Zonal Centrifuges and Other Separation Systems

New methods are being developed at Oak Ridge for fractionating human cells.

Norman G. Anderson

Molecular anatomy is concerned with the description, at the molecular level, of the structure and organization of cells and tissues. It is the logical extension of microscopic anatomy, and it will ultimately be the basis of a molecular pathology of human cells and tissues.

Progress in molecular anatomy has been, and is, critically dependent on the development of techniques for cell dissection and fractionation which make it possible to isolate subcellular formed elements in quantity, and to disassemble these elements into their constituent molecular species. While a very large literature and a vast storehouse of information are available concerning subcellular particles and enzymes, much remains to be discovered.

Since cells and their aggregates are the most complex systems with which modern science deals, molecular anatomy will ultimately require more sophisticated research instrumentation and techniques than are now available. Since the development of the necessary analytical tools, the demonstration of their validity in measurements made on animal and human cells, and their application in broadly based experimental and clinical studies will be costly, the program can be justified only if the ultimate objective is the dissection and analysis of normal and abnormal human cells.

The need for a detailed exploration and cataloging of human cell constituents arises from the belief that most human diseases are ultimately to be understood at the molecular level, and that treatment, if it is to be rationally evolved, must function and be understood at this level. For only a few human diseases is the ultimate molecular lesion known; for only a few of the many drugs in common use has the molecular target or site of action been discovered. Unlike human blood plasma, which has been almost completely fractionated, which can be separated into constituent proteins on a large scale for therapeutic purposes, and which can easily be studied by immunoelectrophoretic methods, the molecular constituents of human cells have been only partially charted. Matters are put in perspective when one attempts to draw up a balance sheet listing the amount of protein in each human-cell fraction accounted for by well-characterized molecular species. The available data are insufficient for this purpose.

The question has been asked (*1*, *2*), Should a systematic attempt be made to develop high-resolution methods for sorting out cell molecular constituents and to catalog them in an orderly fashion?

In an attempt to answer this question experimentally, a small-scale Molecular Anatomy Program has been organized at Oak Ridge (*3*).

Choice of Initial Problems

For feasibility studies, difficult key problems are generally chosen. The isolation of subcellular organelles is a necessary first step and, therefore, the logical area for initial studies.

The choice of the subcellular particle to be emphasized initially was based on the following consideration. In the development of high-resolution separation systems, test particles are required. If ill-defined and physically heterogeneous particles are used, it is difficult to distinguish between particle inhomogeneity and low resolution of the separation system. It is difficult to find homogeneous particles in the size range of interest; the best known are certain viruses. Isolation of virus from tissues and from large-volume suspensions was therefore emphasized, and centrifuge systems designed to accomplish this are being developed by the Oak Ridge Molecular Anatomy Section in collaboration with the Technical Division of the Oak Ridge Gaseous Diffusion Plant (*4*). This immediate objective has the advantage that the centrifuge systems would be directly applicable in virus vaccine purification (*5*).

At the molecular level certain problems concerned with high-resolution ion exchange separation were chosen (*6*). These are discussed below.

Tissue Fractionation and Virus Isolation

The centrifugal systems now available are capable of making separations on the basis of either particle sedimentation rate or buoyant (isopycnic or banding) density. The question is, What sorts of separations may be expected from the application of high-resolution centrifuges of either of these types in studies of cells and tissues? Obviously a particle having a sedimentation coefficient and a banding density not shared by other subcellular particles can, in theory, be isolated in a pure state. If the sedimentation coefficients of a number of viruses and subcellular particles are plotted against banding densities, a plot like that of Fig. 1 is obtained (*7*). Such a plot led to the suggestion that most (but not all) viruses fall in a clear space or "virus window" corresponding to densities intermediate between those of endoplasmic-reticulum fragments and polysomes, and that they could be isolated by a combination of rate-zonal and isopycnic-zonal centrifugation. Even if a virus does not have a unique sedimentation rate and banding density, if it is relatively homogenous and not bound to other subcellular particles it should be separable from the majority of the cell mass by these methods. An experimental verification of the plot of Fig. 1 can be made with the earlier swinging-tube centrifuges. However, we con-

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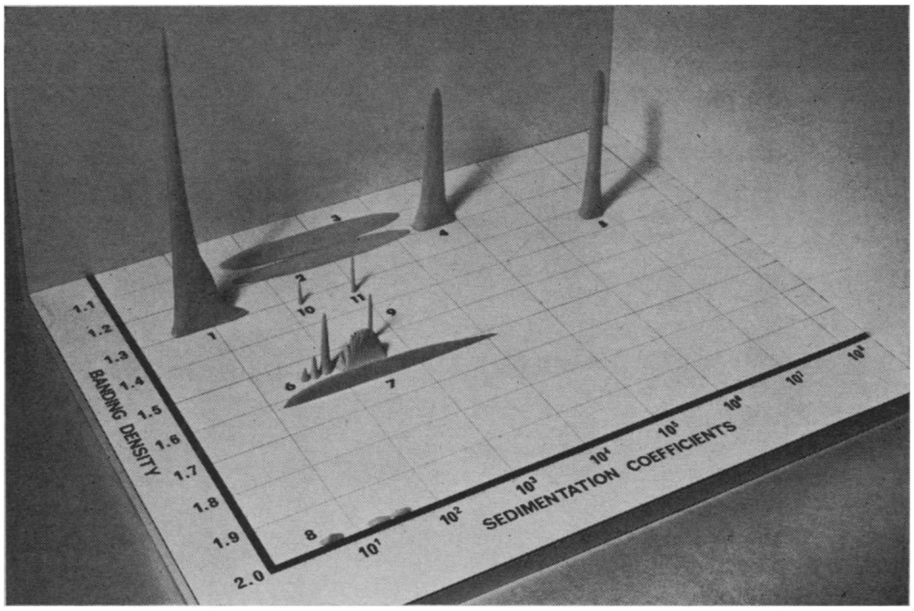


Fig. 1. Diagrammatic presentation of distribution of subcellular components as a function of sedimentation rate and banding density. Components are: 1, soluble proteins; 2, membranes with attached particles; 3, smooth membranes; 4, mitochondria; 5, nuclei; 6, ribosomal subunits, ribosomes, and polysomes; 7, glycogen; 8, RNA; 9, poliovirus; 10, T3 bacteriophage; 11, adenovirus, type 2. Banding densities for most particles are those for cesium chloride. Note that viruses fall in a clear space—the "virus window." The positions of additional viruses are given in 7. [Adapted from Anderson *et al.* (*7*)]

sider isolation of cell components or viruses a first step toward a more complete molecular fractionation, and, for such studies, quantities sufficient for purely analytical purposes are insufficient. Large-scale separations based on sedimentation rate and on banding density are therefore required.

