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**FINAL REPORT
1972 SUMMER INSTITUTE FOR
BIOMEDICAL ENGINEERING**

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FEBRUARY 1973



**GODDARD SPACE FLIGHT CENTER
GREENBELT, MARYLAND**

FINAL REPORT

1972 SUMMER INSTITUTE FOR BIOMEDICAL ENGINEERING

Under Contract No. NASW-2386

Eugene M. De Loatch, Ph.D.
Howard University

February 1973

GODDARD SPACE FLIGHT CENTER
Greenbelt, Maryland

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1972 SUMMER INSTITUTE IN BIOMEDICAL ENGINEERING

INTRODUCTION

Biomedical Engineering, one of the younger members of the engineering family, has struggled hard during the past decade to survive as an accepted and meaningful discipline. While its continued existence in the academic community is highly dependent on the number of trained people available to structure it and to adequately define its objective, its need by the national health care community has become increasingly obvious.

The cost of routine medical care, as experienced by many working families in this country, has been recognized as an undue and often crushing burden. In some of these families, it takes only one major lingering illness to completely liquidate a modest life savings. Congress is aware of this situation, and studies are being undertaken with the intent of alleviating these problems and reacting to public concern.

One consequence of spiraling medical costs is that out-patient clinics have become overcrowded and emergency rooms are being used, inadvisably, by those attempting to minimize the cost of essential medical services. The increased strain on these parts of the hospital system has served to expose an increasing shortage in the number of practicing physicians, nurses and para-professionals prepared to perform high-demand patient services. It is postulated that this dichotomy has contributed to still higher costs of medical care.

To help offset shortages in trained personnel, hospitals have been greatly tempted to increase automation and to employ more semi-automatic attending devices. While this approach will undoubtedly become increasingly practical, at present, improper analysis and coordination have left us with many devices and systems which have served, indirectly, to increase the unit cost to the patient.

The Technology Utilization Office at the Goddard Space Flight Center has sought to find ways to involve itself via programs funded through NASA Headquarter's University Affairs Office. The Center was specifically interested in finding new ways to close the gap between the current technical needs of the medical community, as centered on clinically-oriented devices, and the application of the existing knowledge of technologists attempting to fulfill those needs. Through the expertise developed during the years of the space program, it is felt that NASA possesses a prolific "stock pile" of knowledge and methodology which, if properly directed, could make deep inroads into existing clinically-oriented medical problems.

This report is submitted as the record of a specific project in which NASA expertise and facilities were coordinated with a part of the medical-academic community as a test of the hypothesis advanced above. It is hoped that the results will encourage others to believe that team efforts between medical and technical personnel are not only possible, but hold forth potential for unique and strikingly positive results, if properly coordinated and synchronized.

THE PROGRAM

The focus of this program was a ten (10) week design-oriented work session spanning the period of June 19, 1972 through August 25. A total of ten (10) engineering/science students were employed in a research environment and given maximum freedom to seek solutions to five previously unsolved clinically-based problems. The students, recruited through an open invitation, were representative of a total of eight (8) undergraduate colleges and universities. Selection of the students was based on their own expressed interest in Biomedical studies as well as their academic preparations. A copy of the program announcement and the student application form are included in the appendix of this report.

MEDICAL AND TECHNICAL PERSONNEL

Early in the planning stages it was projected that a close cooperation between select clinically-oriented medical doctors and technical personnel at the Goddard Space Flight Center (GSFC) would have to be established and maintained if the program was to succeed. Under the capable and generous guidance of Mr. Wayne T. Chen and Mr. Donald S. Friedman of the Technology Utilization Office (GSFC), key persons in the NASA organization were introduced to their Howard University counterparts. As the program objectives became clearer to the medical doctors (all from Howard), suggestions for problems to which the Institute could address itself, began to emerge. Among the doctors who responded with unconstrained enthusiasm were:

Dr. Ulrich Henschke	Radiotherapy	Freedman's Hospital
Dr. Edward Briscoe	Anesthesiology	Freedman's Hospital
Dr. Robert Hudson	Sickle Cell Foundation	Freedman's Hospital
Dr. Roland Scott	Sickle Cell Foundation	Freedman's Hospital
Dr. Marjorie Cates	Hematology	Freedman's Hospital
Dr. John Downing	Pediatrics	Freedman's Hospital
Dr. Robert Curry	Pediatric Cardiology	Freedman's Hospital
Dr. Milton Bernard	Oral Surgery	Freedman's Hospital

As a result of their belief in the program, several extremely challenging problems were identified from which five were finally selected for our intense study.

Selection of the five problems was based on two considerations:

1. Projects that had the greatest potential for the national health care community.
2. Projects for which NASA technical advisors were available to help scale the technical barriers which the student might face.

The advisors who agreed to take on these responsibilities at the Goddard Space Flight Center were:

Mr. Lawrence Kobren	Mr. Maxim Aleksandrov
Mr. Joseph Epstein	Mr. Frederick Gross
Mr. Leonard Kleinberg	

THE INSTITUTE STAFF AT HOWARD UNIVERSITY

Just as it is believed that a medical research team which includes technical persons should view all team members as contributing equals, it was believed that the staff of the Biomedical Institute should have a minimum pyramidal structure. For this reason, the staff of the Institute was broadbased.

Dr. Eugene M. DeLoatch, Principal Investigator under this contract, was the only full-time staff member from Howard University. His responsibilities were to see that the Howard University portion of the contract was fulfilled and that results obtained during its tenure were properly reported.

The active number of staff members of the Institute swelled preceding and during the ten-week period of actual problem studies. During that time, Professor Anna J. Coble, a lecturer in Biophysics, served as an assistant program coordinator. Among her duties were: a) preliminary studies on the scientific feasibility of some of the proposed problems; b) the continued guidance of the students in their search for pertinent reference materials.

Due to an early display of interest, Dr. Edward Briscoe (Anesthesiologist) and Dr. Robert Hudson (Pediatrician) accepted assignments and were added to the advisory staff of the Institute. Their available time for actual work on Institute matters was naturally limited, but they showed eager willingness to cooperate in directing us to the personnel and facilities which we needed that existed in the Howard University Hospital Center/Medical School environment.

Ms. Paulette A. Payne, a junior student in Electrical Engineering, provided an invaluable service in her capacity as an assistant to the professional staff.

Her duties were numerous and the fulfillment of them served to unite the desires of the staff and the efforts of the students into an exciting and rich educational experience.

THE PROBLEMS SELECTED FOR STUDY

A total of twelve task descriptions were submitted for review as possible problems for study by the Institute. This number was larger than expected, based on the amount of time which the doctors were given to prepare them. After a screening process, conducted by all concerned parties, five problems which appeared sufficiently challenging and bearing the greatest potential for wide-range impact were selected.

The problem titles, the medical investigators who originally stated the problems, and the technical advisors who volunteered their efforts for coordination are as follows:

"RAPID SAMPLING OF RELATIVE VISCOSITY OF BLOOD SAMPLES"

Stated by:

Dr. Roland Scott, M.D.

Dr. Robert Hudson, M.D.

Technical Advisors:

Mr. Leonard Kleinberg

Mr. Joseph Silverman

Mr. Maxsim Aleksandrov

"EXHAUST SYSTEM FOR ANESTHESIA APPARATUS IN OPERATING ROOMS"

Stated by:

Dr. Edward Briscoe, M.D.

Technical Advisors:

Mr. Lawrence Kobren

Mr. Frederick Gross

"IMPLANT GUN FOR IRIIDIUM 192 SEEDS"

Stated by:

Dr. Ulrich Henschke

Technical Advisor:

Mr. Joseph Epstein

"AN IMPROVED NON-INVASION METHOD OF OBTAINING PULSATILE PRESSURE DATA FROM INFANTS & CHILDREN"

Stated by:

Dr. John Downing

Technical Advisors:

Mr. Leonard Kleinberg
Mr. Joseph Silverman

"IMPROVED DESIGN OF ELECTROPHORETIC EQUIPMENT FOR RAPID
SICKLE CELL ANEMIA SCREENING"

Stated by:

Dr. Roland Scott
Dr. Robert Hudson
Dr. Marjorie Cates

Technical Advisors:

Mr. Lawrence Kobren
Mr. Frederick Gross

The problems, as stated, were presented to the students, who were to work in teams of two. These teams were assembled by the students themselves, matching each student with the problem of his interest. Any early conflicts such as several students desiring to study the same problem, were resolved with agreements made through mutual accord and minimal amounts of "arm twisting."

Once under intensive study by the student teams with medical/technical monitoring and guidance, the problems were reshaped to better fit clinical needs as well as technological feasibility. The original descriptions are placed at the beginning of each report. The revised descriptions and scope as finally agreed to by all parties, are included as part of the narrative. In most cases, it will be noted that the original problem was modified.

THE TEN-WEEK PERIOD: JUNE 19 - AUGUST 25

The ten students participating in this program were granted weekly stipends to study the pre-selected problems during the ten-week period of June 19 - August 25. Following a two-day orientation, students were asked to make their own problem preferences known. During this orientation, the students were exposed to the design of the program, the key people and divisions of the participating organization, and given a briefing on the problems to be studied. On the morning of the third day, matches between the two-student teams and problems for study were effected.

Acting as project engineers, the students were given full responsibility for identifying and gathering all the information and items needed to effectively research their problems. Any reasonable requests for assistance in securing materials or gaining access to key personnel were immediately acted upon by the program coordinators at Goddard and/or Howard as required. This was

done with the realization that tight scheduling would be required to achieve reasonable results in the ten-week period.

In former years of this program, weekly lectures were used to expose students to various aspects of medicine under study. The staff decided to replace these lectures with allowing the student greater flexibility in selecting those things which he or she deemed necessary to arrive at an adequate solution of the problem. As a result, local travel between Goddard and Howard was increased as compared to previous years and lectures or conferences were arranged according to request.

To trace the progress of the students, frequent informal student group assemblies were held. This allowed the students to exchange ideas and discover areas of common difficulty which they may have been experiencing. It was found that this exchange served to add to the over-all efficiency of the Institute as well as to periodically reinforce the students' confidence.

At the end of the seventh week, a preliminary presentation of their progress was given at a meeting at the Howard University School of Engineering. The medical doctors were here invited to observe the direction in which their problem was being taken and to receive projections of the results that were expected in the ten-week period. It was later found that this session served as an ideal forum in which to prepare for presentation of the final reports.

CLOSING THE PROGRAM: THE TECHNICAL REPORT

Results of the studies were presented at a public meeting held at the Goddard Space Flight Center on August 25th. Each of the five teams was given twenty minutes to condense and present its problem, outline the solution and chart the future course of the development of its device as collectively perceived. A question and answer period followed each oral presentation.

As a result of the audience's response and the desire to see the original ideas and designs continued, it was decided to extend the Institute on a greatly reduced scale into the Fall of 1972. It was tentatively agreed to keep all problems alive at some level of observation, with two of them getting a closer look by the Howard students who were original members of the program. The intent of this follow-up process was to establish a continued liaison between the technical advisors at Goddard and the medical advisors at Howard, with the students serving as a catalyst. For the other three problems, it was hoped that the Principle Investigator would coordinate the Howard effort while the NASA coordination would come from the Technology Utilization Office.

The section which follows consists of the technical reports submitted by the students as by-products of their study. Each report is prefaced with the problems as originally stated. Following these statements are the reports as written by the students. Some reorganization of the materials has been done for continuity, but for the most part, with a minimum amount of editing. This was done purposefully because of the educational nature of this program, and in an attempt to preserve the general purpose of the Summer Institute.

Central to this report are the assembled works of ten (10) student participants of the 1972 Summer Institute.

- 1) Eugene M. DeLoatch — Principal Investigator, Howard University
- 2) Wayne T. Chen — Coordinator, GSFC
- 3) Jurgen Pohly — Representative, NASA Headquarters

STUDENT PARTICIPANTS

<u>Name</u>	<u>College</u>	<u>Area of Study</u>
Walter Carpenter Lansing, Mich.	Rose-Hulman Institute Terre Haute, Ind.	Mechanical Engineering
Clifton Cole Baltimore, Md.	Howard University Washington, D.C.	Electrical Engineering
Irvin Hirsch New York, N.Y.	City College of New York New York, N.Y.	Electrical Engineering
Larry Hum Troy, N.Y.	Rensselaer Polytechnic Inst. Troy, New York	Mechanical Engineering
Judy Kosovich Madison, Wisc.	University of Wisconsin Madison, Wisc.	Chemical Engineering
Joseph Meyer Reading, Penn.	Lehigh University Bethlehem, Penn.	Chemical Engineering
Judy Reddick New Orleans, La.	Federal City College Washington, D.C.	Chemistry
Charles Smoot III West Lafayette, Ind.	Purdue University Lafayette, Ind.	Electrical Engineering

STUDENT PARTICIPANTS (Continued)

<u>Name</u>	<u>College</u>	<u>Area of Study</u>
Delma West New Bern, N.C.	Howard University Washington, D.C.	Electrical Engineering
David Wodicka Terre Haute, Ind.	Rose-Hulman Institute Terre Haute, Ind.	Biological Engineering

SECTION 1

DESIGN MODIFICATION OF ELECTROPHORETIC EQUIPMENT

Submitted by:

Judy M. Reddick
Irvin Hirsch

Medical Advisors:

Dr. Roland Scott
Dr. Robert Hudson

Technical Advisors:

Mr. Lawrence Kobren
Mr. Frederick Gross
Mr. Edward King

ORIGINAL PROBLEM STATEMENT

As paraphrased by:

Written by:

Dr. Roland Scott
Dr. Robert Hudson

Eugene M. DeLoatch, Ph.D.

