. The same holds true for mammalian cells which are actively growing in tissue culture. The quantity of denatured DNA is proportional to growth rate; a stationary bacterial or tissue culture shows very little or no denatured DNA. In synchronous bacterial cultures the denatured DNA increases just before the onset of DNA synthesis. This, together with other labeling data, shows that the metastable transient state is a template for DNA replication and possibly for RNA synthesis.

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