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Summaries from
ORNL-4273
Special

THE MOLECULAR ANATOMY PROGRAM

(The MAN Program)

SECOND BIENNIAL PROGRESS REPORT

For Period March 1, 1967, to February 29, 1968

N. G. Anderson
Program Coordinator



OAK RIDGE NATIONAL LABORATORY

operated by

UNION CARBIDE CORPORATION

for the

U.S. ATOMIC ENERGY COMMISSION

THE MOLECULAR ANATOMY PROGRAM

(The MAN Program)

Cosponsored by the National Cancer Institute,
The National Institute of General Medical Sciences,
The National Institute of Allergy and Infectious Diseases,
and the U. S. Atomic Energy Commission

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NOTICE

During this report period the reporting schedule to NIH has been changed. One major report will be prepared annually to cover the entire year's work through February. This is the first such report. An interim report will appear at midyear (November). The major report covering work through June 30, 1967, therefore is appended (Appendix 27) as part of this new schedule report. In order to have the reports ready by November 1 and May 1 each year, the reports will cover the period through August 30 and February 28, respectively.

This report is submitted to fulfill the following interagency agreements:

- 1) Joint AEC-NIH Zonal Liquid Ultracentrifuge Development. Interagency Agreement NCI-FS-(64)-7.
- 2) Joint AEC-NIH (NIAID) Research and Development Program, entitled "The Isolation of Viruses and Viral Subunits."
- 3) AEC-NIH/NIGMS Interagency Agreement for the Molecular Anatomy Program.

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Appendix 5. Annual Progress Report to Oak Ridge National Laboratory from Eli Lilly and Company.

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- Appendix 13. Elrod, L. H., N. G. Anderson, L. C. Patrick, and J. C. Shinpaugh. Isolation of Nuclei Using Reoriented Gradients. Abstract for Tennessee Academy of Science Meeting, November 17-18, 1967.
- Appendix 14. Cotman, Carl, H. R. Mahler and N. G. Anderson. Isolation of a membrane fraction enriched in synaptic membranes from rat brain by zonal centrifugation. Submitted to Biochim. Biophys. Acta.
- Appendix 15. Anderson, N. G., R. H. Stevens, and J. W. Holleman. Analytical Techniques for Cell Fractions. X. High Pressure Ninhydrin Reaction System. Submitted to Anal. Biochem.
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- Appendix 22. Network Satellite Laboratory, NIAID, Bethesda, Maryland.
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- Appendix 24. Program and list of attendees conference on "Centrifugal Techniques for Vaccine Purification."

- Appendix 25. Abstracts of the conference on "Viral, Tumor, and Transplantation Antigen Isolation." Submitted to Transplantation.
- Appendix 26. Program on "Symposium on Urinary Constituents of Low Molecular Weight."
- Appendix 27. The Molecular Anatomy of Cells and Tissues (The MAN Program) Annual Report, For period July 1, 1966, to June 30, 1967. ORNL-4171 Special.

ABSTRACT

The Joint NIH-AEC Molecular Anatomy (MAN) Program is concerned with (a) the development of the biophysical tools required to dissect human cells to the molecular level, (b) the application of these to current biomedical problems, and (c) the systematic application of these tools to the problem of writing a molecular anatomy of human cells.

During this report period, the major effort has been the completion of the development of the K-II centrifuge and the application of it to the large-scale purification of commercial vaccines. For cell, tissue and plasma fractionation on a large scale, additional rotors (K-IV, V, and VI) have been developed. This work provides a direct example of the research and development pattern being followed in the entire MAN program where a present requirement (in this case a purified vaccine) is used to evaluate and guide the evolution of a new system ultimately required for human cell fractionation. Using the K-V rotor, five gram samples of highly purified human α_2 macroglobulin have been prepared for radiation protection experiments.

In the smaller B series, rotors for rate zonal separations which may be either center or edge unloaded have been developed (B-XXIII and B-XXIX). These have been successfully applied to the problem of separating mitochondria, lysosomes, and peroxisomes. A rotor modification having a large center line, B-XV, has been found useful for rate separations of DNA up to 130 million molecular weight. Theoretical and experimental studies are continuing on the development of gradients for the isopycnic banding and separation of quantities of human serum lipoproteins. A method has been developed for isolating the membranes from brain synaptosomes.

The isolation of cell surface membranes and transplantation antigens from these is of interest in connection with attempts to produce antitumor vaccines. An adenovirus 31 tumor membrane fraction has been prepared which affords marked protection against subsequent tumor challenge.

Studies on virus-like particles in human leukemic plasmas and in control sera using negative staining showed no correlation between the disease or treatment and particle incidence. Additional studies using sectioned pellets should be done.

A group of low-speed rotors (A-XVI, XVII, and XVIII) have been developed specifically for the isolation of nuclei in gram quantities. These rotors use either static loading and reorienting gradients, or dynamic loading and unloading with scoops in place of seals.

The separation and analysis of cell macromolecules will require a number of analytical systems now under development, including an Edman protein sequenator (90% completed), a miniaturized amino acid analyzer, a nucleic acid sequenator, high resolution electron microscopy, and new techniques for automated enzyme assay. Reports concerned with the status of each of these are included.

The development of analytical systems for low-molecular-weight organic (mesomolecular) compounds is centered chiefly on substances which absorb in the ultraviolet and on sugars. The problem chosen for emphasis is the analysis of human plasma and urine. High resolution systems capable of detecting up to 140 compounds in urine have been developed and the systematic identification of these substances is continuing.

Production of human cells for fractionation studies has been started and will be scaled up as required. Design studies for a virus isolation and vaccine purification laboratory for the National Institute of Allergy and Infectious Diseases have been started. Three conferences were held on the following topics: (1) "Centrifugal Techniques for Vaccine Purification," (2) "Viral, Tumor, and Transplantation Antigen Isolation," and (3) "Urinary Constituents of Low Molecular Weight."

Planning network techniques, with or without computerization, are being adapted to the problem of planning and managing the MAN program. Reports are being revised to include only summaries and preprints of open literature or ORNL and ORGDP reports.

INTRODUCTION

The major objective of the Molecular Anatomy or MAN Program is (a) the development of methods of separation and analysis applicable to human cells and tissues, and (b) the systematic application of these to the writing of a molecular anatomy of human cells. For the first few years the program has been, and will be for the next year, almost entirely devoted to the development of techniques. As these are perfected, efforts will gradually shift to experimental studies.