TITLE: IMPROVED DESIGN OF ELECTROPHORETIC EQUIPMENT FOR
RAPID SICKLE CELL ANEMIA SCREENING

STATEMENT OF THE PROBLEM

Electrophoresis has played a major role in the discovery and the characterization of different types of hemoglobins. Included among these is Hemoglobin S, the hemoglobin responsible for the sickling of red cells. The disease follows a Mendelian pattern which could have a catastrophic effect on the community health. It is therefore desirable to perform continual mass screening for the trait and the disease. One major constraint on present electrophoretic methods is the time required to complete a single test. To effectively accomplish mass screening, minimization of the electrophoretic run should have major advantages.

SCOPE OF THE WORK

Zone electrophoresis which involves the use of various media in number of shapes and forms, including sheets of paper or cellulose acetate, columns, plates, blocks, or slabs of starch, acrylamide or agar gels, has attained a position of importance in analytical chemistry. A new look at the problems surrounding heat production due to molecular migration under high voltages along with other constraints serving to increase the time of analysis, are in order. As a result, a novel design of electrophoretic apparatus and/or the process specific to large batch analysis of hemoglobin samples would greatly assist the task of the mass screening of various hemoglobin abnormalities.

IMPROVED DESIGN OF ELECTROPHORETIC EQUIPMENT FOR
RAPID SICKLE CELL ANEMIA SCREENING

STATEMENT OF THE PROBLEM

The immediate need for the establishment of sickle-cell screening programs and enlargement of existing centers has been well-documented in recent years. Such centers provide the basis for medical and genetic counseling which is the key to prevention of the disease. To facilitate much of the large-scale analysis at screening centers, an apparatus suited for mass screening would be a valuable asset.

Three major phases are involved in the electrophoretic screening process. They include the preparation of the hemolysate, its application onto the medium of migration and the actual electrophoretic run.

After a series of laborious steps required to prepare the hemolysate, the hemolysate is generally applied to the migration media in a sequential (individual sample) manner. The duration required for a desired separation is, at the shortest, 10-12 minutes using the technique described by Barnes, Lomarmy, and Novac while most commercially available devices require 20-50 minutes.

Thus we can see that, in view of the time consumed in these three stages of the process, present procedures can hardly allow for rapid screening conditions.

APPROACH TO TREATMENT OF PROBLEM

In orienting our approach to the problem, it was decided that adherence to conventional procedures of cellulose acetate electrophoresis and modifying it whenever expedient, would be the most appropriate course to follow. The reason for this lies in the fairly high resolution ability of this medium which therefore enables the tests to be quite comprehensive.

The goals of our project are to center on each of the three stages. This includes the investigation of the feasibility of consolidation and/or elimination of some of the preparatory steps, the designing of a multisample applicator capable of simultaneous application of 20 blood samples on a single cellulose acetate strip and the development of an electrophoretic chamber capable of processing large numbers of samples in a rapid fashion. These changes will serve to eliminate unnecessary handling during preparation and application as well as to reduce the overall time to run a given batch of samples.

HEMOGLOBINS

Hemoglobins are a group of blood proteins responsible for combining reversibly with and transporting oxygen from the lungs to the tissues. Red cells contain about 32% hemoglobin; this translates into approximately 14.5 g of hemoglobin per 100 ml of whole blood. Of this amount, 2% binds iron in the ferric form (methemoglobin) and does not combine with oxygen. Hemoglobin in arterial blood is approximately 98% saturated with oxygen (oxygen tension = 94 mm Hg), while that in venous blood is approximately 75% saturated (oxygen tension = 40 mm Hg).

Each molecule of hemoglobin consists of one molecule of globin to which are attached four (4) molecules of heme. Investigation has shown clearly that

it is in the globin part of the hemoglobin molecule that changes occur which ultimately result in sickle cell anemia.

Globin is composed of two alpha chains and two beta chains. Each alpha chain consists of 141 amino acid residues and each beta chain has 146 amino acid residues. One gene, called a structural gene, codes for the number and arrangement of amino acid in the alpha chains and another codes for the arrangement in the beta chains.

Pauling and his coworkers in 1949 were the first to suggest that sickling might be due to a chemically different type of hemoglobin molecule which would on deoxygenation aggregate into rods and thereby twist the cell out of shape. In a classic experiment, they discovered that sickle cell hemoglobin carried a higher positive charge than normal hemoglobin. They postulated that this difference was due to either a change in the number or in the kinds of ionizable groups in the hemoglobin (16). An alteration in molecular structure of the hemoglobin molecule on deoxygenation could account for the disease state.

By chromatography and peptide "finger prints," Ingram showed that in the sickle hemoglobin molecule, two charged glutamic acids were replaced by two uncharged valines. This finding both substantiated the theory of a molecular chemical change and determined what the change was.

Ingram's initial theory postulated that a single gene mutation, resulting in the replacement of no more than one base pair in the DNA chain of the gene responsible for the synthesis of sickle hemoglobin molecules, could be responsible for the chemical change (10). Hunt and Ingram subsequently provided evidence that in sickle cell hemoglobin, glutamic acid is replaced by valine at the sixth position from the amino acid end (N-terminal) in each beta chain (9).

The amino acid sequence of the beta chain depends on the structural gene which codes for the synthesis of beta chains. The way the structural gene codes, in turn, depends on the arrangement of chemicals which compose it. A change in the arrangement of the chemicals in the structural gene, therefore, would account for the altered amino sequence of the beta chains. The sequence of chemicals on the structural gene coding for glutamic acid is GAG; that for valine is GUG. By substituting one base, "u" (uridine) for the "A" (adenine), in the structural gene which codes for glutamic acid, a structural gene which codes for valine instead of glutamic acid is produced. This change in the DNA codon is responsible for the synthesis of abnormal globin and hence abnormal hemoglobin.

SICKLING PHENOMENON

Kohn and Gillespie, in 1927, reported that sickling occurs only with low oxygen tensions and that sickled cells can revert to normal shape when they are exposed to sufficient oxygen (6). Subsequently, other investigators found that oxygenated normal adult hemoglobin and sickle cell hemoglobin have the same solubility, but, on deoxygenation, the solubility of normal hemoglobin falls by one-half, whereas, that of the sickle cell hemoglobin becomes 50 times less soluble. With lower oxygen saturations, a marked increase in the viscosity of sickle cell hemolysates occurs and the normal biconcave discs of erythrocytes assume the shape of sickles, holly wreaths, and straight tubes. Stetson has demonstrated that the altered shape of sickled cells is due to crystallization of sickle hemoglobin on deoxygenation.

The concentration of sickle hemoglobin is another important condition for sickling. A mean concentration of at least 15 to 25 grams of sickle cell hemoglobin per 100 ml of blood increases the likelihood of sickling.

The mechanism underlying the sickling process was first suggested by Pauling (16). He postulated that in a non-specific way, the sickle hemoglobin molecules might be able to aggregate, or stack together, in long chains because of their complementary surface configurations. Ingram pointed out that the low solubility of a protein depends on the distribution of positive and negative charges upon its surface (10). A change in surface charge pattern through the removal of a glutamic acid could result in a change of solubility.

NORMAL HEMOGLOBINS

Hemoglobins have been described which differ in regards to spectroscopic behavior, immunologic specificity, oxygen dissociation characteristics, isoelectric point, and electrophoretic mobility.

Hemoglobin A is found in normal adults. It has an isoelectric point of 6.87 and it migrates on electrophoretic media with the mobility of human beta globulin. Only one change in the amino acid sequence of its two alpha — and two beta polypeptide chains is required to transform Hemoglobin A into a mutant type.

Hemoglobin A₂, a minor component which comprises about 2.5% of the hemoglobin in normal adults, was first isolated and described by Kunkel and Wallenius (12). It is made up of alpha and delta chains. Hemoglobins A and A₂ have identical molecular weights and ultraviolet absorption spectra but can be differentiated electrophoretically. At pH 8.6, Hemoglobin A₂ migrates more slowly than does Hemoglobin A. Hemoglobin A₃ can be differentiated from both of these by virtue of having a higher electrophoretic mobility than Hemoglobin A.

ABNORMAL HEMOGLOBINS

Abnormal hemoglobins are expressions of mutations which are inherited as autosomal genes. In 1949 Pauling and his colleagues demonstrated that patients with sickle cell anemia had a form of hemoglobin which was electrophoretically distinct from normal hemoglobin. Neel (11) reported that the genes responsible for red cell sickling, in heterozygous individuals and for severe sickle cell anemia in individuals homozygous for the factor, was inherited in simple Mendelian fashion.

All of the abnormal hemoglobins examined by fingerprint method (electrophoresis followed by chromatography) have shown a single amino acid difference with Hemoglobin A. Since the hemoglobin molecule consists of two identical parts, each having two different peptide chains, substitution of a normal amino acid by one which is more acidic or less acidic will change the structure of both halves of the molecule and thereby change its charge and hence its electrophoretic mobility.

Hemoglobin F has a structure comprised of alpha and gamma polypeptide chains and is the principal component of human fetus hemoglobin. It is distinguished from other hemoglobins by its resistance to alkali denaturation. It was first found in the umbilical cord and separated by means of paper electrophoresis by Smith and Conley (19). These investigators used a veronal buffer of PH 8.6 and ionic strength 0.06 to separate Hemoglobin F from Hemoglobin A.

Hemoglobin F is present in some adults affected by hereditary hematological disorders, including sickle cell anemia and thalassemia major and by certain acquired disorders, including pernicious anemia and leukemia. The level of Hemoglobin F in thalassemia major may exceed 90% of total hemoglobin. Hemoglobin F comprises from 55-85% of total hemoglobin at birth, but this level falls rapidly and reaches the adult level of approximately 1% at one year of age. As Hemoglobin F falls, it is replaced by Hemoglobin A.

Thalassemia is a hereditary anemia which occurs commonly in the Mediterranean region or in persons of Mediterranean origin. Thalassemia minor which occurs in individuals heterozygous for the thalassemic gene, is characterized by the presence of target cells and by stippling of erythrocytes. It produces a mild anemia which may be asymptomatic. The level of Hemoglobin A₂ is frequently elevated in these patients. Thalassemia major, or Cooley's anemia, occurs in the homozygous individual and is usually expressed in the first year of life. It is a severe hypochromic anemia characterized by marked changes in red cell morphology accompanied by alterations to the skull and bones due to bone marrow hyperplasia.

Hemoglobin S, the hemoglobin responsible for the sickling of red cells, was discovered by Pauling and associates and is an outstanding example of profound changes in physiologic function which can be brought about by alterations in molecular structure. As with other abnormal Hemoglobins, Hemoglobin S is characterized by the substitution of a single amino acid (valine for glutamine) at one locus of the beta-polypeptide chain of normal hemoglobin (Hb A). Hemoglobin S (Hb S) is occasionally found in association with other abnormal forms of hemoglobin. Individuals homozygous for Hemoglobin S have severe anemia and experience neurologic and intestinal symptoms.

Production of Hemoglobin S in affected individuals reaches adult levels approximately 4-1/2 months after birth. Sickling of red cells is found more commonly in adult Negroes than children. The frequency of Hemoglobin S may reach 10-20% in some East African populations. In American Negroes, the incidence of homozygous inheritance is approximately 0.2%; the heterozygous condition occurs with a frequency of approximately 7%.

Hemoglobin C produces a mild form of anemia. It was first isolated by Itano and Neel and is distinguished by a low rate of electrophoretic migration. It is found more frequently in Africa and also becomes more apparent in adults than in the newborn. Hemoglobin C arises from the substitution of lysine for valine of Hemoglobin S. In Ghana, it may reach a 20% frequency; in American Negroes, the incidence of Hemoglobin C is 2-3%. Hemoglobin C is often present *in association with Hemoglobin A or with Hemoglobin S.*

Hemoglobin D may contain alpha, beta, or gamma polypeptide chains and has the same electrophoretic mobility as does hemoglobin S on most supporting media. Distinction between Hemoglobin S and Hemoglobin D depends upon a test for red cell sickling and also by the greater solubility of Hemoglobin D. This variant occurs in some parts of India with a frequency of 1%. Differences in peptide amino acid composition of Hemoglobin D among several populations have been observed.