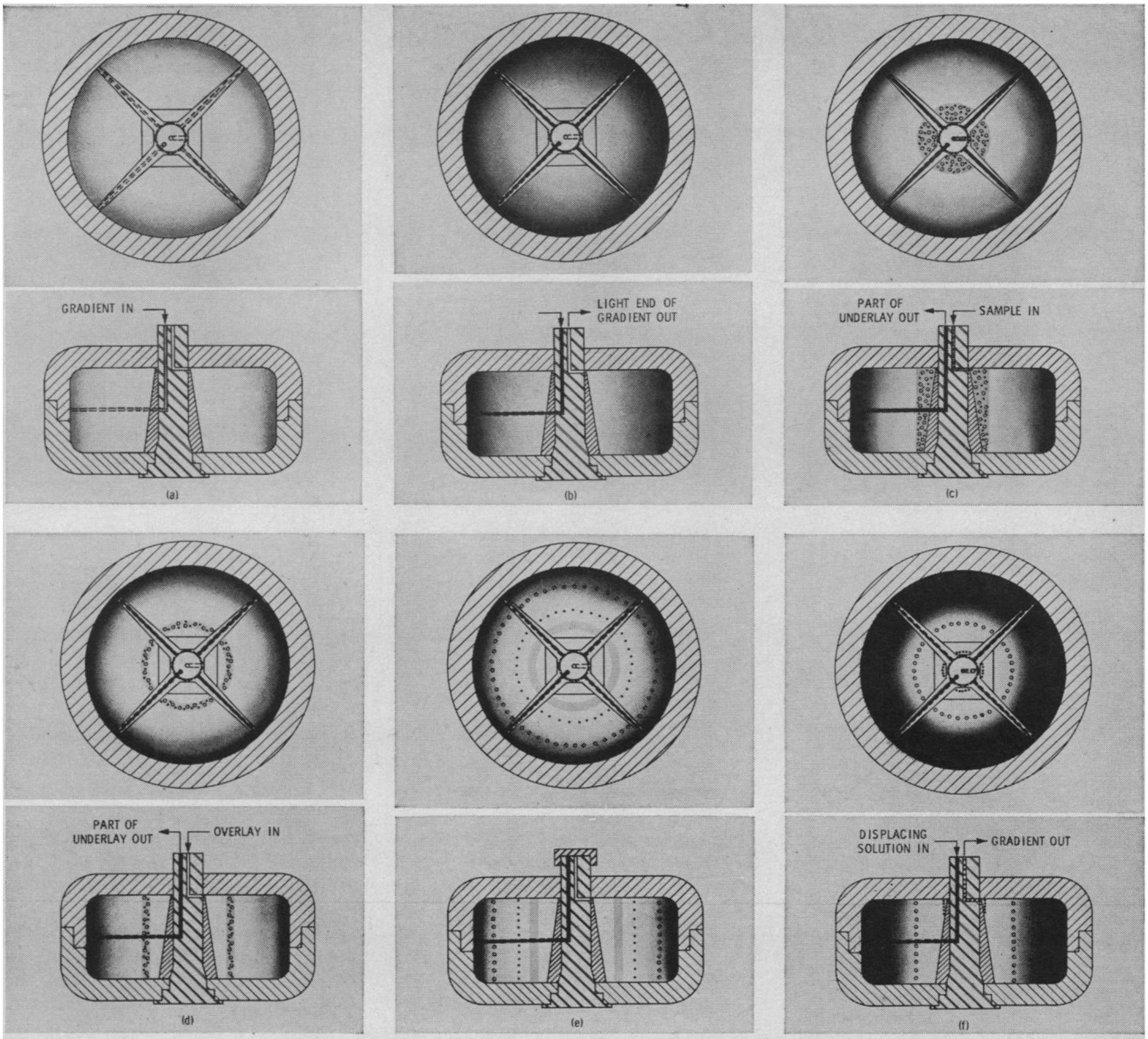
Development of Zonal Centrifuges

The basic problem is that of arranging for separations to occur in liquid density gradients in the strongest large-volume rotors that can be designed. Swinging-tube rotors do not fill the requirement, for the simple reason that the capacity of such tubes is severely limited by the strengths of available materials. Swinging tubes, and tubes in any form, can be eliminated if use is made of centrifugal force to stabilize gradients and sample layers during loading and unloading —that is, if the rotor is loaded and unloaded during rotation (*8*). Loading and unloading during rotation requires a seal system which makes it possible to attach two fluid lines to the rotor. These may be left attached to the rotor during high-speed operation (*9*), or they may be detached after loading and reattached for unloading (*9–11*). Sector-shaped compartments are required to avoid wall effects (*12–14*) and to prevent swirling due to Coriolis forces (*13*) or to change in rotational speed.

To date, over 40 rotor systems and rotor modifications have been constructed in the development of zonal centrifuges (*14*). These have been grouped into classes on the basis of their speed ranges and uses. Thus the A-series rotors are for operation at relatively low speeds (*15*); the B-series, for operation at intermediate speeds (*16, 17*); the C-series, for experimental work at speeds between 70,000 and 150,000 revolutions per minute; the D-series for use above 150,000 revolutions per minute; the F-series, for rapid centrifugal freezing (*18*); and the K-series, for large-scale vaccine purification with batches of 100 liters and above (*19*). The A-XII (*15*), B-IV (*16, 17*), and B-XIV and B-XV (*11*) rotors are now routinely used for rate zonal separations. The salient characteristics of rotors B-XIV and B-XV are shown in Table 1. The loading and unloading sequence of the B-XV rotor is shown in Fig. 2, and the rotor components are shown in Fig. 3.

With such large gradients, accurate measurements of the shape of the gradient may be made. When gradients are loaded into the rotor and then recovered during rotation, little change between the introduced and the collected gradients is seen (*9*). A 25-milliliter sample is recovered in a volume which occupied a zone less than 2 millimeters wide in the rotor. Theoretical studies by Berman (*13*) suggest that the gradient capacity (the mass of material which may be placed in a zone of a given width without disturbing the gradient) may vary markedly as sedimentation occurs, the variation depending on the physical properties and shape of the gradient. Berman's work suggests that the small gradient capacity reported experimentally (*20*) may be due to anomalies in the gradient used. In swinging buckets, for example, it is difficult to control the shape of the gradient immediately below the sample layer.