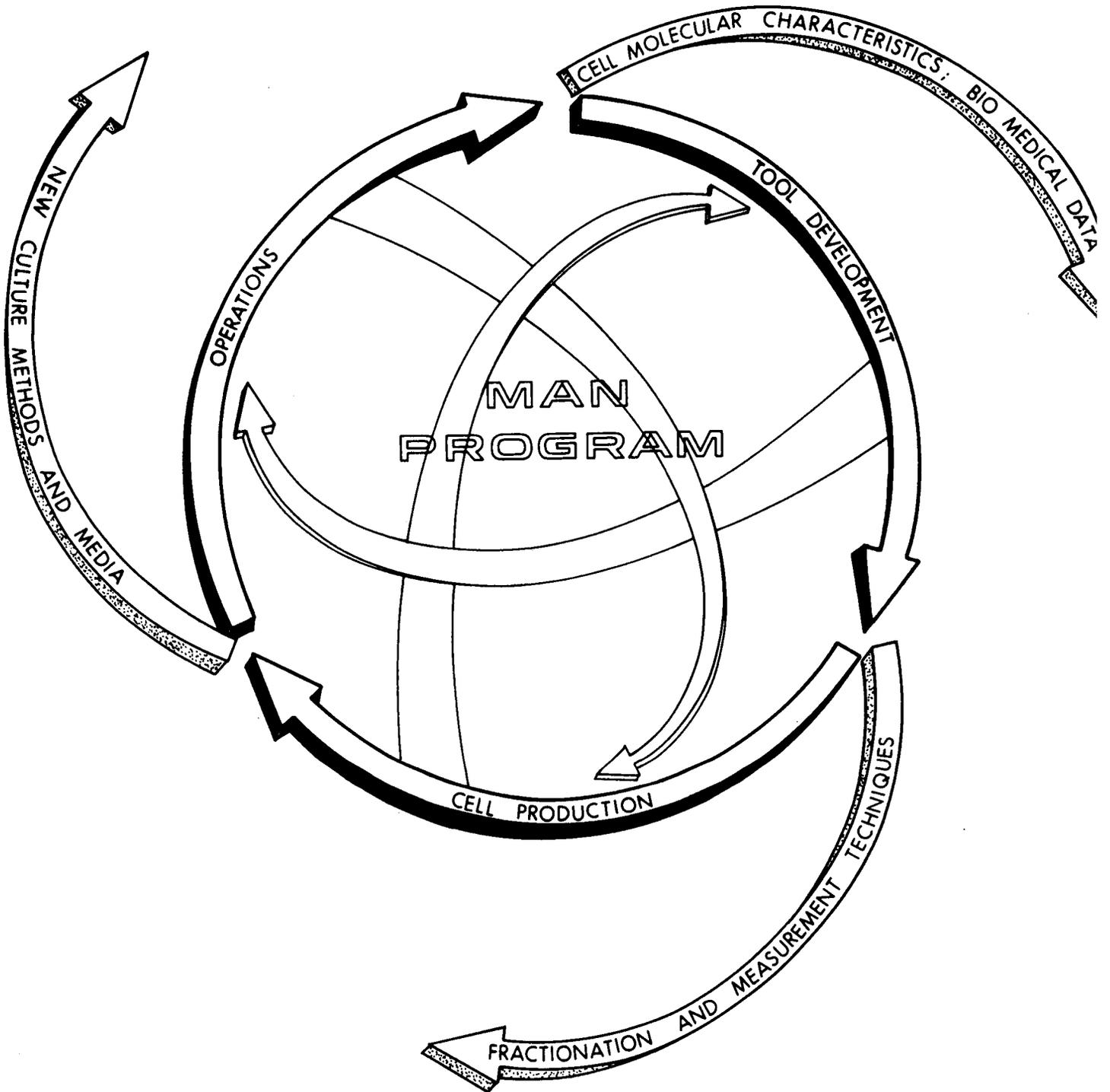
The major effort during the past year has been directed toward completion of the K-II vaccine centrifuge. Extensive collaborative testing was done with a vaccine manufacturer, and a purified influenza vaccine taken all the way to large-scale trials. This was done in an extremely short period of time and required very close collaboration between the ORNL and ORGDP developmental staffs and industrial participants.

During 1968, a number of significant changes and developments have occurred in the Joint NIH-AEC Molecular Anatomy Program. The method of reporting has been changed so that one major report will be prepared each year covering work ending in February, and a minor progress report will cover work ending in August each year.

Two additional changes have been made. Writing, except for publication in the open literature, will be kept to an absolute minimum. Reports, therefore, will consist almost entirely of preprints of papers prepared for publication. Planning and transfer of information relative to planning and progress will be done in terms of probabilistic networks adapted to decision

making and computerization. A program entitled Decision Evaluation Control Involving Doubtful Elements (DECIDE) is being adapted for this purpose.

Since familiarization with this network system is essential to an understanding of this report, the basic elements are included here.



I. DECIDE

J. T. Huffstetler, F. T. Snyder and N. G. Anderson

The problem of exploring AEC-related technologies and capabilities involves by its very nature much exploratory research and development. As new directions and possibilities occur, small efforts are mounted, expanded if successful, discontinued if not. The time scale involved is often measured in weeks, sometimes in days. To make such the subject of a proposal, review, site visit, budget request, etc., would expand the time scale to years and defeat the entire program purpose. On the other hand, constant internal review and budgetary control are essential for the efficient management of the program.

The technique chosen is to budget only between probabilistic nodes in a planning network. In this way, programmatic and budgetary decisions are interlinked.

An additional problem concerns the very early development of ideas and concepts. The most successful developments thus far in the program have, in several instances, been reviewed at a very early stage with specialists outside the laboratory working in the same research area. The views expressed before definitive studies had been done were very often completely negative. We therefore divide the exploration of a new approach to a technical problem broadly into two parts. The first post-conception phase is the embryonic phase. Removal of a fetus for complete examination is not considered good medical practice, and even X-raying it in the very early stages can interfere with normal development. In the beginning we often do not know precisely what area a new development will contribute to, and what institute or review group should consider it. In the post partum (or post-patent) phase a more searchi

examination is indicated along with a more formal schedule of nutrition (or funding).

In the design of an effort such as the MAN Program, where many unfilled requirements exist, the problem of stimulating a flow of new ideas and applications, and of nurturing them to birth (even stillbirth) must be faced directly. Basic decisions must be made by or through individuals who can and who do the experimental work themselves. The DECIDE network is designed to take these factors into consideration.

Network Notation and Definitions

An activity is represented by an arrow directed from event i to event j , as shown in Fig. 1. For each activity j must be greater than i . An event will be represented by a node, and each node may have one or more activities incident upon it. Each end event number must be greater than the number of any interior event. The limitations of the present program are: no more than 200 events, no more than 15 end events, no more than 300 activities plus end events, and no activity may have zero duration.

Note that each activity is assigned a probability of occurrence value; that is, a value of 1 if the activity is undertaken with certainty and a value between 0 and 1 if it is probabalistic. Time and cost estimates are assigned as in conventional network analysis.



Fig. 1. Activity Notations

The node at the head of an arrow is a "receiver," and the node at the tail is a "source." Snyder et al consider three types of receiving events as follows (illustrated in Fig. 2).

1. The "AND" event will occur if all the activities leading into it occur. The event occurrence time will be the completion time of the activity completed last. The event cost will be the sum of the incident activities.

2. The "INCLUSIVE-OR" event will occur if one or more activities leading into it occur. The event occurrence time will be the completion time of the activity completed first. The event cost will be the cost associated with the activities that occur.