Hemoglobin E migrates to the region of Hemoglobin C, and is more commonly found in Southeast Asia. In Burma, Hemoglobin E has been reported to reach a frequency of more than 10%.

Hemoglobin H is a fast moving hemoglobin present in patients heterozygous for the thalassemic gene. It is distinguished by having a structure composed of four beta polypeptide chains. It is readily denatured by freezing and thawing. Hemoglobin H has the same electrophoretic mobility as Hemoglobin I which occurs in individuals with no sign of disease. Hemoglobin I is distinguished by the presence of tryptophan.

Hemoglobin M is found in individuals with methemoglobinemia where the prosthetic group contains iron in the ferric form, and the resultant hemoglobin does not combine with oxygen. The occurrence of hemoglobin M is therefore associated with cyanosis. This variant shows a characteristic absorption peak at 600 $m\mu$ and is found near the origin on electrophoresis.

Barts hemoglobin is a fast moving form of fetal hemoglobin which is present in thalassemia. It consists entirely of polypeptide chains of gamma configuration. A Table listing all the normal and abnormal hemoglobins, mentioned above, is displayed in Table 1.

Table 1

Normal and Abnormal Hemoglobins

Hemoglobin	Abnormal Chain	Designation	Abnormality
A		$\alpha_2^A \beta_2^A$	None
A ₂		$\alpha_2^A \alpha_2^A$	Normal γ chains in place of β
C	β	$\alpha_2^A \beta_2^6 \text{lys}$	lys replaces glu in residue #6
D _{Punjab}	β	$\alpha_2^A \beta_2^{\text{T-13}}$	
E	β	$\alpha_2^A \beta_2^{26} \text{lys}$	lys replaces glu in residue #26
F		$\alpha_2^A \gamma_2^F$	None
G _{San José}	β	$\alpha_2^A \beta_2^7 \text{gly}$	gly replaces glu in residue #7
G _{Philadelphia}	α	$\alpha_2^{68 \text{lys}} \beta_2^A$	lys replaces asp-NH ₂ in residue #68
H		β_4^A	α chains absent
I	α	$\alpha_2^{16 \text{asp}} \beta_2^A$	Asp replaces lys in residue #16
N _{Boston}	α	$\alpha_2^{\text{T-7}} \beta_2^A$	Tyr replaces his in tryp. peptide 7
M _{Emory}	β	$\alpha_2^A \beta_2^{\text{T-7}}$	Tyr replaces his in tryp. peptide 7
M _{Milwaukee-1}	β	$\alpha_2^A \beta_2^{\text{T-9}}$	glu replaces val in tyrp. peptide #9

Table 1 (Continued)

Hemoglobin	Abnormal Chain	Designation	Abnormality
S	β	$\alpha_2^A \beta_6^{val}$	Val replaces glu in residue #6
Norfolk	α	$\alpha_2^{57-asp} \beta_2^A$	asp replaces gly in residue #57
Barts		γ_4^F	α & β chains absent

PRINCIPLES OF ELECTROPHORESIS

Electrophoresis, an electrokinetic phenomenon, involves the migration of particles under the influence of an electric field. The speed and direction of movement are dependent on their size, surface charge, and the physical and chemical properties of the medium in which they migrate.

Among the various techniques of protein separation, the one of major clinical importance is zone electrophoresis in which a solid stabilizing medium serves as the matrix that supports the migrating proteins. In addition to others, the most widely used media are agar, starch, and polyacrylimide gels and cellulose acetate; all of which are noted for their high resolving ability of biochemical substances.

In attempting to speed the rate of migration, our attention was focused on the factors governing electrophoretic mobility. Those factors are buffer pH, particle migration and the heat absorbed by and absolute temperature of the buffer and medium.

1. Buffer pH. The first condition for a protein to be electrophoresed is that it carry (or be induced to carry) an electric charge. Proteins being amphoteric may be positively or negatively charged, according to the pH of the buffer solution immersing the medium. In an alkali solution hemoglobins develop a negative charge if the pH is above their isoelectric point (pI) and will migrate in the direction of the anode, given the presence of an electric field.

The converse is true if the buffer is acidic. It is by virtue of the characteristic isoelectric point for each hemoglobin that differences in electrophoretic mobilities are exhibited. It is in this way that hemoglobin components lend themselves to electrophoretic separation. Negatively charged proteins exhibit less absorption than do positively

charged ones, and consequently a pH of 8.6-8.8 was found to offer optimum results in our work. This is exemplified in Figure 1-1.

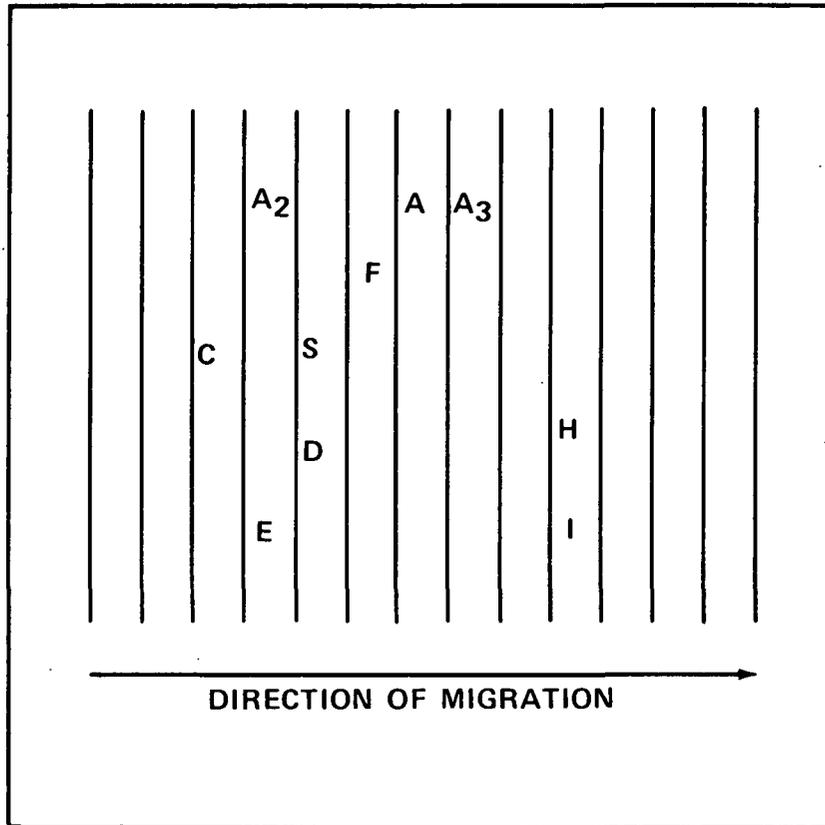


Figure 1-1. Relative Mobilities of Several Hemoglobins at pH 8.6

2. Particle Migration. The factors determining the rate of migration of hemoglobins are analogous to those affecting any ion in an electric field. The action on the ion includes both a driving force due to the electric field:

$$F_D = qH$$

(where q is the net charge of the particle and H the electric field) and a resisting force given by Stoke's law for a spherical particle of radius r :

$$F_R = 6\pi\eta r v$$

(where η is the coefficient of viscosity and v , the electrophoretic velocity).

At the stage of equilibrium between the two forces $F_D = F_R$ and a steady state particle velocity is attained.

Moreover, $qH = 6\pi r\eta v$ and an expression for the electrophoretic mobility is obtained which shows that electrophoretic mobility is dependent on the size and charge of the particle and the viscosity of the medium.

$$\mu = \frac{v}{H} = q/6\pi r\eta$$

Of particular interest is the influence of these parameters on the time (t) required for a given migration distance (d):

$$t = \frac{d}{H\mu} = \frac{6\pi dr\eta}{Hq}$$

All parameters may be treated as constants except for the field intensity H which may vary according to the distance between the electrodes and the potential impressed across them. This fact observed, we electrophoresed our samples at a potential gradient of 167 v/cm of bridge width and obtained good results in five minutes.

3. Heating and Temperature. Electrophoretic mobility increase with temperature. The heat generated both in the buffer and the cellulose acetate strip results in increased conductivity since ions move faster in warmer solutions.

Higher voltages afford a more marked movement of the sample but also minimizes diffusion by sharpening separation.

Excessive heating has, however, adverse affects on the system particularly as temperature approaches 50°C causing denaturation of the hemoglobin molecule. Evaporation from the strip may cause local areas of discontinuity within the medium but the problem did not arise in our investigation. Having monitored the temperature of the cellulose acetate strip by mounting a thermocouple on it, we noticed that the strip temperature did not exceed 33°C indicating no danger of denaturation.

Since the heat generated in the strip is related to voltage, current, and time, the absence of excess heat production may be attributed to the short running period.

CELLULOSE ACETATE MATRIX

Several different types of media may be used depending on the resolution desired. The high resolution media such as the various types of gels entail far too lengthy a procedure for screening purposes. Cellulose acetate, on the other hand, is the medium of choice for such purpose owing to the short duration required for an adequate separation.

Cellulose acetate is the acetylated product obtained by the action of acetic anhydride on cellulose and was first introduced as a stabilizing medium by Kohn in 1957. The degree of acetylation of cellulose may vary from 0-44% and thus a wide variety of this medium is available. We have used Celegram (Shandon Scientific Co.) and Sepraphore (Gelman Instrument Co.) with satisfactory results.

Both quantitative and qualitative advantages are offered by cellulose acetate. It is non-adsorptive, chemically pure material stable in aqueous solvents and resistant to dilute acid or alkali.

Separation is rapid and well defined and the accuracy of quantitative determination is thereby improved.

The highly porous matrix resembles a sponge-like structure and manifests only minor variations in such physical properties as pore size (0.4 in diam.) and density (80% by volume). Water molecules attached to the structure serve both to lubricate the pore openings and depress buffer movement.

ELECTROPHORETIC METHODS AND MATERIALS

The chief aim of this section is to outline the methodology of both the present electrophoretic procedure used in the Howard University Medical research center and a modified version of this electrophoretic procedure, which we proposed as an improved technique.

The first two methods for electrophoretic separation of hemoglobin, which are specifically geared toward minimization of mass screening programs, will be described briefly. Method I, includes a ten minute cellulose acetate electrophoresis. Method II, a five minute cellulose acetate electrophoresis, a modified version of Method I, places special emphasis on its approach at optimizing the entire electrophoretic procedure.

METHOD I. A TEN MINUTE CELLULOSE ELECTROPHORESIS

Apparatus

The completed electrophoretic apparatus is shown in Figure 1-2. The body of the cell is constructed from 1/4" clear acrylic sheet. The electrophoresis chamber contains anode and cathode compartments with gold alloy electrode wires. Acrylic bridge supports for the cellulose acetate strips form the inner walls of the anode and cathode buffer compartments.