Representative results of separations made with zonal centrifuge rotors are shown in Figs. 4–6. In the A-XII rotor, aging human-heart pigment (*21*), rat liver nuclei and mitochondria (*15*), spinach chloroplasts (*22*), rat myofibrils (*23*), rat skeletal-muscle relaxing particles (*24*), and oral structures from *Tetrahymena* (*25*) have been separated. The B-IV and B-XV rotors have been used to isolate nuclei (*26*), mitochondria (*27*), microsomes (*9*), ribosomes and polysomes (*28*), ribosomal subunits (*16, 29*), and ribosomal RNA (*30*), and to clearly separate mitochondrial and lysosomal activities (*31*). The isolation of serum macroglobulin (*32*) demonstrates the lower limits of resolution of these rotors. Rather large amounts of virus have been isolated, including poliovirus (*9, 33*), influenza virus (*34*), echo 28 virus (*35*), T2 and T3 bacteriophage (*7, 35*), and tobacco mosaic virus (*36*). The B-IV or the B-XV rotor is capable of handling more than a million doses of a given strain of influenza virus at one time (*37*). Two types of viruses having different sedimentation coefficients, such as T2 and T3, may be cleanly separated (*7*). Concentration of virus from large fluid volumes is a necessary prerequisite for this work and is discussed below. A computer program for calculating the equivalent sedimentation coefficients (S^*) for ideal particles (particles which are spherical, impermeable, and nonosmotic) sedimenting in the B-IV, B-XIV, and B-XV rotors has been written (*38*); equations developed by E. J. Barber (*39*) for sucrose density and viscosity as functions of concentration and temperature were used in the calculation.



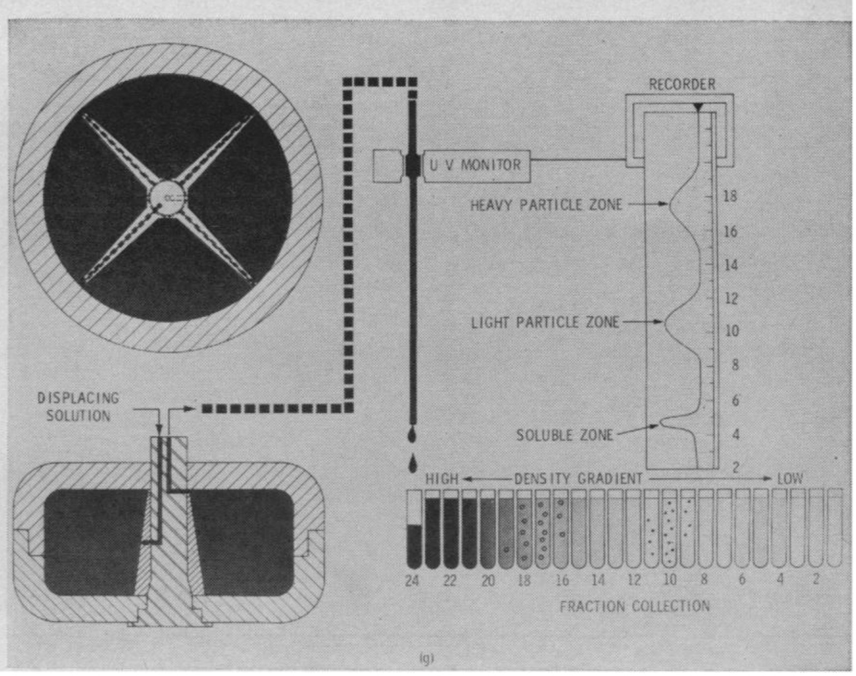


Fig. 2. Loading and unloading of a B-XV rotor. The rotating rotor, viewed in top and side section at each stage, is shown (*a*) partially filled and (*b*) completely filled with a density gradient, which is pumped to rotor edge with the low-density end first. In *c* the direction of flow through rotor is reversed and the sample is introduced through the center line. To move the sample free of the core, a low-density fluid, the "overlay," is pumped in, as shown in *d*. These steps are taken at relatively low speed, usually 3000 revolutions per minute. The seal is removed and a cap is attached to the rotor, which is then accelerated in a vacuum to operating speed to achieve the separations shown in *e*. The rotor is then decelerated to ~3000 revolutions per minute, the vacuum chamber is opened, and the fluid-line seal is reattached. In *f*, a dense displacing solution is pumped to the rotor edge, displacing the gradient, and the particle zones contained in it, toward center core, and pumped out of the rotor. In *g*, fractions are monitored and recovered.

Table 1. Operating data for B-XIV and B-XV zonal rotors.

Model	Weight (empty) (kg)	Volume (cm^3)	Speed (rev/min)	Maximum (g)	Maximum radius (cm)	Maximum stress (tensile) (atm)	Maximum radial growth (cm)	Maximum pressure estimated (atm)	Maximum end load (kg)
B-XIV									
Aluminum	3.571	649	30,000	60,000*	6.62	3,400	0.033	340	34,000
B-XV									
Aluminum	7.439	1,666	21,000	45,000†	8.79	3,400	.044	367	56,000
Titanium	12.7	1,666	26,000	60,000‡	8.79	5,575		544	79,400

* At 29,400 rev/min. † At 21,500 rev/min. ‡ At 24,000 rev/min.

In virus isolation a relatively homogeneous species is usually to be separated from contaminants having a range of sedimentation properties. Quite a different problem is the separation of a continuous spectrum of particles into a series of relatively homogeneous fractions. This has been done with liver glycogen (*7, 40*). With the rotor systems now available, rate separations of 1- to 10-gram quantities of mixtures and 0.1- to 1.0-gram quantities of relatively homogeneous materials may be effected. Since larger capacity will be required for routine fractionation for the Molecular Anatomy Program, a series of much larger centrifuges (the K series) are now under construction (*19*).

Isopycnic Zonal Centrifugation

The same rotor systems used for rate-zonal separations may also be used for separations based on buoyant density, in which particles are driven to their isopycnic or isodensity level in a gradient by centrifugal force. This method has been used with the B-IV rotor to separate smooth rat liver endoplasmic reticulum from rough (*41*); to isolate mitochondria (*27*); to isolate subcellular fractions, including paramylum, from *Euglena* (*42*); to isolate bacterial cell walls (*43*); and to separate nuclei by means of sucrose and D_2O (*26*). In the B-XV rotor (*35*), viral subunits from alkali-disrupted adenovirus preparations have been isolated by isopycnic banding. With large gradients (up to 1.7 liters in the B series), prohibitively large quantities of gradient solutes such as cesium chloride are required. Methods for reprocessing cesium have therefore been developed (*44*).

To explore the possibilities suggested by Fig. 1, a separation based on sedimentation rate and then on isopycnic banding of all the fractions recovered is required. [The reverse procedure, that of making a large isopycnic separation followed by a large number of rate separations, is technically difficult (*7*) and requires dialysis or dilution of all the recovered fractions.] Since the separations depend on the sedimentation rate (on s) and on the buoyant density (ρ or ρ_B), the technique has been termed the s-ρ method (*7*).