3. The "EXCLUSIVE-OR" event will occur if one and only one activity leading into it occurs. The event occurrence time will be the completion time of the activity which occurs. The event cost will be the cost associated with the activity which occurs.



AND



INCLUSIVE-OR



EXCLUSIVE-OR

Fig. 2. Types of Receiving Events

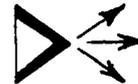
Two types of source events are defined below and illustrated in Fig. 3.

1. The "DETERMINISTIC" event is one from which all emanating activities must (from the internal logic of the network, but not necessarily from a budgetary viewpoint) occur, assuming that the event has occurred.

2. The "PROBABALISTIC" event is one from which an emanating activity may occur with a known probability less than one, assuming the event has occurred. The sum of the known probabilities of all emanating activities must be equal to one.



DETERMINISTIC



PROBABALISTIC

Fig. 3. Types of Source Events

Except for start and end events, each event will be both a receiver and a source, resulting in six distinct types of events as shown in Fig. 4. A start event is a source only, and an end event is a receiver only.

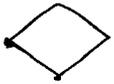
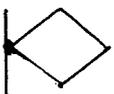
<u>Symbol</u>	<u>Receiver</u>	<u>Source</u>
	AND	DETERMINISTIC
	INCLUSIVE-OR	DETERMINISTIC
	EXCLUSIVE-OR	DETERMINISTIC
	AND	PROBABALISTIC
	INCLUSIVE-OR	PROBABALISTIC
	EXCLUSIVE-OR	PROBABALISTIC

Fig. 4. Types of Events

The computer program to analyze networks containing activities and events of both probabalistic and certain occurrence is described by Snyder et al (1968).

Note that in the presentation of planning diagrams for report purposes the retrospective nets include only actual occurrences and do not include probability, cost, etc. In prospective portions of the net (beyond the point marked PRESENT POSITION POINT) probabilities are sometimes indicated, cost and time are usually omitted. This is because planning tools are active instruments to be updated at very frequent intervals. Therefore, they rapidly become out of date.

- Appendix 1. Snyder, F. T., W. C. Stoddart, and P. E. Scott. Decide: A Computer Solution of Probabalistic Networks to Aid Decision Making Involving Uncertainty. A 46-page ORNL report No. ORNL-TM-2096, 1968.
2. Huffstetler, J. T. and F. T. Snyder. Proposed Management System for the MAN Program. A 45-page proposal.
3. Network for Operations - MAN Program.
4. Anderson, N. G. and J. L. Liverman. Planning for the MAN Program. Scientific Research, in press. A 22-page manuscript.

II. Development of the K-II Centrifuge

D. A. Waters, R. F. Gibson, C. E. Nunley and N. G. Anderson

The K-II centrifuge was developed specifically for large-scale vaccine production and represents the major effort of the past year.

Both the K-II A and K-II B centrifuge armor proved to be inadequate in destructive tests. The K-II C centrifuge having cylindrical shielding of naval armour was therefore constructed and tested using a rotor specifically designed for explosion testing. The armour of the K-II C was not damaged in these studies, and work on the remainder of the problems associated with the development of this version of the instrument has been largely completed. A complete set of drawings was prepared and made available to industry. [Available as CAPE Package No. 1681 from the Clearing House for Federal Scientific and Technical Information, 5285 Port Royal Road, Springfield, Virginia.]

Arrangements to furnish these instruments to the pharmaceutical industry have been completed. By obtaining increasing numbers of parts and accessories from industry, the time required to produce by industry complete, tested systems may be reduced. Detailed operation experience is described in the Appendices.

Appendix 5. A 22-page annual progress report to Oak Ridge National Laboratory from Eli Lilly and Company.

6. A 6-page trip report to Oak Ridge National Laboratory from Abbott Laboratories.

7. Waters, D. A., R. F. Gibson and E. F. Babelay. The K-II Zonal Centrifuge for Vaccine Purification. Fed. Proc., in press.

Appendix 8. Denny, E. C., R. F. Gibson and C. E. Nunley. A 31-page Instruction Manual for the K-II Centrifuge. K-L-2532 Revised, 1968.

Publication: Reimer, C. B., R. S. Baker, R. M. van Frank, T. E. Newlin, G. B. Cline, and N. G. Anderson. Purification of Large Quantities of Influenza Virus by Density Gradient Centrifugation. J. Virol. 1: 1207-1216, 1967.

III. K-III Rotor

R. F. Gibson, D. A. Waters, and C. E. Nunley

Corrosion problems have been experienced with aluminum-alloy rotors after extended use with solutions containing sodium or potassium citrate. A titanium version of the K-II continuous-flow-with-banding rotor would not only minimize the corrosion problem, but would allow higher operating speeds to be obtained. Design studies have been initiated during this period and will be completed before June, 1968, at which time fabrication will be initiated. As with other rotors, computer programs are used to determine optimum wall thickness, critical speeds, and performance.

Appendix 9. Listing of existing and proposed K series centrifuges.

IV. K-IV Rotor

C. E. Nunley and N. G. Anderson

The K-IV rotor is designed for rate-zonal separations using a core similar in principle to that of the B-IV rotor, but having six septa in place of four. The total internal volume is 6.7 liters. The rotor is dynamically loaded and unloaded and requires a new large exponential gradient pump which was designed and constructed during this period. Vibrational problems were encountered during loading which changed the shape of the gradient. The problem could be solved by loading the rotor with distilled water before rotation started and then displacing it with the gradient during rotation. Successful separation of macroglobulins from human plasma was achieved with this rotor.

Since the K series centrifuges were originally designed for continuous flow work with the flowing stream prechilled, the cooling wall used in the centrifuge was not designed to operate below 0°C. For prolonged runs without fluid flow, as required with the K-IV rotor, a new refrigeration system capable of operation to -30°C and with rotor temperature sensors will be required.

V. K-V Rotor

N. G. Anderson, C. E. Nunley, and J. N. Brantley

Unlike continuous-flow-with-banding rotors, which may be loaded or unloaded either during rotation or at rest, cylindrical rotors for rate separations cannot now be made to operate in either mode. Design of a rotor for rate studies which could be loaded and unloaded statically was therefore undertaken.

Experimental studies with this rotor showed that minimal gradient degradation occurred during reorientation. The major immediate interest in rotors of this configuration is in the isolation of human α macroglobulin. The macroglobulin fraction may be readily isolated from ² 250-ml samples of plasma or from 15 grams (the largest sample thus far used) of Cohn Fraction III. The separated fractions are concentrated by pelleting in a titanium rotor at 50,000 rpm and are dissolved in buffer and frozen until used. Bacteriological studies indicate the product is sterile. Studies are in progress to determine whether the postirradiation protection provided in mice by this material is equal to that of small-scale preparations made in the B-XV rotor. If sufficient human material can be prepared, studies on radiation protection in man may be considered and the possibility of increasing radiation doses in human cancer examined. We believe this effort should receive special emphasis and support. Should successful results be obtained in man, arrangements for commercial production will be arranged.