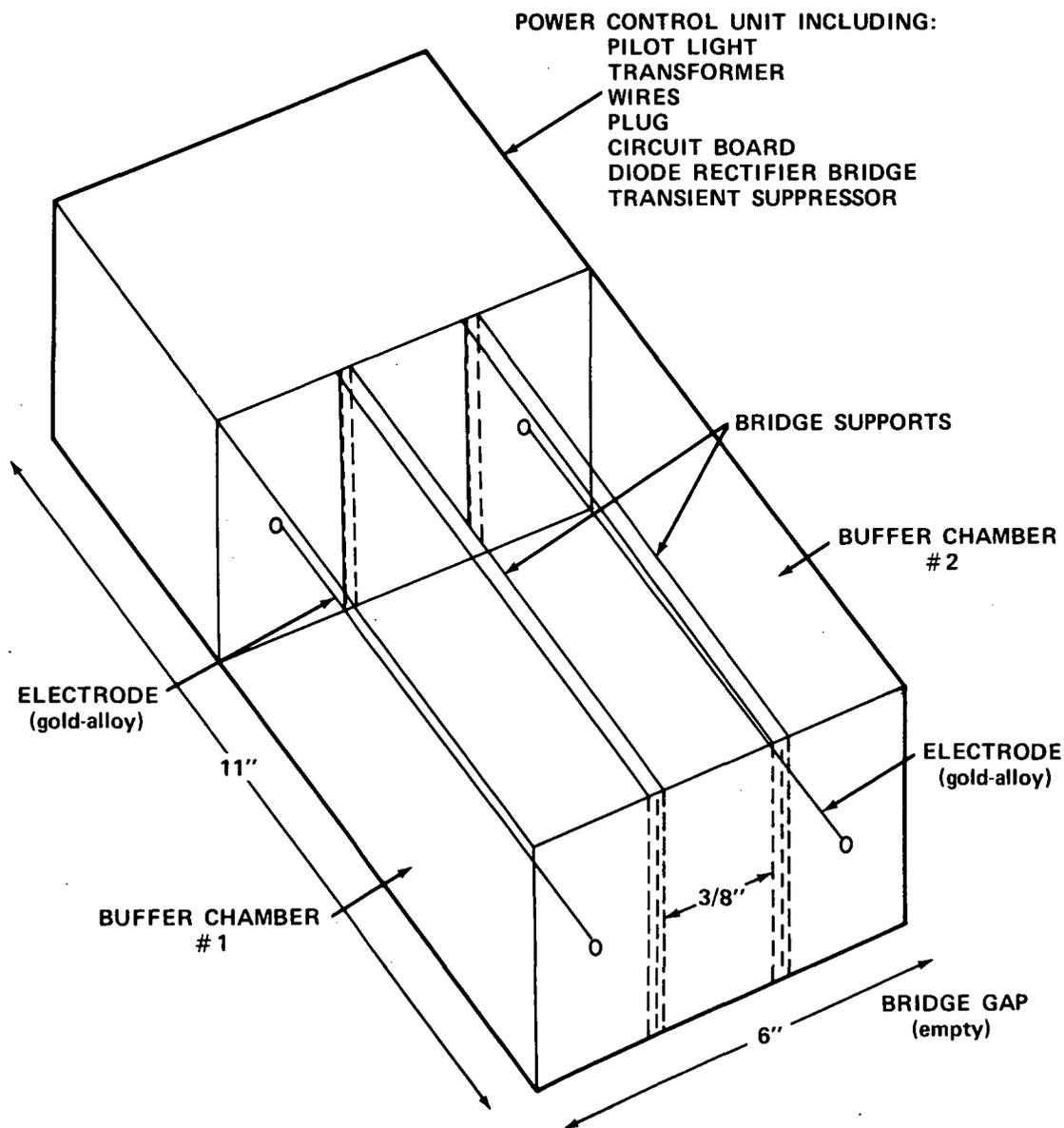


Figure 1-2. Electrophoresis Apparatus (designed by Yale-New Haven Hospital)

Power Supply

The rear of the cell contains a 250 volt pulsed d.c. power supply sealed with an acrylic chamber. A transformer initially boosts line voltage from 120 a.c. to approximately 250 a.c.

Preparation of the Hemolysate

Capillary blood is collected in 2 75 mm × 1.3 mm heparinized microhematocrit tubes. One end of each is sealed by either heating in an open flame or with hematocrit sealing clay. The two microhematocrit tubes are centrifuged for 10 minutes in a standard microhematocrit centrifuge. Following centrifugation, the microhematocrit tubes are scored with an ampule file about 3 mm below the buffy coat. The tubes are broken on the segments containing plasma and buffy coat are discarded. The portions containing packed red blood cells are broken in two parts and placed in a 12 × 75 mm test tube containing 0.1 ml of a 0.01% saponin solution (detergent used for lysing the cells). Mixing for 30 seconds on a vortex mixer lyses the red cells and releases the hemoglobin. No attempt is made to remove the red cell stroma.

Buffer

A discontinuous buffer system is utilized with solutions prepared as follows:

+Anode Buffer - 0.26 M Tris, pH 9.1

Tris (hydroxymethyl) aminomethane	25.2 gm
EDTA (ethylenediamine-tetraacetic acid)	2.5 gm
Boric Acid	1.9 gm
Distilled H ₂ O to	1,000 ml

-Cathode Buffer - 0.03 M barbital pH 8.6

Sodium diethylbarbiturate	5.15 gm
Diethylbarbituric acid	0.92 gm
Distilled H ₂ O to	1,000 ml

Preparation of the Cellulose Acetate Media

Strips of cellulose acetate, 50 × 200 mm (Celagram) are cut into two 50 × 100 mm strips to facilitate handling. The cut strips are floated on top of a mixture of equal parts of the two buffers until saturated. The strips are then blotted between two sheets of ordinary blotting paper. The moist strips are ready to accept the sample.

Application of Hemolysate to the Cellulose Acetate

The sample is applied to the moist strip by using a Millipore applicator. The applicator tip is formed of two closely spaced stainless steel strips, held exactly parallel and molded into a weighted handle. The sample applicator holds a quantity of $\approx 0.3 \mu\text{l}$ of hemolysate. The procedure for application of the hemolysate involves the following tedious steps:

- a. Sample hemolysate is drawn into a Pasteur pipette.
- b. Hemolysate is, next, loaded onto applicator by placing the tip end of the pipette, containing sample hemolysate, between the two parallel wires beginning at one end and streaking the sample along the wires to the other end.
- c. Hemolysate is then transferred onto the middle of the moist strip (previously mentioned) by slightly pressing applicator to the paper delivering a narrow line of ≈ 4 mm long.

As many as six hemolysates are applied to a single strip (50 × 100 mm) utilizing this same time consuming step by step procedure. A Khon AS or AC hemolysate is included as a control to aid in identification.

Preparation of the Cell

Anode and Cathode buffers are poured in their respective right and left chambers to a depth just sufficient to cover the electrode wires. The buffer should be replaced after every 3-4 runs. Strips of ordinary laboratory tissues are used as buffer wicks and are draped from the top of the bridge supports down into each buffer. The 3 cellulose acetate strips with samples of hemolysate applied are then placed on the supports of the cell with the bridge gap adjusted to 3.5 cm.

Electrophoretic Run

Electrophoresis is carried out at 250 volts for 10 minutes utilizing the power supply described above.

Staining and Destaining Procedures

Following electrophoresis the strips are stained for 3 minutes in a 0.5% solution of Ponceau S in 7.5% Trichloroacetic acid. Destaining is accomplished in 3 rinses of 2% acetic acid. Strips are then air dried on sheets of blotter paper and examined.

METHOD II. A MODIFIED VERSION OF METHOD I WITH AN IMPROVEMENT IN METHODOLOGY

Apparatus

The electrophoretic cell used in this study is a modified design of the cell described in Method I. The changes made in the cell are the following: Bridge Gap dimensions (outer width - 3 cm.; inner width 2.57 Electrodes consisted of 60% platinum and 40% Rhodium). The effectiveness of the bridge gap adjustment is discussed in a later section.

Power Supply

Power Supply used in our experiments was a standard power supply which provided maximum current and voltage.

Preparation of the Hemolysate

Whole blood, from finger puncture, is collected in two 75 mm × 1.3 mm heparinized microhematocrit tubes. One end of each is sealed in hematocrit sealing clay. The 2 microhematocrit tubes are centrifuged for only 15 seconds in an international microhematocrit centrifuge. Following centrifugation the tubes are scored, right above the sealing clay, with an ampule file. The tubes are broken and the segment containing the sealing clay is discarded. The remaining segment contains plasma and packed red blood cells. The plasma is then discarded by either slightly tilting the hematocrit tube and allowing plasma to flow out freely or extracting the plasma off with a syringe, (Insert syringe into plasma end of the hematocrit tube), leaving only the packed red blood cells. Two cc of a 2% aqueous solution of Triton X 100 (detergent used as a lysing reagent) is induced into the hematocrit tubes on the end containing the packed red blood cells. Mixing for approximately 15 seconds by slightly tilting the hematocrit tube back and forth lyses the RBC and releases the hemoglobin.

The sample is applied directly from the hematocrit tube. This procedure is discussed later.

Buffer

Vernol (barbital) buffer of pH 8.8 and ionic strength 0.06. The buffer was diluted with distilled H₂O to 1 liter.

Preparation of the Media

Two brands of Cellulose Acetate Membranes of varying dimensions were used as a supporting medium in our experiments. Cellulose acetate (celagram) 50 mm × 100 mm and Cellulose triacetate (Separapore) 6 in × 4 in. However, we found that Celagram cellulose acetate membranes were most suitable for the high voltage technique. Before the membrane was prepared for electrophoresis, each strip was marked with a bic pen or marking pen to enable identification of the samples being analyzed. Impregnation of the cellulose acetate membranes was carried out in the same manner as mentioned in Method I.

Application of the Hemolysate to the C.A. Membrane

The sample is applied to the moist strip by using the sample applicator that we designed, shown in Figure 1-1. The applicator was designed specifically for the purpose of multisample application.

The microhematocrit tubes, containing the hemolysate, are inserted in the grooves which are drilled into the applicator. The hematocrit tubes rest on two narrow parallel wires transferring the sample between these parallel wires. The applicator is then pressed lightly to the moist strip delivering a desired amount of sample.

This procedure involves no tedious steps. It is simple, reliable and practical. As many as 10 samples can be applied simultaneously to a strip of paper.

Preparation of the Cell

Same preparatory methods were used as described in Method I.

Electrophoretic Run

Electrophoresis is carried out at 500 volts for 5 minutes utilizing the power supply described above.

Staining and Destaining Procedures

Same procedures described in Method I.

ADDITIONAL TECHNIQUES FOR ELECTROPHORETIC

HEMATOLOGICAL EVALUATIONS

A careful hematological evaluation should be made when the presence of an abnormal hemoglobin is suspected. The techniques discussed below will include "the demonstration of sickle cells in peripheral blood," "the stains for the demonstration of inclusion bodies," and "the demonstration and estimation of fetal hemoglobin-containing red cells.

Sickle Cell Preparation

When the oxygen tension of blood from a patient with a sickle cell trait or sickle cell anemia is lowered by oxygen-removing substances, such as, ascorbic acid or sodium bisulfite, many red cells appear as elongated cells with pointed ends. The amount of Hb-S present in each red blood cell correlates more or less with the time it takes for the cells to sickle. Blood cells from patients heterozygous for Hb-S will sickle under reduced oxygen tension in vitro, but sickled cells are usually not seen in the peripheral blood smears of these trait patients as is common in the blood of the homozygous sickle cell anemia patient. A fresh solution of sodium bisulfite is prepared daily. Equal amounts of fresh blood and sodium bisulfite are mixed on a glass slide and covered with a cover slip. The preparation is sealed with paraffin or petroleum jelly and allowed to stand for 15 minutes. The slide is then inspected for sickled red cells.

Stains for Inclusion Bodies

Inclusion bodies such as Howell-Jolly bodies and Cabot's rings, which are nuclear fragments, may be stained with a polychromatic stain such as Wright's or Leishman's stain. Heinz bodies are denatured and precipitated hemoglobin. These bodies can be demonstrated on slides which are prepared from a mixture of 1 drop of blood and 4 drops of a 0.5% methyl violet solution in physiological saline. The smear is covered with a cover slip and examined under high power. The bodies appear as refractile bodies around the periphery of the red blood cell. The bodies stain deep purple, while Howell-Jolly bodies stain almost black.

The Demonstration and Estimation of Fetal Hemoglobin Inside Red Blood Cells

To evaluate the distribution of fetal hemoglobin within the red blood cells, a procedure developed by Botko and Kleihauer is described below.

Reagents

- a. Citric acid - 0.1 M, i.e. 21.015 in 1,000 ml aqua. dest.
- b. Disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$)-0.2 m, i.e. 53.63 g in 1,000 ml aqua. dest.

Add 73.4 parts solution A to 26.6 parts solution B.

The resulting PH is 3.3

Erythrosin B-0.1 percent in aqua. dest. is used as the hemoglobin stain.

Method

1. Fix thin blood film in 80% ethanol for 5 minutes.
2. Rinse thoroughly in aqua. dest. (5 minutes).
3. Immerse for 5 minutes in citric acid - sodium phosphate-buffer PH 3.3
4. Rinse thoroughly in aqua. dest. (10 minutes).
5. Allow to dry thoroughly to avoid staining artifacts.
6. Stain for 3 minutes in Mayer's haematoxylin.
7. Rinse in tap H_2O to blue the nuclei.
8. Counter stain for 4 minutes with 0.1% Erythrosin B.
9. Rinse thoroughly in aqua. dest.

Dry and mount

Estimation of Fetal Hemoglobin—Singer's Test.—Fetal hemoglobin resists denaturation by alkali and since denatured hemoglobin is readily precipitated by

ammonium sulfate, the filtrate from a solution of hemoglobin so treated will contain only alkali-resistant pigment. Not all of this, however, Hb-F. Variable traces of non-haem pigment may also be present and this leads to difficulty in the interpretation of low concentration. Conversion of hemoglobin into the carbonmonoxy form renders it more alkali-resistant.

Concentrations of over 2% in an adult indicate with certainty the presence of an abnormal amount of Hb-F and usually this is also true of concentration between 1 and 2%.

Method

This is a slight adaption of Singer et al. (1951). Use 0.2 ml of a hemoglobin solution (containing about 10g Hb/100ml), and add this to 3.2 ml 0.083 N-NaOH at 30°C. After precisely one minute the reaction is stopped with 6.8 ml of half-saturated acidified ammonium sulfate solution (76g in 200 ml dist. H₂O + 0.5ml 10n HCl) taking 20 seconds to make this addition. Mix and allow to stand for ≈ one minute. The filtrate from this, which must be absolutely clear, and which contains the alkali-resistant fraction is read in a spectrophotometer at 540mm and compared with 0.2 ml of the original hemoglobin solution diluted with 10ml of ammoniated water which represents 100% Hb.