In brief, part of each fraction collected in each run with the s-ρ technique (40 to 42 40-milliliter fractions in the case of the B-IV or B-XV rotors) is layered in an angle-head centrifuge tube (*45*) over either a cesium chloride or a sucrose density gradient. This may be done 12 tubes at a time by means of a gradient distributing rotor (B-XXI) (*46*). To prevent tube collapse and cap leakage, the angle-head centrifuge tubes were redesigned (*47*). When the isopycnic separations are complete the tubes are photographed in a banding camera (*47*) in which bands are visualized by scattered light, and the photographs are cut in strips and mounted together, forming a pycnogram, as shown in Fig. 7 (bottom). Plastic beads of known density (*47*) are used to determine the banding densities in the tubes. The pycnogram for rat liver (Fig. 7, bottom) matches closely the distribution shown in Fig. 1. Added T3 bacteriophage particles were recovered from the band shown at the tip of the arrow, essentially free of cellular contamination. The lower white band in Fig. 7 (bottom) is composed of pure glycogen particles of increasing size, as shown in Fig. 8. The s-ρ technique makes it possible to study the distribution of glycogen-synthesizing enzymes and the rate of synthesis and breakdown of glycogen as a function of particle size (*48*), and it opens up new possibilities in the study of glycogen storage diseases (*49*). The exploration of a variety of cells and tissues by means of the s-ρ technique will probably reveal a number of new subcellular components. In addition, since most virus particles do fall within a so-called "virus window" (*7*), the method should prove useful in the search for virus particles such as the human hepatitis virus.

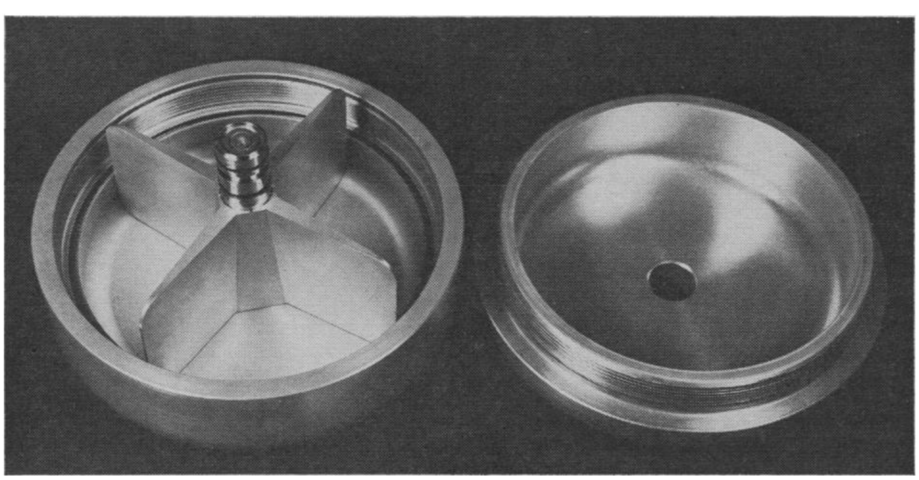


Fig. 3. Components of the B-XV zonal rotor. The rotor chamber consists of two similar halves which thread together. The static seal is not shown. (Scale: ¼ cm = ~1 cm)

Continuous-Flow Centrifuges

For the concentration of viruses from large fluid volumes and the large-scale isolation of subcellular particles, continuous-flow (or, more precisely, continuous-feed) centrifugal separation methods are required. In theory, a cascaded series of such centrifuges should be capable of making separations equal in resolving power to those made by

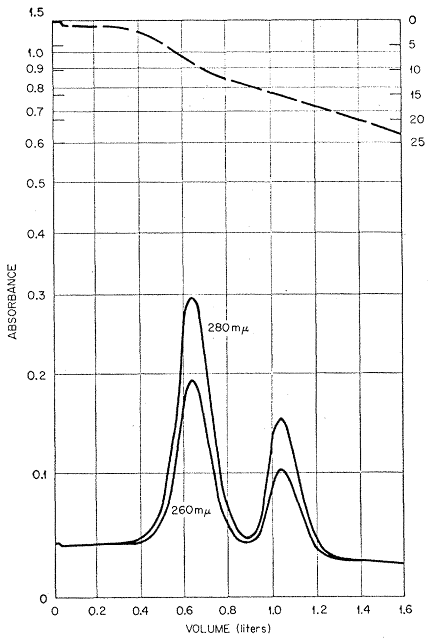


Fig. 4. Representative separations made with the B-IV zonal rotor are shown here and in Figs. 5 and 6. Direction of sedimentation is to the right. Graph shows separation of rat serum macroglobulin from an ammonium sulfate concentrate. The macroglobulin is recovered in the peak at right. [From Fisher and Canning (*32*)]

so-called differential centrifugation in tubes. For isolation of viruses by a continuous-flow method, the B-V rotor was developed (*50*); it has a very narrow annular space and a completely enclosed flow system for isolating small particles which may be present in trace amounts in large fluid volumes. Results obtained with poliovirus (*33*), echo virus 28 (*35*), and T3 bacteriophage (*35*) approach the theoretical limits of performance calculated by Berman (*13*). Since the capacity of the B-V rotor is small, larger-capacity continuous-flow rotors (of the K series) in which the inflow and outflow are at opposite ends of the rotor are now being developed (*19*).

Particles, instead of being sedimented from a flowing stream against the rotor wall (a procedure which produces undesirable particle aggregation), may be sedimented into a density gradient maintained in the rotor. It was thought that, if the gradient could be recovered undisturbed at the end of the run, discrete bands of particles might be isolated. A series of rotor cores (B-VI to IX) were constructed to test this concept (*51*). The B-IX rotor (*52*) has been successfully used to isolate the respiratory syncytial virus (*53*), influenza virus (*35*), and a number of other viruses from multiliter quantities of fluid.

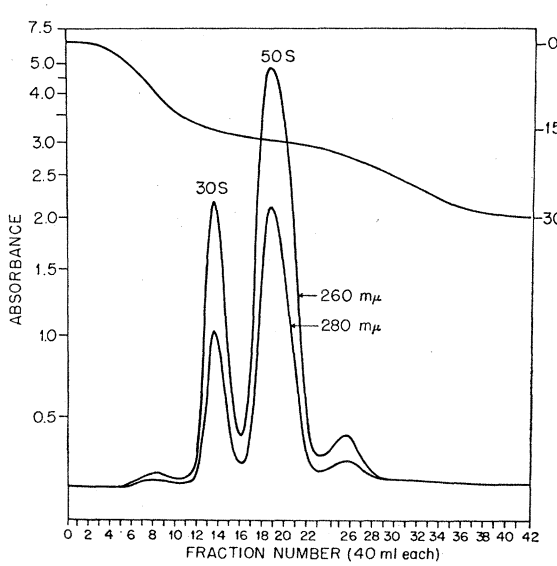
On the basis of this experience, the B-XVI roto core, in which a centrifugal valve is used to prevent stream mixing in the rotor, has been developed (*35*). With this rotor two viruses—such as T3 bacteriophage and adenovirus, type 7—may be removed from a flowing stream and recovered as two well-separated bands (*35*). The application of this rotor system to the isolation of subcellular particles is being explored.