Reference: Hanna, M. G., Jr., Paul Nettesheim, W. D. Fisher, Leona C. Peters, and Mary W. Francis. A serum alpha globulin fraction: Survival-and-recovery effect in irradiated mice. Science 157:1458-1461, 1967.

T. E. Perardi, C. E. Nunley, R. F. Gibson and N. G. Anderson

With many harvest fluids used in the preparation of vaccines, a large spectrum of particles are present having a considerable range of sedimentation coefficients and densities. This is especially true of chorioallantoic fluid from embryonate eggs. Precentrifugation is therefore indicated before passage through a high-speed-continuous-flow-with-banding centrifuge. With unstable materials, it is desirable to do this "on stream" with two centrifuges having matched flow rates. Rotor B-XXVIII was designed and built for this purpose and tested with influenza vaccine at the Lilly Research Laboratories. It was found that between 0.4 and 0.5 grams of sediment per liter of harvest fluid should be removed in the precentrifugation. The problem of matching flow rates made direct flow from the B-XXVIII to the K-II difficult. An alternate approach is to make the pre-cleanup and virus isolation rotors coaxial, i.e., as one double-pass centrifuge. Rotor K-VI was designed for this purpose. The fluid flows initially into a cup in the center of the rotor core where large particles sediment out and are retained. The effluent from the cup then flows out into the main rotor chamber where virus particles are sedimented into a gradient imprisoned in the rotor.

During this report period removal of bacterial cells from the stream into the central cup was successfully performed. Collaborative studies with Eli Lilly & Company are in progress.

VII. Flow Studies in Continuous-Flow Rotors

T. E. Perardi and R. A. A. Leffler

During the operation of a K-II centrifuge rotor part of the density gradient is washed out of the rotor. A series of studies was undertaken using both the aluminum rotor and a specially fabricated Lucite model which allowed flow through the rotor to be visualized. In these experiments, the rotor was partially filled at rest with water and then completely filled with a 60% w/w sucrose solution. The rotor was then accelerated to operating speed (2,000 rpm for Lucite, 20,000 rpm for aluminum). The stream fluid (either top water, dyed water, or an aqueous suspension of E. coli) was introduced after reaching operating speed. Flow rates of 10 to 40 liters/hr were used. At the completion of an experiment the rotor was decelerated to rest and the contents unloaded and analyzed for sucrose.

Gradient decay was studied as a function of flow rate, time of operation, volume of feed solution, rotor speed, rate of loading and unloading, and gradient reorientation. Decay of the gradient was found to depend primarily on the time of operation and also on the feed rate and operating speed. The gradient profile recedes and changes slope slightly as time of operation increases, but a usable sucrose gradient was maintained for 10 hours at a flow rate of 20 liters/hr. The slope of the lower part of the profile (the portion of the gradient next to the flowing stream) depends on sample flow rate, especially for short experiments, with high flow rates causing greater wash out of sucrose. A flow rate of 40 liters/hr was found to cause rapid gradient decay at 2,000 rpm, but much less decay at 20,000 rpm.

Band dispersion was studied as a function of number of reorientations and of movement up and down the rotor. Surprisingly, up to three reorientations caused little or no dispersion of band formed with red blood cells banded in sucrose. A band of E. coli cells did not widen noticeably during movement from the bottom to the top of the rotor, but changed markedly to at least double its original width as a result of movement down the rotor from top to bottom. Both bands were synthetic in that they were loaded in and were not true isopycnic bands formed during centrifugation.

Appendix 10. Perardi, T. E. and R. A. A. Leffler. Flow Visualization Studies in the K-II Continuous Flow Centrifuge. A 38-page report, ORNL-MIT-38.

VIII. B-XXIII Rotor

N. G. Anderson, C. E. Nunley, and D. H. Brown

The zonal rotor systems developed previously were unloaded through the center core line. If rotors could be developed which may be unloaded from the edge, a number of new and useful separations could be made. These include combined rate-isopycnic experiments done in one rotor. The procedure is to unload the lower (outer) portion of the gradient and then replace it with fresh gradient material. In this way large or rapidly sedimenting particles which approach their isopycnic points rapidly may be removed, while smaller particles are still moving on a rate basis in the lighter portion of the gradient. Mitochondria may be thus removed while lysosomes and peroxisomes are still sedimenting. Of special interest is the purification of virus particles which may be separated from larger subcellular particles and then banded isopycnically in the replaced portion of the gradient. Edge unloading rotors are also useful for the concentration of virus from liter volumes of solution into short, steep gradients next to the rotor wall. If CsCl is used for this purpose, only sufficient salt to make the short gradient need be used. For center unloading, sufficient dense solution is required to displace the entire rotor contents. Edge unloading therefore results in a very considerable saving of expensive gradient materials. It also solves the problem of concentrating virus from liter quantities of solution, thus providing an intermediate capability between that of swinging-bucket centrifuges and continuous-flow machines. It is this intermediate range that is of greatest interest at present to virology laboratories.

The B-XXIII rotor was constructed in aluminum alloy with a 20° wall taper. Experimental studies showed that only a small decrement in resolution was observed in wall unloading as compared with center unloading. A preprint of a paper on the performance of this rotor is included in the Appendix. On the basis of these studies rotor B-XXIX was designed.

Appendix 11. Anderson, N. G., C. T. Rankin, Jr., D. H. Brown, C. E. Nunley, and H. W. Hsu. Analytical Techniques for Cell Fractions. XI. A Zonal Centrifuge Rotor for Center or Edge Unloading. Submitted to Anal. Biochem. A 13-page manuscript.

IX. Rate-Zonal Fractionation of DNA

J. P. Breillatt, Jr. and V. N. Schumaker

A. Rotor B-XXV

Rate-zonal fractionation of DNA is of interest in connection with attempts to detect viral-host DNA aggregates. However, as the size of a DNA molecule increases, both the lability to hydrodynamic shear and the concentration dependence of the sedimentation coefficient increase markedly. Because of the former effect, shear forces during loading and unloading of zonal rotors must be decreased if large size DNA is to be fractionated therein. A modification of the B-XV rotor having a large diameter center line has been constructed in titanium for exploratory studies in this field. A mixture of calf spleen RNA, DNA, and bacteriophage T₄ DNA has been fractionated in this rotor. The sedimentation coefficient of the T₄ DNA was that of the undegraded molecule after passage through the rotor; therefore, the rotor design is satisfactory for double-stranded DNA with molecular weights to 130 million.

B. Detection of DNA at extreme dilution

The marked concentration dependence of the sedimentation coefficient of large DNA molecules raises three obstacles to rate-zonal fractionation of DNA: (1) to determine what is, in fact, the sedimentation coefficient in the absence of particle-particle interaction, (2) to ascertain whether the shape of a band is due to inhomogeneity or to concentration-dependent sedimentation of a homogeneous species, and (3) to detect the DNA at extreme

dilution without resorting to radioisotopes, which would introduce breaks in the strands.