PHYSIOCHEMICAL PROCEDURES FOR THE DETECTION AND QUANTITATION OF HEMOGLOBIN TYPES

This topic will be limited to the discussion of only a few of the various procedures utilized in Detecting and Quantitating hemoglobin types.

Preparation of a Red Blood Cell Hemolysate

A blood sample of about 5 ml is collected with oxalate, heparin, or sequestrene as anticoagulant. The red blood cells are washed at least 3 times with .9 gm/100 ml NaCl. The washed erythrocytes are hemolyzed by addition of an equal volume of distilled H₂O and 1/2 volume of toluene. The mixture is shaken for approximately 5 minutes and stored in the refrigerator overnight. The hemoglobin solution is separated from the toluene layer and the insoluble debris by centrifugation for 20 minutes. A clear hemoglobin solution is then obtained. The hemolysate can be obtained immediately after a period of shaking for 15-20 minutes. Isolation of the hemoglobin solution is also facilitated by the fact that it can be pipetted directly from the top of the CCl₄ layer. It is recommended that inorganic ions and small molecules be removed by dialysis against a weak (0.02M) sodium phosphate buffer, pH 7.2 to 7.6, for 24 hours at 4°C. The dialyzed hemolysate should be stored at 4°C.

Electrophoretic Techniques Using Stabilizing Media

The following procedures will be described: starch block electrophoresis, starch gel electrophoresis and agar gel electrophoresis.

Zone Electrophoresis in Starch.—The discovery of minor hemoglobin component Hb-A₂ by starch block electrophoresis (Kunkel and Wallenius, 1955) initiated the wide use of this technique. The hemoglobin can be studied as oxyhemoglobin, carbon monoxyhemoglobin, cy and ferri-hemoglobin and as ferrihemoglobin, the latter form being necessary for the detection of abnormal forms of ferri-hemoglobin. Hemolysates have to be clarified by centrifugation in the cold at high speed (20,000 - 25,000 rpm). Potato starch of different sources can be used. The material should be washed repeatedly with a barbital buffer (pH, 8.6, 0.05 M) and not with H₂O; the pH of the effluent should approximate the pH of the washing buffer. The starch is resuspended in the buffer in order to make a thick semi-liquid suspension. The starch paste is poured into a mold of specific size with thick filter paper wicks inserted in each end. After the granules settle (10-15 min) the excess buffer is removed by blotting. The starch block should be approximately 4 mm thick. A suitable number of slits can be made with a metal spatula at the center of the block at right angles to the long dimension of the block. Small amounts of hemolysate (50 m liters of an ≈ 10% hemoglobin solution in a slit of 1 cm length) are pipetted in these slits. The slits are sealed by dropping several drops of buffer on each side of the slit. The mold should be completely covered with an evaporation chamber. The large buffer vessels contain the same buffer as used for the preparation of the starch suspension; connection with the starch block is made through the wicks. The electrophoresis at 4°C requires a current of 30-60 mA at 150 -300v. The length of the run depends on the apparatus used. The Hb. components can be eluted from isolated starch sections with small volumes of buffer. The elutes are cleared by centrifugation at 3000 rpm for 10 minutes. The optical densities are determined at 415 mμ after suitable dilution of each fraction. The method lends itself to preparative work. Since the size of the sample applied to the starch must be limited, the preparation of larger amounts of a specific hemoglobin component requires the use of several large starch blocks.

Zone Electrophoresis in Starch Gel.—The method using starch gel as a supporting medium was developed by Smithies (1955). A vertical electrophoresis as described by Smithies (1959) is most commonly used. Electro-starch, prepared by O. Hiller, (P.O. Box 1294, Madison, Wis.); has certain physical properties which makes it a most desirable product for hemoglobin separations. Covers producing gels with either 8 or 16 slits can be used, preferably the first type. The Gels can be prepared with different buffer solutions. For most purposes a Tris-EDTA boric acid buffer, pH 8.1, is recommended. This buffer is

prepared by dissolving 60.5 gm tris and 7.8 gm EDTA in approximately 1 liter of distilled H₂O, whereafter concentrated boric acid solution is added until a p.m. of 8.1 is obtained. The volume is finally brought to 20 liters and the pH adjusted to 8.1. A boric acid NaOH buffer of pH 9.0 (185.52 gm boric acid and 24.0 gm NaOH dissolved in 10 liters of distilled H₂O) is placed in the compartments of the electrophoresis chambers.

Hemoglobin solutions (25 μ liters of a 10% solution) are pipetted in the slits. Next, each slit is completely filled with the tris-EDTA-borate buffer, pH 8.1 to which a small amount of KCN (0.2 ml 2% KCN in 10 ml buffer) is added. The slits are finally covered with petroleum jelly, heated to about 35°C, to seal the slits. The remainder of the gel surface is covered with Saran Wrap. A voltage gradient of 175 V across the gel is applied for 16-20 hours. At the end of the electrophoretic run the gel can be sliced according to the procedure described by Smithies (1959). One half of the gel is stained with amido black 10B (a saturated solution of the dye in a mixture of methanol-H₂O-glacial acetic acid, 50:50:10 by volume) for 5-10 minutes. Excess dye is removed by repeated washing with the methanol-H₂O-acetic acid mixture. The second half is incubated in an o-dianisidine. H₂O₂ solution (100 mg o-dianisidine dissolved in 70 ml of ethanol, 30 ml of 0.5 M sodium acetate buffer pH 4.7 and 0.1 to 0.2 ml 30% H₂O), for approximately 1 hour. The gel is finally washed with distilled H₂O and, when advisable, made transparent by immersion in undiluted glycerol overnight in the cold.

Zone Electrophoresis in Agar Gel.—The method of Robinson et al. (1957) has the advantage of a complete separation of Hb-A and Hb-F while many other hemoglobin variants are also detectable with this technique. The agar gel is prepared from a 1% of agar (Bacto-Agar, Difco Laboratories) in a 0.05 M citric acid-sodium citrate buffer solution, pH 6.2. The heated agar solution is poured on thin glass plates and allowed to gel. A small amount of hemolysate is introduced into a thin slot cut in the agar gel. Electrophoresis is carried out at 350V for 16 hours using the same buffer solution in the electrode vessels as was employed for the preparation of the agar plates. The proteins can be stained with a saturated solution of amido black 10B in methanol-H₂O-glacial acetic acid mixture (50:50:5 v/v/v) for 20 minutes. After careful washing of the plate with a 2% acetic acid solution, the agar is dried at room temperature. Semi-quantitative determinations are possible with the use of a densitometer.

Column Chromatography

Separation of different hemoglobins depends on the charge on the ionizable groups of the exchange resin on the surface of the inactive polymer matrix

of the column. Thus an ion exchange resin is first of all equilibrated with buffer so that all ionizable groups are at the same charge, e.g. for the carboxyl groups of carboxymethyl-cellulose on Sephadex, a poly dextran matrix, all groups will be negatively charged (fully ionized) at pH 6.8; this is then placed in the column. Next the hemoglobin solution equilibrated with the buffer is allowed to flow down through the column. The pH is then slowly changed by running buffer of gradually rising pH through the column. The charge on the resin cannot change as it is already fully ionized but as the pH rises from 6.8 the charge on the hemoglobin changes through its isoelectric point from + to - and thus is no longer attracted to the resin but becomes detached and so is eluted. The pH at which it no longer adheres depends on its amino acid composition and thus varies for different hemoglobins and so separation into different fractions can be achieved.

EXPERIMENTAL DATA

Experimentation was approached so that screening conditions could be simulated, the reason being that the degree of similarity of the experiments to actual screening conditions would be a measure of the significance of the data obtained. As a result the voltage rating of the power supply, for instance, was to be determined when the buffer cell was fully loaded with 3 strips (as it would in a screening situation) rather than a single strip.

We proceeded to determine the bridge width that would maximize the potential gradient across it. At first a cell having a bridge width of 10 cm was procured. It was found that at 513 V close to 30 min. were required for a desired migration.

<u>pH</u>	<u>Bridge width</u>	<u>volt</u>	<u>Time required</u>
9	10 cm	513 V	30 min.

Next, the bridge width was decreased to 3 cm. and far more encouraging results were obtained. It was felt that this width would maximize the potential gradient (at 502 V) while, at the same time permit a migration distance of 1 cm.

The power of the input was measured by placing an ammeter in series with the electrophoresis cell. Current drawn from the supply, voltage, time and temperature of strip were monitored during the electrophoretic phase and the following data were obtained (Table 2 and Figure 1-3).

The rise in current drawn from the power source with time should be noted. This may be attributed to the increasing ionization of the buffer solution as well as a rise in temperature of the medium as electrophoresis proceeds.

Table 2

	Minutes	Voltage (V)	Current (ma.)	Temp. (°C)
Test 1	2	502	23.2	23
	4	502	23.3	25
	5	502	23.1	26
Test 2	3	502	24.7	27
	5	502	25.7	29
Test 3	2	502	23.9	27
	4	502	24.1	29
	5	502	24.2	27
Test 4	2	503	21.0	27
	5	503	24.2	29
Test 5	2	502	22.5	25
	5	502	26.4	27
Test 6	3	502	24.6	28
	5	502	26.9	30
Test 7	2	502	24.0	25
	4	502	27.0	28
	5	502	29.3	29
	7	502	30.5	30

DESIGN

The major criteria for designing the device was its amenability to screening conditions. When large scale screening is concerned, a suitable apparatus must be capable of accommodating a large volume of samples and process them rapidly. To meet these ends we have proposed a high voltage multicell device consisting of 4 cells, each of which is capable of accommodating 20-25 samples, requires 5-6 minutes electrophoresis period, and may be activated individually or all four simultaneously, depending on the need (Figure 1-4). Included in the device is a timing mechanism that automatically deactivates the cell by cutting the flow of current to the cell. A pilot light located along the side of each chamber indicates when each is in operation (not shown on schematic). Safety features should be included in view of the high voltage. Magnetic switch opened and closed by removing or firmly placing the lid in place. Arcing should be avoided.

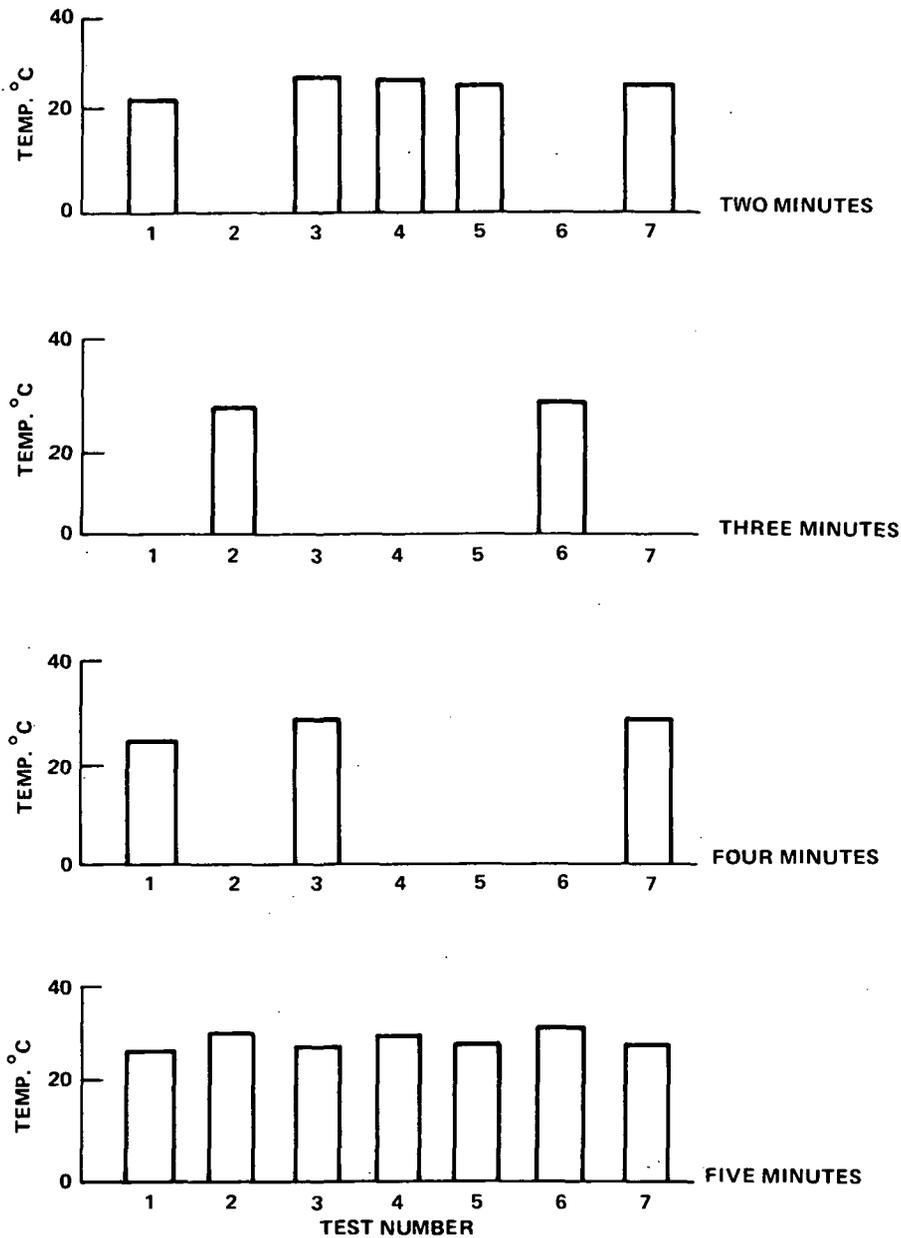


Figure 1-3. Temperature vs. Time was Monitored by Mounting a Thermocouple onto Strip

We have not to this point verified whether 500 V pulsed d.c. or 500 V filtered d.c. offers better resolution or determined which is better suited in the following areas: heat production mobility etc. The design of the power supply is contingent upon this determination.