Scale-up of Techniques

While the B-series rotor systems increase by a considerable factor the amount of material which may be fractionated with high resolution, even larger centrifuges will be required. This is especially true when mitochondria or nucleoli, for example, are to be used as starting materials for the isolation of trace constituents of these structures. The shell of the K-II rotor (total internal volume, 8.9 liters) may be used, with rotor cores similar to those of B-IV, B-V, B-IX, and B-XVI rotors, to scale up each type of separation mentioned.

Other New Gradient Techniques

During the feasibility studies for the Molecular Anatomy Program, centrifugal fractionation of suspensions of subcellular particles, particularly those containing viruses, has been the central theme. However, a number of new techniques have also been developed for molecular separations in liquid gradients under conditions where the centrifugal field is insufficient to appreciably sediment the molecules in question when they are not aggregated (*14*). One of these methods is termed gradient resolubilization and is illustrated diagrammatically in Fig. 9. It depends on the fact that a finely divided precipitate will dissolve rapidly when it is returned to conditions under which it is soluble.



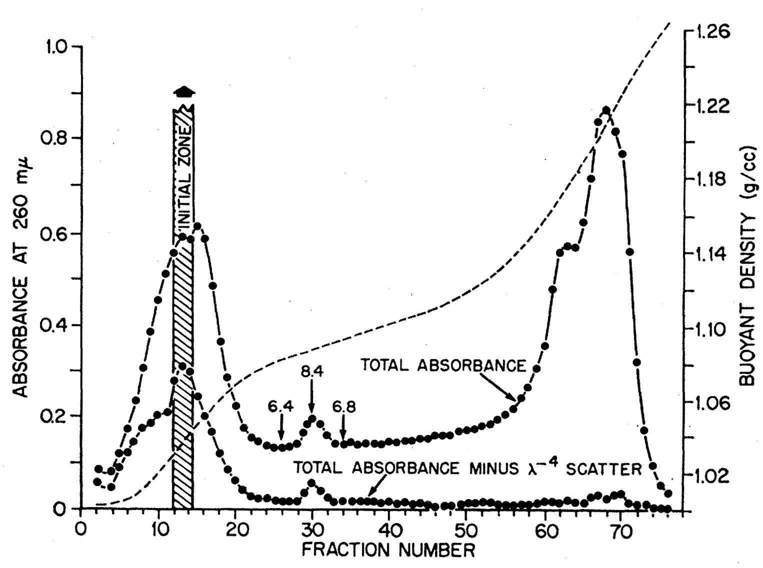


Fig. 5 (left). Separation of 30*S* and 50*S* ribosomal subunits from *Escherichia coli*. Direction of sedimentation is to the right. [From Anderson *et al.* (*16*)]; Fig. 6 (right). Sedimentation profile of a 50-milliliter sample of a poliovirus concentrate prepared in the B-V continuous-flow rotor and centrifuged for 90 minutes at 30,000 revolutions per minute. Direction of sedimentation is to the right. Titers, in $\log_{10}$ of the number of infectious particles per milliliter, are indicated for three 20-milliliter fractions. [From Reimer *et al.* (*33*)]

(The rate at which equilibrium is approached is more rapid when a fine precipitate is added to a nearly saturated solution than when the experimenter starts with a supersaturated solution and waits for sufficient precipitation to occur to produce an equilibrium between the dissolved and precipitated phases.) Experimentally a protein is precipitated with alcohol or other precipitant, and is then passed through the B-XVI rotor over a gradient which is positive radially with respect to density and negative with respect to the precipitant used. If heavy metals are used for precipitation, a chelating agent may be included in the gradient; if lowering or raising the *p*H is the method used to produce precipitation, then suitable buffers are incorporated in the gradient to return the sedimenting precipitates to a *p*H range in which they are soluble. As the floc is centrifuged out of the flowing stream it sediments through the gradient until it reaches a level where it can return to solution and be recovered in solution in the gradient at the end of the experiment. This technique has been used to concentrate adenovirus subunits in this laboratory and is currently being used to fractionate cytoproteins.