If the third problem can be solved so that meaningful experiments can be carried out at concentrations below those where particle-particle interactions occur, then the solution of the first becomes straightforward and the second ceases to exist. We have sought the DNA level where the sedimentation coefficient loses its concentration dependence by moving-boundary sedimentation velocity experiments in the Spinco analytical ultracentrifuge equipped with a photoelectric scanner. The scanner sensitivity has been increased by reading the signal out on an X-Y recorder after passage through a 10 X operational amplifier. The sedimentation coefficient of intact T⁴ DNA has been determined at 1.5 µg/ml in a 12 mm lightpath cell. The present signal to noise ratio will permit measurements at one-third that concentration and the use of a 30 mm cell will increase the sensitivity to 0.2 µg/ml. Tentative results indicate that particle-particle interactions diminish in intact T⁴ DNA solutions below 2 - 3 µg/ml. Detection of DNA in the effluent of zonal rotors will be accomplished by use of a stream splitter and reaction of DNA with ethidium bromide in a Technicon Autoanalyzer coupled to a fluorometer flow cell. This method offers sensitivities to 0.1 µg DNA/ml.

Concurrent with modification of the photoelectric scanner of the analytical ultracentrifuge, we have automated data acquisition on computer cards. Retransmitting slidewires on the X-Y recorder are read by a

Benson-Lehner decimal converter, Model F, which punches out, through an IBM printing card punch, 100 pairs of X-Y coordinates per cell scan. A computer program is being written to accommodate moving-boundary and zone centrifugation data acquired in this way. An $\omega^2 dt$ integrator has been installed on the analytical centrifuge to facilitate extrapolation of sedimentation coefficients to zero centrifugation.

X. B-XXIX Rotor

N. G. Anderson and C. E. Nunley

Previous studies with the B-XXIII rotor have shown that a number of new and very useful separations can be achieved in rotors which can be unloaded from either the center or the edge. The basic problem is to design the edge rotor surfaces in such a manner as to achieve maximum resolution during unloading. In the B-XXIX, a 10^0 taper has been used to funnel zones to the rotor equator where a narrow super elliptical (an ellipse with four foci instead of two) groove channels fluid to small nipples at the edge of the septa. The position of the threads has also been changed and the resulting configuration is thought to be stronger than previous rotors of this series.

During this report period, construction of three rotors of this type was begun. The chief interest in this rotor is in the isolation of cell plasma membranes as a first step in the isolation of tumor transplantation antigens.

Appendix 12. Anderson, N. G., C. E. Nunley and C. T. Rankin, Jr. New Zonal Rotors for Antigen Fractionation. Fed. Proc., in press.

XI. A-XVI Rotor

Theoretical and experimental studies have demonstrated that the major problems with reorienting gradients occur at very low speed. A test rotor, A-XVI, for use at speeds up to 5,000 rpm was constructed of polycarbonate plastic and attached to a very large (25.4305 kg) flywheel to help achieve very slow acceleration and deceleration. Studies with sucrose gradients showed very little alteration through the body of the gradient after one or more reorientations. At the top (light end) of the gradient mixing into the overlay was observed. This effect is minimized by including a small gradient in the overlay. The rotors have been used to develop a very rapid method for isolating nuclei from tissue homogenates.

Appendix 13. Elrod, L. H., N. G. Anderson, L. C. Patrick, and J. C. Shinpaugh. Isolation of Nuclei Using Reoriented Gradients. Abstract for Tennessee Academy of Science Meeting, November 17-18, 1967.

XII. A-XVII Rotor

L. H. Elrod, N. G. Anderson, and C. E. Nunley

From studies with rotor A-XVI it became apparent that a very simple rotor could be constructed which allowed nuclei to be removed from a sample layer in a matter of minutes. The technique proposed was a hollow core plastic rotor with no seals which could be loaded and unloaded during rotation or at rest. Dynamic unloading would be with suction scoops. To be maximally useful, the sample should be simply poured into the spinning rotor and recovered as soon as the nuclei have sedimented out of the sample zone, leaving the nuclei in the gradient which remains in the rotor. In this manner nuclei may be recovered from a series of homogenates as rapidly as they are prepared.

The majority of animal tissue studies are done with rat liver, and the rotor is scaled to handle samples of homogenate each containing up to 10 grams of tissue. During this report period the rotor was constructed and successfully tested.

XIII. A-XVIII Rotor

L. C. Patrick, N. G. Anderson, and J. C. Shinpaugh

Recent studies by Mathias (personal communication) has shown that diploid, tetraploid, and octaploid nuclei could be separated in the A-XII zonal rotor. For routine work the seals used with this system present maintenance and operational problems. A rotor using direct sample introduction and gradient recovery through scoops would allow rapid and easily controlled operation. During this report period, one A-XVIII was designed and constructed. Experimental studies showed that several modifications in the core would be required. These were completed by the end of this report period.

The objective of the A-XVII - A-XVIII combination is the rapid isolation of mammalian nuclei, and their separation into different size classes as a prelude to studies on the sedimentation rate of mammalian DNA in the B-XXV rotor. It should be noted that tumor nuclei generally have physical properties different from those of most normal cells. Methods for separating them from other nuclei are of interest from a variety of viewpoints.

XIV. Design of Gradient for Lipoprotein Fractionation

J. P. Breillatt, Jr. and W. K. Sartory

Before the development of zonal rotors, the serum lipoproteins were fractionated by a series of differential centrifugations, each requiring about 20 hours. In a previous report from this laboratory (ORNL-4171, Special), the resolution of serum lipoproteins in the B-XIV and B-XV rotors was presented. However, it was not possible to band all three classes in a single centrifugation: only the very low and low-density lipoproteins (VLDL and LDL) or the LDL and high-density lipoproteins (HDL) could be obtained in any run. This report covers the design of a gradient that allows resolution of the three groups in existing rotors.

Because the VLDL have a density range of 0.96 to 1.02 g/cc, true isopycnic banding is not possible in aqueous gradients; therefore, a diffusion gradient formed from an initial density step was chosen.

Gradient materials were evaluated on the basis of diffusivity and viscosity. KBr and CsCl were chosen. Using computers, diffusion in the B-XIV and B-XV rotors was simulated and the B-XV-Ti was found to be capable of 50% more g-hrs in the time required to form a gradient than the B-XIV-Ti. It was therefore chosen to carry out further simulation studies of diffusion from various initial step gradients. When optimal diffusion conditions had been obtained, sedimentation, floatation, and band formation of lipoproteins in the diffusion gradient were simulated. It was shown in these studies that the HDL could be resolved from the serum albumen and globulin, necessitating

the removal of all proteins of density > 1.20 g/cc from the sample.

Using simulation methods, it has been possible to design a gradient which permits resolution of all three classes of lipoproteins during a single centrifugation in an existing rotor. Experiment has confirmed the theoretical conclusions.

XV. Development of an Edman Protein Sequenator

J. W. Holleman

A modified Edman protein sequenator for the determination of amino acid sequences in proteins automatically is being developed. To date no commercial version of this instrument has appeared. It will be required for determining the primary structure of tumor antigens and of protein subunits of viruses.