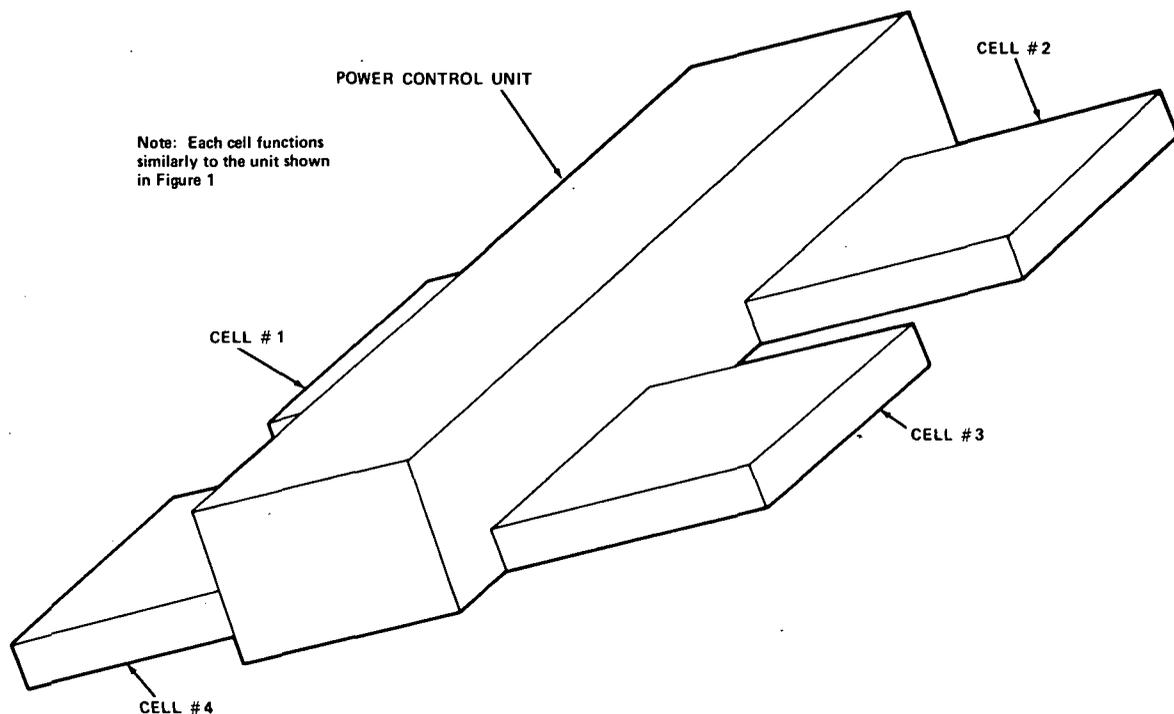


Figure 1-4. The Four-Cell Electrophoresis Apparatus

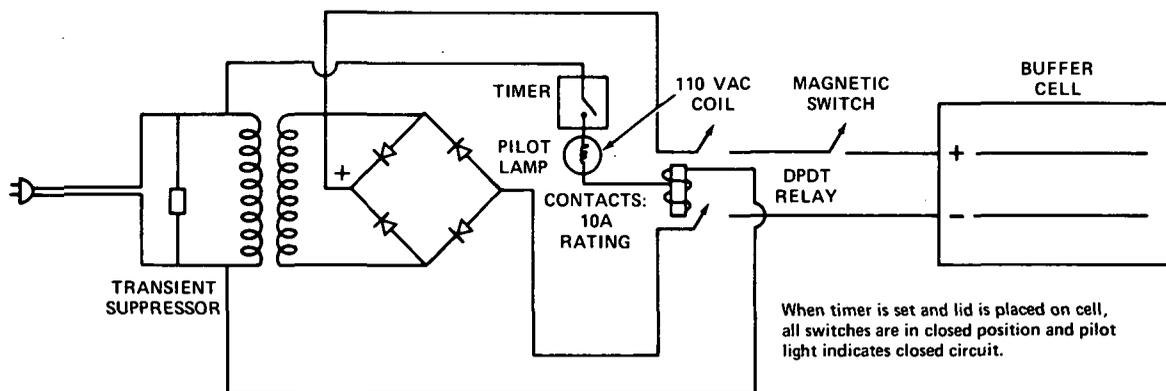


Figure 1-5. Circuit Typical of Four Buffer Cells

A schematic is included with the temporary assumption that 500 V pulsed d.c. is delivered to the buffer cell.

APPLICATOR

Until automated methods are employed to sample application, a multisample applicator will be useful in standardizing the amount of sample applied, and its position at the origin with respect to the others on the strip.

A cross section view is shown in Figure 1-6. Narrow cylindrical slots are drilled into a plexiglass block to accommodate the hematocrit tube, which transfers the hemolysate to the two narrow parallel wires. 20-25 samples may be applied simultaneously to a single large strip. One strip per cell during electrophoretic run.

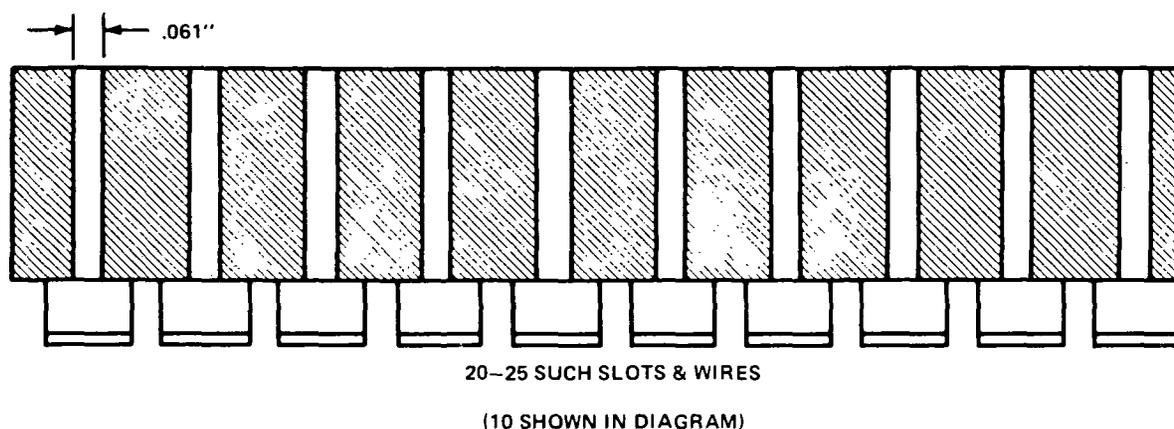


Figure 1-6. Hemoglobin Multisample Applicator

Note how the applicator is directly geared to the method of preparation. Our preparation technique attempts to eliminate constant transferring of the hemolysate by adding the reagents to the hematocrit tube. The idea of doing the preparation in the hematocrit tube is then followed by application with the hematocrit tube (inserted into multisample applicator).

CONCLUSIONS AND RECOMMENDATIONS

If a screening center is to provide meaningful feedback to patients it must utilize comprehensive screening methods. Tests must not be specific to only a single hemoglobinopathy e.g. as are the various solubility tests for Hb-S, but rather be capable of indicating the presence of the hemoglobinopathies with the highest incidence so that medical and genetic counseling can be more conclusive and definitive. Cellulose acetate electrophoresis is ideally suited for this end.

To satisfy the immediate demand for improving screening techniques the modifications that we proposed result in a significant decrease in processing time over procedures presently used. Mass screening can be accomplished with greater speed without detracting from the quality of results.

Mention should be made of the fact that our proposals are far from the ultimate scheme of large batch processing of blood samples. In the final analysis, the hemolysate preparation and application must be done in some sequential automated fashion. Only in this way will a multi-cell device be operated with maximum efficiency with a minimum number of technicians.

Characteristics of the Various Hemoglobins

<u>Hemoglobin</u>	<u>I E P_a</u>	<u>F S H_b</u>
A	6.87	High
S (B)	7.09	Low
C	7.30	High
D	7.30	High
E	7.30	High

(a) Isoelectric points obtained from literature.

(b) Ferrohemeoglobin solubility, modified Itano method.

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SECTION 2

OPERATING ROOM ENVIRONMENT CONTROL
(A Three-Part Report)

PART A—A Valve Cannister System For Anesthetic Gas
Adsorption

PART B—A State-of-the-Art Survey of Laminar Flow
Operating Rooms

PART C—Three Laminar Flow Experiments

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ORIGINAL PROBLEM STATEMENT

As paraphrased from:

Written by:

Dr. Edward Briscoe

Eugene M. DeLoatch, Ph.D.

TITLE: EXHAUST SYSTEM FOR ANESTHESIC APPARATUS IN OPERATING ROOMS (Problem A)

STATEMENT OF THE PROBLEM

Nurses and technicians who administer anesthesia, and to some extent, doctors, are often subjected to an operating room environment which is contaminated with varying concentrations of anesthetic gases. Studies have indicated that the long term effect of these gases on personnel located in the immediate vicinity of the anesthetic apparatus, can be deleterious. An improved method of detecting these gases and/or removing them from the operating field, is desired.

SCOPE OF WORK

Present semi-closed systems of anesthetic apparatus central to mask or endotracheal application techniques could benefit from redesign. An examination of the schemes, the equipment and the techniques may point up areas where either sensory devices or preventive controls will serve to protect operating room personnel. The constraints against additional large equipment and long exhaust tubes make some vacuum systems less than desirable.

TITLE: EXHAUST SYSTEM FOR OPERATING ROOMS (Problem B)

STATEMENT OF THE PROBLEM

The use of gases for anesthetizing patients results in environmental problems for personnel working in the operating room during the course of an operation. Exhaust schemes are constrained by the necessity to limit cross currents in the vicinity of the field of operation. These currents may serve to transport bacteria into the sterile field or cause excessive heat loss from the body of the patient, due to convection.

SCOPE OF WORK

The design of the system must incorporate the major constraint of cross currents as mentioned above. It must also be effective, if using a scrubbing

technique, in removing upwards to eight different gases from the field of operation. These gases arise as a result of the venting of the patients' exhaled gases directly into the atmosphere. A system which responds to predesignated concentration levels or predetermined (concentration) x (time) exposures, may be needed. Some of the gases used are halothane, nitrous oxide, ethylene, cyclopropane, chloroform and metholane.

PART A—A VALVE-CANNISTER SYSTEM FOR ANESTHETIC GAS ADSORPTION

STATEMENT OF THE PROBLEM

Long-term exposure to anesthetic gases has been shown to adversely affect hospital personnel. The two major sources of these gases are the anesthetic equipment in the operating room and the patient's breath in the recovery room.

During an operation, excess anesthetic gases are exhausted to the operating room through a pop-off valve (a one-way, spring-loaded release valve), which is located on the anesthesia machine and is adjusted by the anesthetist.

After the operation, anesthetic gases present in the dead space of the lungs, the blood stream and in the tissues of the patient are exhaled into the atmosphere of the recovery room. Conventional ventilation is not sufficient to remove these gases.

Our objective is to eliminate, or substantially reduce, the anesthetic gases in the operating and recovery rooms. Constraints in the design of apparatus applicable to this problem are:

1. size
2. cost
3. compliance with operating room safety standards
4. maximum self-containment and minimum obstruction

ANESTHESIA EQUIPMENT

Figure 2-A-1 is a flowchart which illustrates a standard anesthesia system. The rate of gas flow in the system is usually 5/min. with the carrier gas composed of 60% N₂O and 40% O₂. The anesthetic gas (e.g. halothane) is usually 1% or less of the total gas.