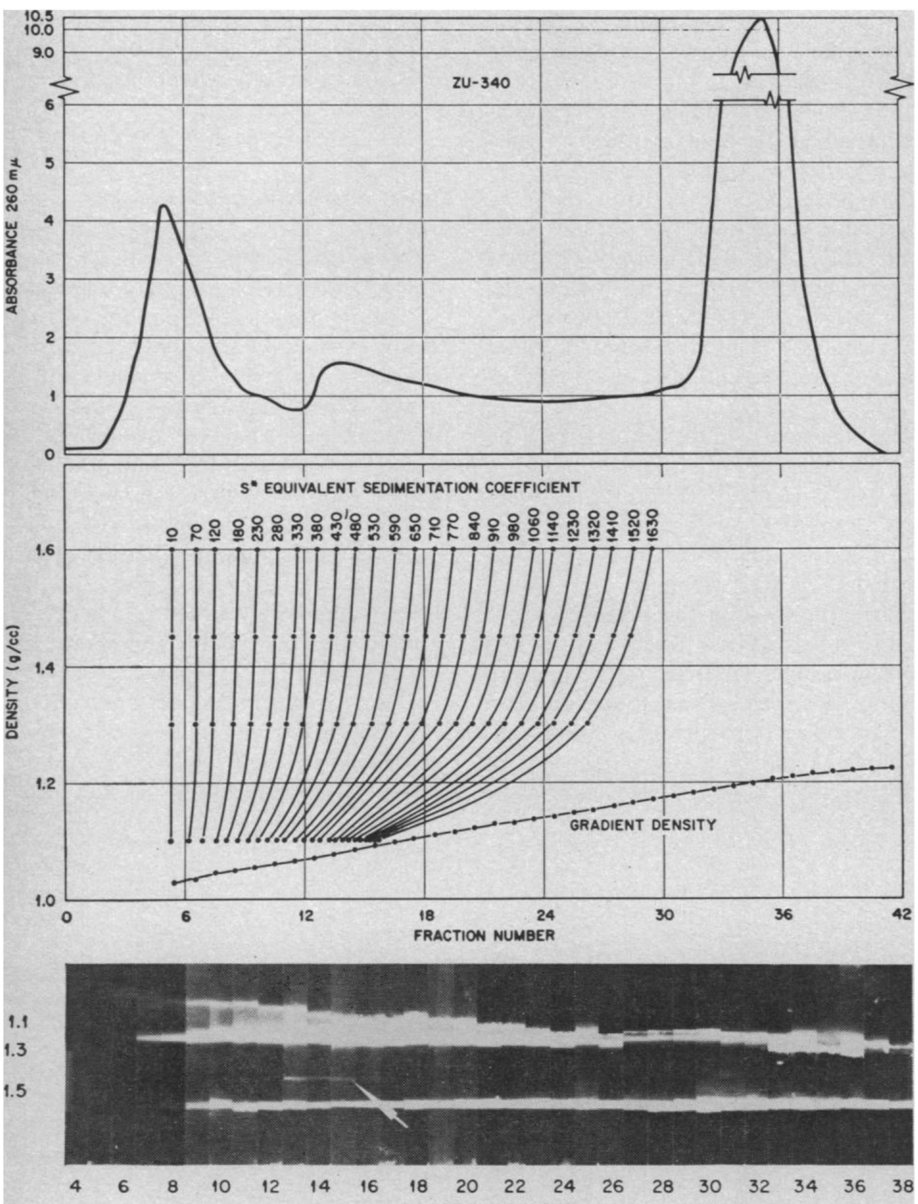


Fig. 7. Combined rate-zonal and isopycnic-zonal separation of a rat-liver homogenate containing particles of T3 bacteriophage. Bacteriophage particles were recovered in band (at tip of white arrow) in pycnogram at bottom. The sample layer was 20 milliliters of a 20-percent (weight to volume) homogenate of fresh rat liver in 8.5 percent sucrose. (Top) Results of rate-zonal centrifugation in the B-IV rotor; the absorbancy at 260 millimicrons (uncorrected for scatter) was determined with a 0.2-centimeter light-path cell, and the observed absorbance was multiplied by 5. (Middle) Computer output for determining equivalent sedimentation coefficients. The positions of hypothetical particles having the densities indicated along the ordinate are plotted, together with the density gradient. (Bottom) A pycnogram, or composite of a series of photographs of individual density-gradient tubes, showing results of isopycnic banding with cesium chloride as the gradient solute. [From Anderson *et al.* (*7*)]

Another technique of interest involves the immobilization of reagents of relatively low molecular weight in a density gradient so that they react with larger particles sedimenting through the gradient. If, during passage through reagent zones, small fragments or molecules are dissociated from the larger particles, these tend to remain behind and be recovered with the gradient. A sedimented particle may thus be sequentially exposed to enzymes, detergents, salts, or changes in *p*H. Additional methods for using zonal centrifuge rotors for molecular separations have recently been described (*14*).

Macromolecular Separations

Developmental studies on methods for making macromolecular separations from cell fractions are dependent on a continuing supply of starting materials in relatively large quantities; hence the initial emphasis on subcellular particle fractionation methods. However, the greatest technical challenge is in the area of macromolecular separations, and it is in that area that most future effort will be expended. Current programs are concerned with the development of chromatographic and electrophoretic separation techniques (*54*) and with means for detecting proteins and other substances in column effluents (*55*). The problem is to develop, in parallel, analytical and preparative methods which depend on the same principles. For maximum resolution, methods which depend on several different processes in sequence will be required. The sequential use of chromatography and electrophoresis for protein separation—a technique originally used by Sober and Peterson (*56*) and recently brought to a high resolution (*57*)—appears to be the most promising procedure, when used in combination with zonal centrifugation, for preparing fractions having molecular weights within a limited range.