The miniaturized amino acid analyzer described in another section is being designed to match the output of the sequenator. Both Edman's machine and ours work on the principle of forming a derivative of a protein polypeptide chain at the N-terminus. The presence of the derivative group allows intra-molecular rearrangement at the first peptide bond, which can be caused to release the end amino acid, by suitable treatment, as a thiazolinone, leaving behind the residual peptide which can be derivatized again for a repetition of the cycle. The thiazolinone is extracted away and converted to a more stable derivative and identified outside the machine.

During this report period 90% of the components were completed. Titanium, Kel-F, and pyrex spinning reaction vessels were constructed and will be subjected to corrosion tests during the coming period.

XVI. Isolation of Brain Synaptosome Membranes

Brain nerve end particles or synaptosomes have been previously isolated by Whittaker, de Robertis, and others using differential centrifugation and isopycnic banding in swinging-bucket rotors. When a synaptosome-rich concentrate prepared from rat brain was osmotically shocked and then centrifuged through a sucrose density gradient, a new absorbance peak was obtained. Electron microscopic observation showed it to consist largely of synaptosomal membranes, a conclusion supported by the high concentration of sodium and potassium activated ATPase in this fraction.

Appendix 14. Cotman, Carl, H. R. Mahler and N. G. Anderson. Isolation of a membrane fraction enriched in synaptic membranes from rat brain by zonal centrifugation. Submitted to Biochim. Biophys. Acta. A 10-page manuscript.

XVII. Miniaturized Amino Acid Analyzer

N. G. Anderson, R. H. Stevens, and J. W. Holleman

Theoretical considerations suggest that resolution in an ion exchange chromatographic system is improved by decreasing the resin bead size, and, within certain limits, column diameter. The possibility of using miniature columns is a very real one, but it raises the difficult problem of effecting constant flow at very high pressure and low rates of flow. In previous studies on high-pressure systems, very large pumps were required for high-pressure work, thus partially defeating the advantages of miniaturization. In addition, the present ninhydrin system contains too large a volume of fluid and produces too large a decrement in resolution, again defeating the objectives of column miniaturization.

We have achieved a simultaneous solution to both of these problems by a completely new design of the entire fluid handling system. The basic concept is to use a constant-rate piston to maintain the flow of waste fluid out of the colorimeter at a constant rate. The waste piston drives a ninhydrin piston in at the same rate thus providing a direct coupling between column effluent volume and ninhydrin delivery. Pumps are eliminated entirely by pressurizing the entire system using gas pressure applied through a plastic "bellophragm." The gas pressure is controlled by a sensor at the low pressure (colorimeter) end, and is maintained at a level high enough to allow the ninhydrin reaction coil to be run at high temperature and therefore reduced to less than 12 inches. The entire reaction system therefore is approximately the size of two small match boxes.

A study of the ninhydrin reaction at high temperatures (up to 150°C) and pressures has been completed. Components for the complete instrument are now in the final design stage.

Appendix 15. Anderson, N. G., R. H. Stevens, and J. W. Holleman.
Analytical Techniques for Cell Fractions. X. High Pressure
Ninhydrin Reaction System. Submitted to Anal. Biochem.
A 23-page manuscript.

Appendix 16. Network for miniaturized amino acid analyzer development.

XVIII. Immunization of Hamsters Against Adenovirus Tumors
by Tumor Cell Fractions

J. H. Coggin, Jr., N. G. Anderson, and L. H. Elrod

A course of investigation has been initiated to attempt to immunize hamsters against homologous tumor cell challenge employing whole irradiated Adenovirus 31 tumor cells and selected cell fractions from non-irradiated cells as vaccine. The methods employed and the results obtained are described below.

Cells. Adenovirus 31 hamster tumor cells (Syrian golden hamster) passaged routinely in vivo were used as the source of all cells and cell fractions.

Hamsters. Syrian golden hamsters were obtained from Lakeview Hamster Colony, Newfield, New Jersey, and were used at 3 to 4 weeks of age.

Preparation of Irradiated Cell Vaccine. The preparation of whole irradiated cells from tumor passaged in vivo has been previously described. Washed disaggregated cells suspended in Medium 199 were subjected to 8000 r of X-irradiation for use as vaccine.

Preparation of Cell Fractions. Thirty-six grams of fresh whole Adenovirus 31 tumor was placed in a container and the volume was brought to 720 ml (5% w/v) in 8.5% sucrose for homogenization. The tumor was homogenized for 2 min in an omni-mixer and then frozen to -20°C . The suspension was then slowly thawed to 25°C and homogenized again for 2 min whereupon it was again refrozen to -20°C over a 3 hr period. The homogenate was thawed again slowly to 25°C and the blending and freezing cycle repeated a third time. The homogenate

was again thawed slowly, homogenized for 2 min and centrifuged at 4°C in a 30 rotor ($10^7 \omega^2 t$). The supernatants were pooled and centrifuged in a 50 rotor ($5 \times 10^9 \omega^2 t$). The supernatants were again pooled and centrifuged in a 50 rotor to $10^{11} \omega^2 t$. The 10^7 pellet, 5×10^9 pellet, 10^{11} pellet, and 10^{11} supernatant were dialyzed overnight at 4°C against three changes of Miller and Golder buffer pH 7.0 (0.1 M). The following volumes were obtained.

<u>Vaccine</u>	<u>Volume (ml)</u>	<u>Protein (Lowry)</u>	<u>Reciprocal CF "T" Antigen Titer</u>
10^7 pellet (Nuclear Fraction)	170	7.75 mg/ml	20
5×10^9 pellet (Plasma Membranes)	115	10.60 mg/ml	90
10^{11} pellet	112	7.63 mg/ml	60
10^{11} supernatant	325	2.35 mg/ml	18

Table I. The Efficacy of Adenovirus 31 Hamster Tumor
Cells and Cell Fractions Against Homologous
Tumor Cell Challenge

Vaccine Given	Tumors/Survivors*	
	Challenge Level**	
	4×10^6	8×10^6
None	7/8 (87.5%)	7/8 (87.5%)
Buffer Control	6/8 (75%)	7/7 (100%)
Homogenized Tumor Cells	8/12 (66%)	10/12 (83%)
Whole Irradiated Tumor Cells (8000R)	0/12 (0%)	0/10 (0%)
Membrane Fraction (5×10^9 pellet)	1/16 (6%)	2/16 (13%)
Nuclear Fraction (10^7 pellet)	12/16 (75%)	9/14 (65%)
10^{11} Supernatant	7/16 (43%)	15/16 (93%)
10^{11} Pellet	8/12 (66%)	13/16 (81%)

* 156 days postchallenge

** Challenge cells taken from small tumor passaged in vivo.

From these studies it appears that animals can be effectively immunized by the 5×10^9 w²t pellet. Further fractionation aimed at the isolation of the transplantation antigen involved is planned.