Presently, the major source of leaks from the system is in the pop-off valve. This is a spring-load one-way pressure relief valve, adjusted by the anesthetist, and designed to exhaust the anesthetic gases to the operating room. In recent years, some modifications have been made in pop-off valve designs, and attachments are now available to collect and "dispose of" the gas using a vacuum system (1-4).

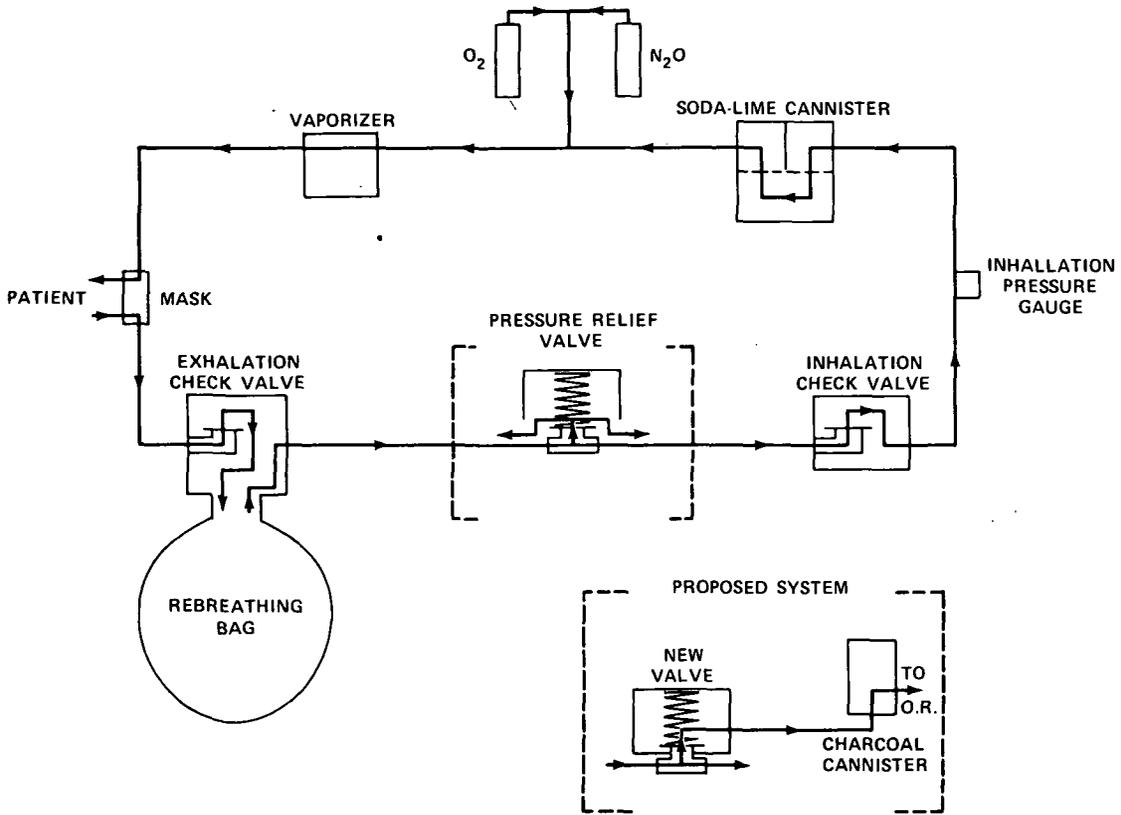


Figure 2-A-1. Standard Anesthetic Circuit

Ohio Medical Products is one of the major manufacturers of anesthetic equipment. Figure 2-A-2 illustrates the pop-off valve that is standard equipment on the Ohio machines. The gases escape from a number of holes surrounding the nylon disk, shown as arrow number 5 in Figure 2-A-2. The attachment available when employing a vacuum system (see Figure 2-A-3) collects the gases which escape through these holes and allows them to be exhausted away. The gases may also be directed to the floor, which is a slight improvement over the original equipment, but not a solution.

The use of the vacuum technique is fairly effective, but it, too has a number of drawbacks:

1. The system is not self-contained, and therefore requires at least one connection between the operating table and the room's exterior.
2. The system is unsuitable for certain types of vacuum systems. At Freedmen's Hospital, a vacuum in the operating room is achieved using a vacuum pump, which dumps the gases right back into the

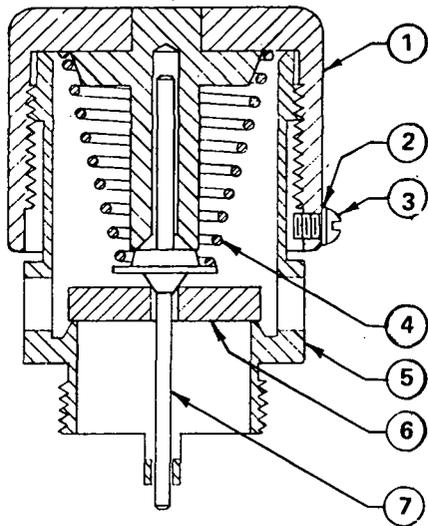


Figure 2-A-2. Ohio's Present Valve

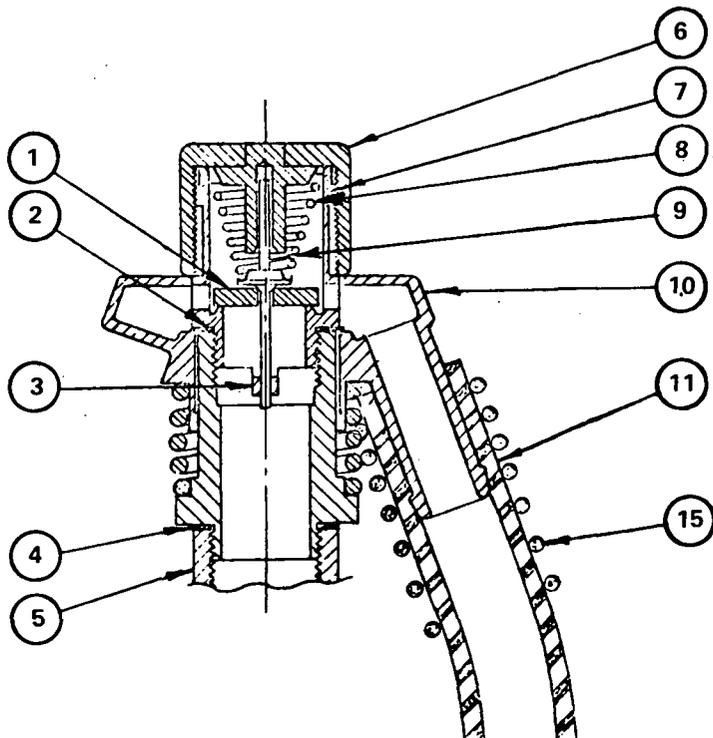


Figure 2-A-3. Ohio's Improved Valve

operating room. For other types of vacuum systems, the extra load could present some problems.

3. Vacuum systems are not permitted with explosive gases, such as ether, cyclopropane, etc. This is a small drawback because the popularity of these gases is rapidly diminishing.
4. The seal between the valve and the vacuum cannot be tight because the relief valve is actuated by the pressure differential across it, and could be greatly affected by a changing vacuum. This is not a drawback of the vacuum system, but rather a limitation of the valves used in the vacuum system. Therefore, we concluded that even though the valve is applicable for use with some systems, it would probably prove to be unacceptable for our purposes.

ADDITIONAL THOUGHTS:

Some other known methods for the removal of gases from a given environmental region are (a) condensation, (b) liquid extraction, (c) dilution and (d) adsorption.

- a. Condensation is not feasible because anesthetic gases are typically used at less than 10% of their saturated vapor pressure, so that very low temperatures would be required before saturation was reached, and extremely low temperatures (liquid Nitrogen range) would be required for significant condensation. Although liquid N₂ is cheap, safe, and relatively easy to handle, the system would probably be less acceptable than a vacuum system, even though it could be self contained. It would probably be bulky, need replenishing many times during the day, and require cryogenic equipment.
- b. Liquid extraction would be even worse, since more than one stage would be required, and the pressure drop would be too high if the gas were bubbled through.
- c. Dilution would be a very costly solution if the anesthetic removal was its only benefit. In recent years, there has been an interest in laminar flow operating rooms due to the decrease in bacteria. If the air coming into the room was anesthetic-free, the dilution effect on the anesthetic exhaust would be a bonus for the system. Since most of the air is recirculated, the anesthetic would have to be removed anyway, which would suggest that the exhaust from the pop-off valve should be treated immediately, as in conventional operating rooms. However, any

secondary leaks such as at the face mask, or from diffusion through the rubber tubing could be treated before recirculating the air, if it was deemed worthwhile. This leads to the question of the merits of laminar flow operating rooms, which was examined in detail, since Freedmen's is in the planning stage for a new hospital. The pros and cons of laminar flow operating rooms are dealt with in Part II of this report.

The fourth approach to the problem is adsorption, which seems like the best immediate solution to a very pressing problem.

- d. Adsorption is a physical phenomenon which is the result of intermolecular forces of attraction between molecules of the solid and the substance adsorbed. It is a readily reversible phenomenon which is somewhat exothermic, with an evolution of heat of the same magnitude as the heat of sublimation of the substance being adsorbed (<10 kcal/mole). It is a surface area dependent phenomenon rather than mass dependent, and care should be taken to distinguish it from chemisorption. Chemisorption is a chemical interaction which occurs on the adsorbent surface, is usually not reversible, and evolves as much heat as a chemical reaction (>10 kcal/mole).

Since adsorption is a surface area related problem, obviously a good adsorbent must have a lot of surface area to be useful. Most of the surface area of granular particles is due to the internal pores. The pores are usually very small, and large in numbers. It is estimated, for example, that a typical gas mask charcoal has an effective surface area of 1×10^3 m²/g.

The method of adsorption seemed to us to possess interesting possibilities as a solution to the problem of halothane contamination. It was further decided that the most important criteria for selecting an adsorbent for this particular application would be to consider the adsorbent's retentivity. Retentivity is simply a measure of the practical saturation limit of the sorbent under conditions common to ventilation. Normally, it is measured by passing clean, dry air through a sample of adsorbent previously saturated with a specific gas or vapor and containing the air flow until the carbon ceases to lose weight. This method determines the weight of the respective gas or vapor that the carbon will retain when exposed to clean air at specified conditions. The retentivity is then taken to be the ratio of the weight of the retained substance to the weight of the charcoal.

For the testing of our specific commercial charcoal, the retentivity was determined using carrier gas (60% N₂, 40% O₂) instead of air, so as to give a

more relevant measurement. For "come as you are" charcoal (straight out of the container as sent by the Atlas Chemical Industries) the retentivity was calculated to be about 0.15. If the charcoal is reactivated at approximately 130°C and cooled in room air, the retentivity roughly doubles.

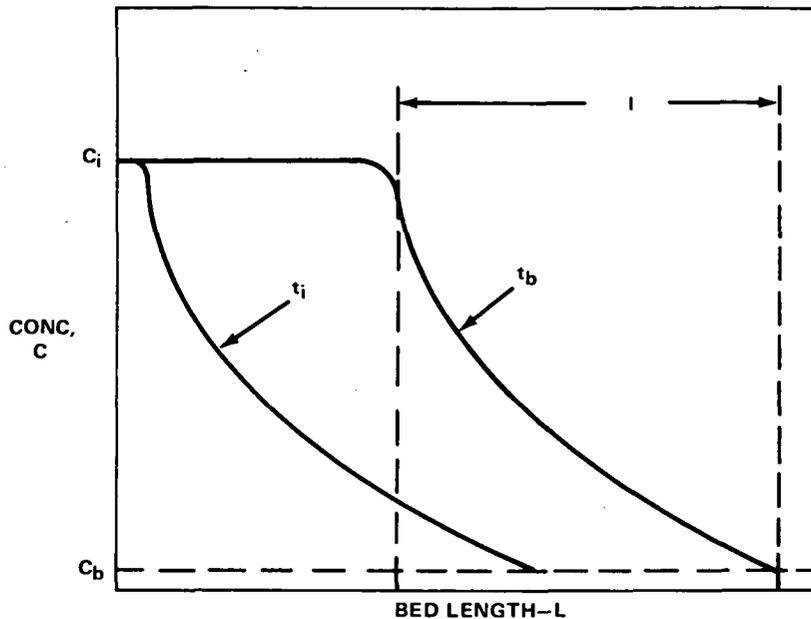
From our limited testing we could only conclude that the retentivity measurements should be the most important design parameter in choosing the adsorbent and that more measurements of better accuracy are required. Also we admit that more commercial charcoals need to be tested, and some regeneration studies should be included. It would be a good idea to get some of the companies actively involved, and get their advice, in addition to free samples of charcoal.