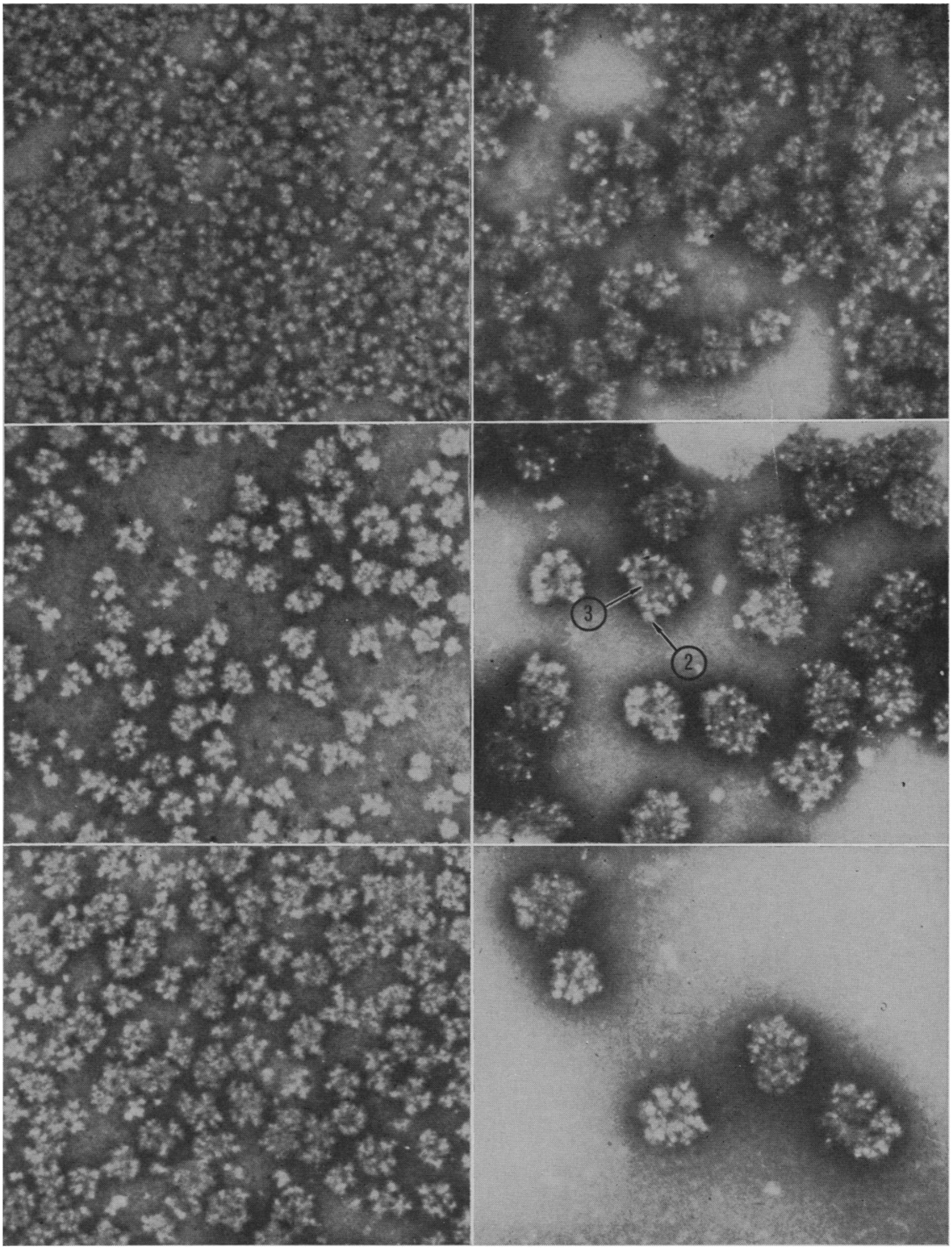


Fig. 8. Rat-liver glycogen separated into discrete fractions by combined rate-zonal and isopycnic-zonal centrifugation, as shown in Fig. 7, where the lower band in the pycnogram is glycogen. Numbers 2 and 3 refer, respectively, to α and β glycogen subunits. [From Barber *et al.* (40)]

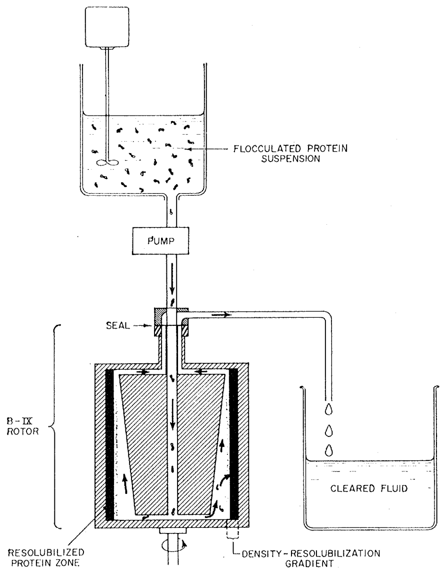


Fig. 9. Schematic representation of the gradient resolubilization technique (see text).

Fortunately, a program concerned with the separation of nucleic acids—first, in large quantities, by means of large-scale precipitation methods and then by advanced chromatographic techniques—has recently been established, as a joint effort, by the Enzymology Group of the Biology Division and the Chemical Technology Division at Oak Ridge. These efforts emphasize the necessity for, and the rewards of, interdisciplinary research programs concerned with the development of advanced separation technologies.

Automated Analytical Systems

Automated analytical systems will be required for each major group of compounds found in cells, or in hydrolyzates of macromolecules. The archetype of such systems is the amino acid analyzer of Spackman, Moore, and Stein (*58*). For the Molecular Anatomy Program an automated system for the analysis of nucleotides, nucleosides, and purine and pyrimidine bases has been developed (*59*). With a high-pressure, high-resolution version of this system, over 100 peaks have been observed in 2-milliliter samples of human urine (*60*). Studies are being made to identify these peaks and to search for disease-correlated changes in them. An automated analyzer for simple sugars has been developed (*61*). An example of the separation which may be achieved with this method is shown in Fig. 10.

In these studies we are concerned with the exhaustive application of presently available technical knowledge and with basic research in areas where technological data do not now exist. Thus, extremely high-pressure chromatographic systems [pressures to 5000 pounds per square inch (340 atmospheres)] and a variety of column sizes, conditions, and packing materials are being examined (*60*).

Molecular Anatomy Program

Interdisciplinary programs requiring large-scale engineering support have been successful in the past only when the objectives could be well defined. Further, the objectives must be amenable to subdivision into specific problems or subprograms.

The Molecular Anatomy Program at Oak Ridge has five basic objectives: (i) to devise sources of cells and tissues suitable for cell-fractionation studies; (ii) to develop high-resolution preparative methods for isolating the major cell components; (iii) to develop methods for separating and isolating the macromolecular constituents of these isolated cell components; (iv) to develop automated systems for analyzing the substances of low molecular weight found either free in cells or as monomeric constituents of macromolecules; and (v), through clinical studies, to establish the relationship of the results of this systematic classification of human cell components to normal and abnormal conditions in man.

We recognize that such a program involves the manipulation of biological materials on a scale much larger than that of work done in the past, and that it will require the active participation of physical scientists and engineers as well as biologists.

The question we have asked is simply this: Is the problem of systematically exploring human cells amenable to the strategy and tactics of previous large-scale scientific programs (*1*)? To find out, we have organized a program in molecular anatomy at an existing large-scale research center where such a massive effort is possible. This experiment is now in progress; however, it is too early for more than partial evaluation of the results.

Since Oak Ridge National Laboratory is a complex research and development organization which traditionally has performed difficult separations on a large scale, it is possible to use skills already developed in nonbiological sciences to develop the high-resolution separations systems required for the Molecular Anatomy Program. The separations which have been first accomplished on a large scale at Oak Ridge include separation of the isotopes of uranium by the electromagnetic process, by gaseous diffusion, by thermal diffusion, and by centrifugation; isolation of plutonium from fission products and uranium fuel, and separation of the fission products themselves; isolation of radioisotopes in pure form for therapeutic and experimental use; separation of experimental quantities of all the major stable isotopes of all the naturally occurring elements of the atomic table; and, within the last year, completion of a unique laboratory and reactor for producing and purifying the transuranium elements. This work has been supported by research groups which include chemists, physicists, mathematicians, biolo-

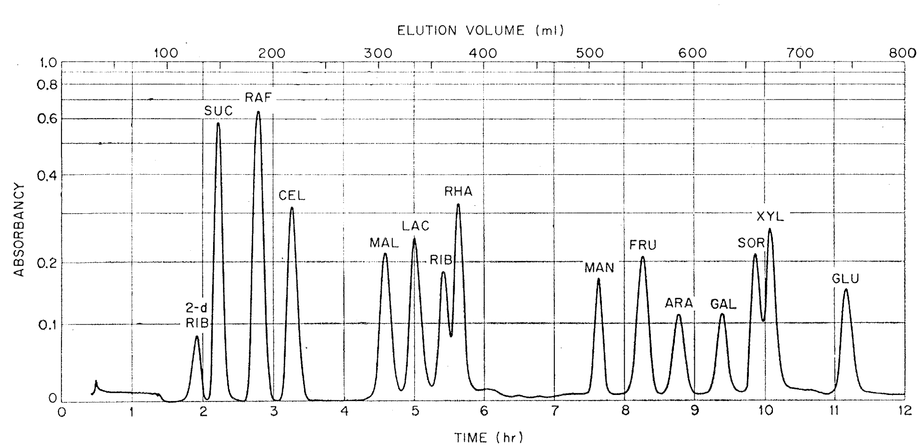


Fig. 10. Separation of simple sugars on a prototype sugar analyzer; 0.75 micromole of each sugar was used. 2-d Rib, 2-deoxyribose; Suc, sucrose; Raf, raffinose; Cel, cellobiose; Mal, maltose; Lac, lactose; Rib, ribose; Rha, rhamnose; Man, D-mannose; Fru, D-fructose; Ara, D-arabinose; Gal, D-galactose; Sor, D-sorbose; Xyl, D-xylose; and Glu, D-glucose. [From J. G. Green (*61*)]

gists, and engineers, whose interests encompass a large part of modern science. The Atomic Energy Commission supports programs to characterize as many subnuclear particles as can be produced; to obtain as much information as possible on the properties of all isotopes of all elements; and to develop the techniques necessary for these studies. The Molecular Anatomy Program is an exactly parallel effort in the biomedical sciences.