XIX. Separation of Mitochondria, Peroxisomes, and Lysosomes
by Zonal Centrifugation in a Ficoll Gradient

David H. Brown

We have developed a procedure for the separation of mitochondria, peroxisomes, and lysosomes by centrifuging a rat liver homogenate in a Ficoll gradient. The separation is performed in a B-XXIII zonal centrifuge rotor, which is a modified B-XX rotor. This is the first instance whereby the mitochondria can be separated from lysosomes with a high degree of purity without requiring the pretreatment of the animals with the detergent Triton WR 1339. (Injection of the animal with the Triton results in a decrease in density of the lysosomes since these organelles selectively take up the detergent.)

Our method also precludes the necessity for preliminary differential centrifugation and, therefore, greatly reduces the time required for the operation.

Appendix 17. Brown, D. H. Separation of Mitochondria, Peroxisomes, and Lysosomes by Zonal Centrifugation in a Ficoll Gradient. Submitted to Biochim. Biophys. Acta. A 7-page manuscript.

XX. A New Automated Micro Analytical System

N. G. Anderson

The purpose of this project is to develop a miniature automated analytical system which would fulfill the following requirements.

1. Analyses of between 1 and 50 samples must be carried out in parallel.
2. The method must be applicable to a large number of different analytical procedures.
3. The amount of reagent used should be directly proportional to the number of analyses run. (Most present automated analytical methods use rather large amounts of reagents in getting set up for even one analysis.)
4. Overall accuracy should be 1%.
5. The method must be capable of being scaled to very different levels using essentially the same procedure so that a technician working in a physicians office may use essentially the same methods used in a large clinical laboratory, thus eliminating the necessity for learning two or more ways to make the same analyses.
6. Changing analytical procedures must be simple and may be done automatically.
7. The methods must require samples in the low microliter range.
8. Methods of solution measurement and transfer must be used which are not affected by change in solution viscosity, surface tension, or the presence of air bubbles.

9. The system must be adaptable to use with small on-board computers for instant and complete data reduction.

10. The basic patents must, if at all possible, fall in the public domain so that competitive sources of instrumentation will exist.

During the past year the basic concepts for this system have been developed and a simple prototype constructed. The basic solution measuring and transfer method has been tested and the results are in press. The problem of complete patent coverage is being taken care of by the AEC. During the next report period the second prototype described in the network diagram will be constructed. We anticipate that this system will find widespread use in both clinical and research laboratories. Since the funds for this project are largely supplied by the National Institute of General Medical Sciences and the AEC, the analyzer is called the GeMSAEC system.

Appendix 18. Network chart of second GeMSAEC prototype.

Reference: Anderson, N. G. Analytical Techniques for Cell Fractions.
IX. Measurement and Transfer of Small Fluid Volumes.
Analytical Biochemistry, in press.

XXI. Analysis of Body Fluids

C. D. Scott

The development of new separation and analytical systems is best done in a program which combines developmental work with experimental investigations using the systems developed. In this way attention remains focussed on the real biological problems. Over the past two decades ion exchange techniques originally developed for fission product analysis have been adapted to the fractionation of mixtures of increasing complexity. Following the pioneering work of Dr. Waldo E. Cohn, high resolution separation systems for nucleotides and related compounds were developed and were found to be applicable to the separation and analysis of a large number of compounds in human urine. In addition, an automated carbohydrate analyzer was developed for analyzing mixtures of mono-, di-, and tri-saccharides. With these systems as many as 140 ultraviolet absorbing peaks, and up to 48 carbohydrates are observed.

The Body Fluid Analysis Segment of the MAN Program is concerned with five problems, which are:

1. Exploration of the limits of resolution of liquid ion exchange systems, both theoretically and experimentally.
2. Development of practical systems for clinical studies.
3. Chemical identification of the separated peaks.
4. Studies on normal and pathological urine samples to determine the reproducibility of the separations, the variations found in normal individuals, and in pathological states.
5. Data reduction techniques.

XXII. Virus-Like Particles in Leukemic Plasma

G. R. Newell, W. W. Harris, K. O. Bowman, C. W. Boone, and N. G. Anderson

The plasmas of 225 patients with lymphoproliferative disorders and 44 controls were screened for "virus-like" particles. The number of patients and controls was large enough for comprehensive statistical analysis. Plasma samples were banded in CsCl under conditions shown to yield known oncogenic viruses, if present. An objective approach to particle screening was used in which representative electron microscope fields were photographed for particle counting without selecting those fields containing "virus-like" particles.

Four categories of particles were described which were (1) spherical, (2) tailed, (3) Q-shaped, and (4) miscellaneous. The number and distribution of these particles were evaluated according to diagnosis, clinical status of the patient, white blood cell count, and platelet count.

No relationship between any of the particle categories and clinical features was found. Large numbers of particles occurred to about the same extent in both control and diseased groups. Patients having high particle counts had no clinical features in common.

We conclude that the "virus-like" particles seen by the negative staining technique in the plasma of patients with lymphoproliferative disorders are unrelated to their diseases. The importance of thin-sectioning in future studies of this kind is emphasized.

Reference: Newell, G. R., W. W. Harris, K. O. Bowman, C. W. Boone, and N. G. Anderson. Evaluation of Virus-Like Particles in the Plasma of 255 Patients with Leukemia and Lymphoma. New England J. of Med., in press.

XXIII. Nucleic Acid Sequenator

Mayo Uziel

From the work of Khym and Uziel (Biochemistry 7:422, 1968) and more recent studies, it appears that a machine allowing the semiautomatic determination of base sequences in ribonucleic acids or polyribonucleotides is feasible. Such a machine would permit sequence determination more rapidly than is now possible and would do for nucleic acid chemistry what the Moore and Stein amino acid analyzer and the Edman protein sequenator have done and are doing for protein chemistry.

The chemistry of the reactions involved have been extensively studied by ourselves as well as others and involves the following five stages:

1. Oxidation of the exposed vicinal hydroxyl groups (glycol) by periodate.
2. Amine-catalyzed cleavage of the (oxidized) base-sugar fragments from the rest of the chains.
3. Removal of the cleaved fragment from the remaining polymers and determination of its base component (the latter is done separately, not in the sequenator as presently envisioned).
4. Release of the terminal phosphate exposed by the cleavage reaction with phosphatase, thus exposing a new terminal glycol group.
5. Removal of the phosphatase and inorganic phosphate, yielding a polynucleotide suitable for recycling, beginning with step (1).

Experimentally we have found that steps 1 - 3, as well as the conversion of the released fragments to a free base for analysis, can be accomplished in

one step, ending with the precipitation of the residual polymer. Step 4 requires the dissolution of this precipitate, an enzyme addition, and a reprecipitation, all of which may be carried out in the same reaction vessel or an additional one. (The base determination is, as noted, done in a separate system.) Thus the major operations consist of two precipitations, for which the principles of the new method of Khym and Uziel would be used, with additions after each of reagents appropriate for the next step. A detailed proposal has been prepared and construction of the first prototype is scheduled for the next six months.