In the past there has been a singular difference between physical-particle and biological-particle studies: the particles of the physicist fitted into a theoretical framework, those of the biologist did not. The discovery of so-called "strange particles" partially obliterates this difference, so that one of the justifications for building larger new accelerators has been the hope of finding particles whose characteristics have not been accurately predicted.

In many respects the Oak Ridge Molecular Anatomy program has been evolving over a period of many years. For example, the direct application to nucleotide separations (*62*) of systems developed at Oak Ridge for fission-product separation is basic to nucleic acid structural studies.

Conclusions

This discussion has included only a partial list of the systems now under development at Oak Ridge as part of the feasibility studies for the Molecular Anatomy Program. It is evident that we are still in the "Robert Goddard" phase of this work. It may not be premature, however, to suggest several conclusions.

Biomedical scientists are discouraged on discovering that developmental efforts cost more, by one or two orders of magnitude, than pure research. In part this is because the full cost of development is generally shown, while in pure research some of the costs may be hidden, or the funds supplied by several sources. Regardless of the reason, the fact remains that development is expensive, as is well understood in nuclear physics and space science.

The role and mission of the large national laboratories, and the kinds of research that should be done in them, have been discussed by Weinberg (*63*). The studies described here were in part stimulated by his ideas. We have been unable to find an environment outside a large national laboratory where a program like the Molecular Anatomy Program could be undertaken at the present rate. It appears that programs which attempt to make use of the multidisciplinary approach characteristic of national laboratories should be carefully designed and should evolve experimentally. There is less chance of success when a program is an administrative invention than when it evolves from scientific invention and discovery.

It has been pointed out (*64*) that most program decisions in science are secret decisions in the sense that the scientific community as a whole does not participate in them. If a choice is to be made at some future time between large-scale expenditures for exploring space, for developing new weapons systems, for constructing new accelerators, for designing large reactors, or for systematically developing methods to explore the molecular basis of human disease, then we will need sufficient information to evaluate each alternative fully, and the information should be generally available. It appears desirable, therefore, to allow the Molecular Anatomy Program to proceed to a point where the full range of its contributions and its inherent limitations may be seen. A rational choice may then be made.

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Image Tubes in Astronomy

William A. Baum

The development of image tubes for astronomy has been a slow and sometime vexing problem. The potential advantage of an image tube over unaided photography was demonstrated more than 20 years ago. Since that time many workers have explored various methods in trying to make image tubes a practical reality for astronomical observation.

Within the past 2 or 3 years efforts to develop image tubes for astronomy have finally begun to bear fruit. Use of an image tube on a telescope is no longer a mere stunt; the technique has become a practical one for making routine astronomical observations. Image-tube papers are beginning to appear regularly in the literature. Thus it may be a good time to review the problem and describe the current state of the art.

The goal is not merely to obtain astronomical pictures in shorter exposure times. A shortening of exposures can be accomplished more easily by ordinary unaided photography at a telescope or spectrograph of shorter focal length than usual, but such a procedure yields a coarser picture containing less information. Similarly, a coarser picture will also result if, instead of a shorter focal length, an image tube of low resolution is used in place of a photographic emulsion; the degradation of the image will partly offset any decrease of exposure time. A comparison of this kind is a good way of judging whether the use of an image tube has really gained something.

The real purpose of using an image tube is to collect more astronomical information per unit time. There are several alternative ways of spending whatever gain is available. Exposure times can be shortened, fainter objects can be reached, or image magnifications can be increased. Suppose, for example, that a particular image tube is found to provide a 10-fold speed gain over unaided photography of equal image quality. Instead of using this factor of 10 to shorten exposure times, we might sometimes choose to raise the signal-to-noise ratio by $10^{1/2}$, or we might choose to increase the resolution by a further magnification of $10^{1/2}$ times. Since the intrinsic resolution of an image-tube system is usually different from that of an unaided photographic emulsion, corresponding images are not ordinarily equal in size when they are equal in image quality.

The concept of information rate has usually been discussed in terms of the amount of picture information per image element. The total number of image elements covered is a separate factor. In this respect, image tubes do not begin to compete with unaided photography. Plates exposed at the 48-inch Schmidt telescope on Mount Palomar, for example, cover about 20,000 by 20,000 resolved image elements, whereas very few image-tube systems exceed 1000 by 1000 elements (2000 by 2000 television lines). Thus, when a very large image of great detail is to be covered, photography wins; but when a gain of threshold is sought, the image tube wins.

The reason that an image tube is potentially able to excel unaided photography is that a photoelectric cathode has a higher quantum efficiency than a photographic emulsion. For a given flux of incident photons, the number of electrons ejected from a good cathode is larger than the number of grains blackened on an unaided emulsion. This ratio of quantum efficiencies is about 30 for blue light and somewhat less for light of longer wavelength. It would directly represent the gain of the image tube if several conditions were fulfilled—namely, if every photoelectron were to produce a grain or grain clump in the final image, if all resulting grains or clumps were of equal size, if spurious background were negligible, and if there were no loss of resolution in comparison with unaided photography at the same focal length. In practice, various image-tube systems do not completely fulfill these conditions; hence the actual gain of the tube over unaided photography tends to be a factor somewhat less than the ratio of quantum efficiencies. We might think in terms of an image degradation factor by which the quantum efficiency ratio must be divided in order to express the true gain of the image tube.

Some types of tubes degrade an image very much more than others. The more times an image is transformed or reproduced, the more the image is likely to be degraded. A television system, for example, tends to introduce a larger degradation factor than a very simple image converter. The preference for simplicity, however, is not in itself a sufficient criterion for the choice of one image-tube system over another. It also turns out that the simplest type of tube from the point of view of physical processes happens to be the most difficult one to operate from the point of view of the observer.

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