XXIV. Large-Scale Cell Culture

N. G. Anderson and P. C. F. Castellani, Jr.

During this report period a tissue culture laboratory has been organized to provide cells for the MAN program. The laboratory was completed at the end of this report period and will be used to explore methods for large-scale cell culture using both spinner culture and monolayer systems.

Appendix 20. Network for culture system development.

XXV. High Resolution Electron Microscopy

N. G. Anderson, R. S. Livingston, W. W. Harris, T. A. Welton and R. E. Worsham

Electron microscopy is within less than one order of magnitude of resolving cell macromolecular constituents at the atomic level. The obstacles to advancement in this field are both biological (specimen purification and preparation) and physical (increasing resolution, image intensification, basic studies in electron optics). The Oak Ridge National Laboratory is a unique setting for an experimental interaction between biologists, physicists, and engineers concerned with all phases of this problem. From previous experience in the Molecular Anatomy Program, maximum progress is made in the development of new biophysical instruments when there is a very close collaboration between specialists from widely different disciplines. It is essential, however, that in the initial phases of the problem they be knit together by a few well chosen basic biological problems.

The usable resolution limit of best available conventional electron microscopes is in the 3-6 \AA range. These operate with a useful objective lens angular aperture of about 10^{-2} radians. If the objective lens can be corrected (primary spherical aberration annulled) to an aperture of 4×10^{-2} radians and chromatic aberration could be made small, both resolution and central contrast would be greatly improved. Calculations show that for a single carbon atom the width of the image at half maximum contrast would be reduced from 2.7 \AA to 1.0 \AA and the central contrast would be increased from 5% to 28%. Substantial evidence now exists that this high resolution and associated high contrast can be achieved by careful application of known

techniques. The objective focal length can be defined to the required fractional precision of 10^{-7} by development of suitable high voltage and lens current stabilization systems, combined with a field-emission electron source and velocity filtering. A simple combination of quadrupole and octupole lenses will annul the primary spherical aberration. Proper design of the illumination system, in combination with advanced image intensification and digital recording systems will sharply reduce radiation damage to the sample.

The High Resolution Electron Microscopy Subprogram is divided into three parts which are concerned with (A) sample purification and preparation, (B) theoretical studies on electron optics, electron diffraction, and high voltage systems, and (C) experimental studies on high voltage stabilization, lens design and performance, heat transfer, and image intensification. Part (A) will be largely done by the Electron Microscopy Group of the Molecular Anatomy Program, while (B) and (C) will be the province of the Electronuclear Division of the Oak Ridge National Laboratory which has been previously concerned with accelerator and cyclotron development and with both high energy and low energy particle physics. This arrangement, sponsored jointly by the National Institute of General Medical Sciences and the Atomic Energy Commission, offers an opportunity to explore the chasm between cell structures, as now seen microscopically, and macromolecular structure, as deduced from chemical analysis.

Appendix 21. A 58-page Addendum to AEC Form 189. The Joint-NIH Molecular Anatomy Program Subsection on High Resolution Electron Microscopy.

XXVI. Satellite Laboratory
NIAID - Bethesda, Maryland

J. L. Gerin

We are in the process of establishing and operating a satellite laboratory in the Bethesda, Maryland, area in further support of the joint NIAID-AEC interagency program in the development of sophisticated biological separations systems. The laboratory will include zonal and analytical ultracentrifuges, electron microscopes and ancillary equipment required for the operation of these and other separation systems developed at Oak Ridge.

The Bethesda laboratory will initiate its program with the purification and isolation of adenovirus Type 7 hexon and fiber antigens using zonal centrifuge techniques.

The technology developed from this exercise in large-scale biophysical separations has application to many of the intramural and collaborative programs of NIAID. Collaborative work with NIAID investigators should result in a better awareness of the potential contribution that these and future developments of the MAN program might have to their particular research needs.

Appendix 22. Network Satellite Laboratory, NIAID, Bethesda, Maryland

XXVII. Design and Maintenance of Equipment

D. D. Willis

During the past year, the original time-force integrators designed and built at Oak Ridge have been updated by Spinco $\omega^2 t$ integrators. The equipment that has been updated, in addition to that at Oak Ridge, is located in Dr. C. W. Boone's and Dr. H. E. Bond's laboratories at NCI, the facilities at the John L. Smith Memorial for Cancer Research, Charles Pfizer and Co., Inc., and the Department of Neurosurgery, Duke University. In addition to the updating of the $\omega^2 t$ equipment, controlled acceleration and deceleration circuits and restart circuits for Spinco centrifuges have been designed, built and installed at Oak Ridge, NCI, and Pfizer laboratories.

A centrifuge (Spinco L-4) and an Oak Ridge B-XVI continuous-flow rotor are being tested and checked out prior to being shipped to Dr. J. L. Melnick's laboratory at Baylor University.

The B-XVI continuous-flow rotor supplied to Roswell Park was returned to Oak Ridge for repairs. The top end-cap could not be removed. It was found that the "O" ring had four cavities on the inside diameter. These cavities permitted potassium citrate to get into the threads where it crystallized out.

XXVIII. Reorienting Density Gradients in Continuous
Sample Flow Zonal Rotors

George B. Cline, J. N. Brantley and J. L. Gerin

Reorientation of liquid density gradients can be done successfully in long cylindrical follow-bowl B-IX, B-XVI and K-II continuous sample flow zonal rotors. Reproducibility of gradient materials is affected by acceleration and deceleration rates of the rotors, viscosity of the material and temperature. Experimental studies with influenza virus and T₄ bacteriophage have shown that fluid gradients will undergo transitions from the vertical to the horizontal and back to the vertical again without appreciable band spread.

Appendix 23. Cline, George B., J. N. Brantley and J. L. Gerin. Reorienting Density Gradients in Continuous Sample Flow Zonal Rotors. Submitted to Anal. Biochem. A 43-page manuscript.

XXIX. Conferences

N. G. Anderson, C. D. Scott, and R. A. Popp

During the past year three symposia were sponsored by the Molecular Anatomy Program.

The first, entitled "Centrifugal Techniques for Vaccine Purification," was held on October 23 and 24 and was attended by 70 individuals from pharmaceutical firms, government or government-sponsored laboratories, and universities. The proceedings are not being published. Rather publication of the results described in standard journals was encouraged.

The second conference, entitled "Viral, Tumor, and Transplantation Antigen Isolation," was held on October 25-27 with approximately 50 people in attendance. The abstracts of the conference were submitted for publication in Transplantation and are included as an appendix.

The third conference, entitled "Symposium on Urinary Constituents of Low Molecular Weight," was held November 30-December 1.

- Appendix 24. Program and list of attendees conference on "Centrifugal Techniques for Vaccine Purification." Eight pages.
25. Abstracts of the conference on "Viral, Tumor, and Transplantation Antigen Isolation." Submitted to Transplantation. 145 Pages.
26. Program on "Symposium on Urinary Constituents of Low Molecular Weight." Four pages.

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