The other item of interest for design purposes, in the adsorption of gases in charcoal beds, is the "length of the adsorption zone." As illustrated in Figure 2-A-4, there are three basic zones in an adsorption operation. (1) The zone which is saturated, i.e., in equilibrium with the incoming gas, (2) the adsorption zone, whose length depends on how the word "saturated" and "phase beginning to release the gas" are defined. When defined in per cents of incoming gas, for different compositions, the length of the adsorption zone is roughly constant. (3) The "empty" zone is the region where, for all practical purposes, adsorption has not yet begun.

One can determine the length of the adsorption zone by passing a gas at constant composition and flow rate through the charcoal, and monitoring what comes out. Using the notation of Figure 2-A-4, the length of the adsorption zone may be expressed as $I = \frac{t_c - t_b}{t_c} L$. t_c is the time it takes for saturation to occur, where the concentration-out equals some percent of the concentration-in. t_b is the length of time it takes for the front of the adsorption zone to reach the end of the sample, or when the halothane is first "detectable," which must also be defined. L is the length of the sample. As one might expect, the length of the adsorption zone is parameter dependent and will increase with either an increase in particle size or an increase in flow rate.

In addition to the parameters of particle size and flow rate, the length of sample (L) will affect the pressure drop through the charcoal bed. This is an extremely important factor because the pressure drop through the treatment apparatus must not be high enough to cause inconvenience (e.g., patient breathing).

According to theory, the pressure drop (P) may be expressed as, $P = P(1/D, V^2, L)$ and the length (L) of the adsorption zone as $L = L(D^{-3/2}, V^{-1/2})$ where L = bed length, D = mean particle diameter, and V = mean particle velocity. The velocity is controlled by the geometry of the bed and the total gas flow rate through it.



NOTES:

- t_i = LOAD PROFILE AT TIME WHEN A BED SEGMENT FIRST REACHES STEADY STATE FIRST OBTAINED
- t_b = BED PROFILE AT SERVICE TIME WHEN BED OUTLET CONCENTRATION EQUALS C_b
- L = ACTIVE ADSORPTION ZONE LENGTH

Figure 2-A-4. Typical Adsorption Profiles

For various models of adsorption theory, the reader is referred to Adsorption, by Mantell, McGraw-Hill, 2nd ed, Mass Transfer, Treyball, McGraw-Hill; Development of Sorber Trace Contaminant Control System Including Pre- and Post-Sorbers for a Catalytic Oxidizer, NASA CR-2027, by Thomas M. Oclott (Lockheed). Precise modeling and optimization is unwarranted in a system with varying composition and flow rate, used intermittently. The design should be based on:

- a. retentivity
- b. desired placement schedule
- c. pressure drop

- d. convenience
- e. length of adsorption zone

LABORATORY TEST METHOD

Some measurements were made at the Goddard Center to determine the length of the adsorption zone using a model constructed as illustrated in Figure 2-A-5. The carrier gas was roughly 60% N₂O, 40% O₂, and the flow rate was adjusted by a needle valve. The carrier gas was then passed through a Matheson 600 flowmeter (rotameter) to get a rough idea of the flow rate.

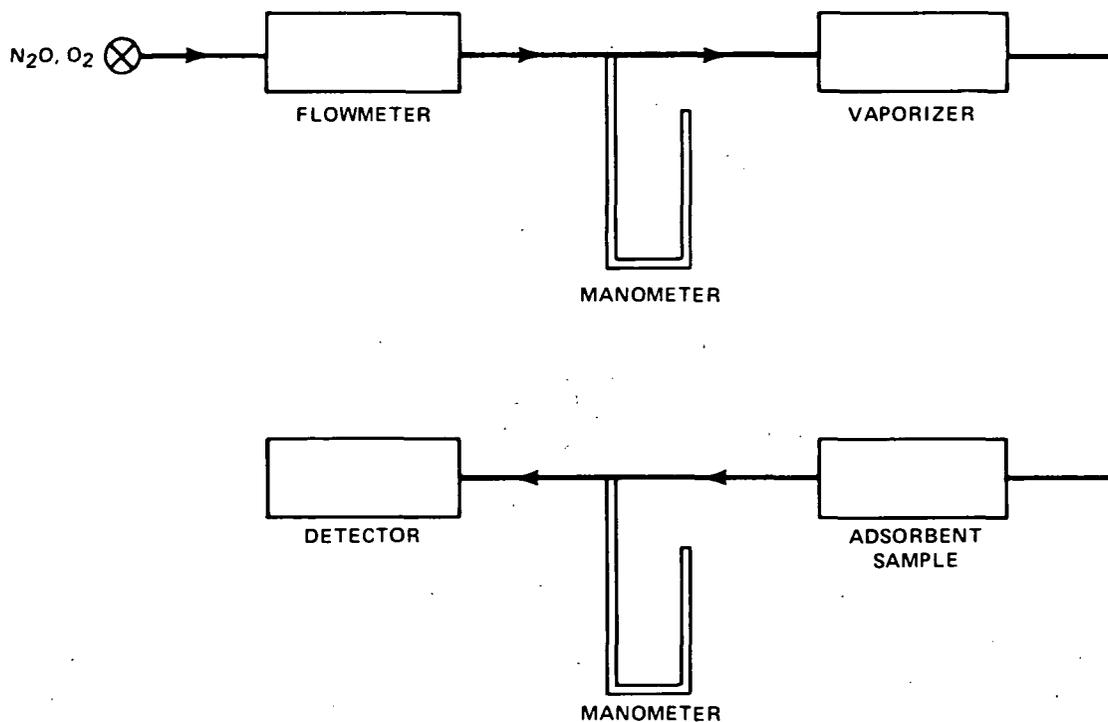


Figure 2-A-5. Adsorption-Testing System

The gas then passed through a wet test meter to determine the volume of gas through it during the run. This volume may have been somewhat erroneous, since the system was operating at a pressure of 8 to 30 in. of water. The gas then went to the vaporizer (the one used on the anesthesia machines), and then to the sample. The dial of the vaporizer was always set at four (4)—highest concentration—so as to keep the runs as short as possible. The pressure of the system was recorded after the wet test meter and after the charcoal sample as well. These samples were placed in drying tubes of varying lengths and of a

fixed diameter. The flow rates through the samples were approximately 100 cc/min. The gas coming out of the sample was then mixed with Helium as a carrier gas, and sent to the Hewlett Packard Flame Ionization Detector. The results were recorded on a strip chart recorder and analyzed using the relation

$$I = \frac{t_c - t_b}{t_c} L.$$

It was found that halothane was decreased approximately by a factor of 50 percent per half inch of charcoal. By going to smaller granule size, the length of the adsorption zone decreased by approximately 25%, but this is a small improvement considering the fact that the total capacity is not changed, and therefore the efficiency of the bed is changed by a few percent—coupled with the fact that the pressure drop is increased, it seems that Barneby-Cheney VG is a good size granule to use.

A SYSTEMATIC APPROACH TO THE PROBLEM

Incorporating the preceding findings into an efficiently operating system became our challenge. As a result, it was our decision to combine the idea of a new valve design with the principle of gas adsorption, to develop a self-contained anesthetic system.

The new valve design, shown in Figure 2-A-6, is airtight and permits the patient to breathe through it directly. Once the release mechanism is operated, under a positive pressure, the exhaust gases are collected at the hose adapter and passed through our activated charcoal bed for adsorption.

The parts list for the valve schematic, displayed in Figure 2-A-6, is as follows:

- A — Adjustment Cover - Brass
- B — Guide Screws - No 1-64
- C — Bellows - 1/2" Normal Length
Bellows Corp. Style 60020-2
- D — Adjustment Plate with Height Indicator
- E — Compression Spring - 1/2" normal, 1/4" compressed @ 40 cm H₂O
- F — Hose Adapter on valve body
- G — Flop Plate with guide pins

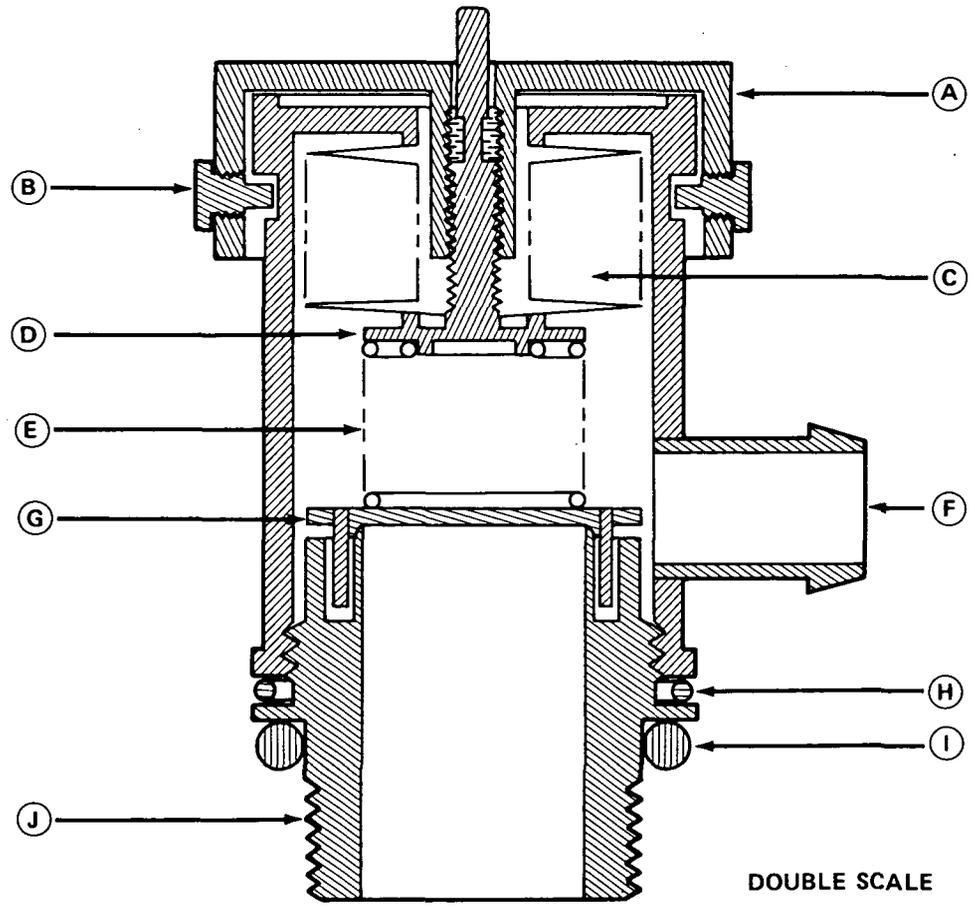
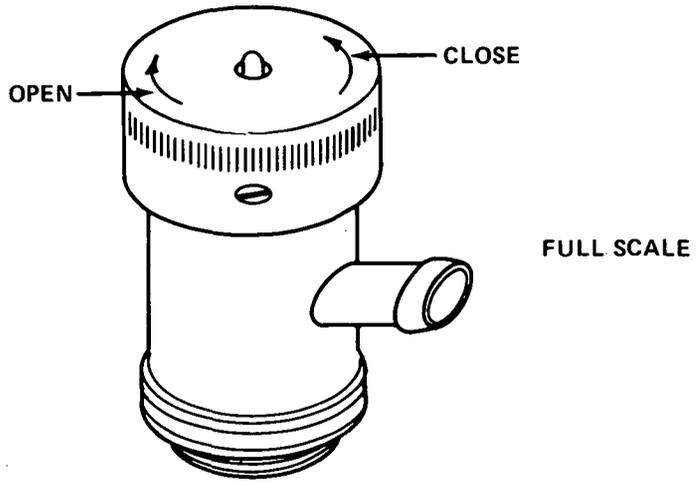


Figure 2-A-6. New Valve Design

H - O - Ring, 1" OD × 1/16" thick - Viton
O - Ring, 1" OD × 1/8" thick - Viton

J - Bottom Carver

Note: All parts are Stainless Steel except as noted.

To justify the feasibility of our conceptual design, we view Figure 2-A-7 which illustrates two basic types of packed beds for adsorption. The top one is poor for our purposes, because flow distribution is a problem, and plenums, distribution plates, and baffles must be incorporated into the system. The pressure drop would also have a tendency to be greater.

The bottom illustration shows the gas coming in through the center through a perforated tube and traveling through an annulus of charcoal, out another perforated tube. This whole system should probably be enclosed to prevent dust problems.

THE RECOVERY ROOM

The adsorption principle may also be applied to the recovery room, but the design approach is altered. In the recovery room, rather than trying to treat the source (the patient's breath) as in the O.R., the room air could be treated by passing large volumes of air through activated charcoal, using a fan.

The first step is to decide on an allowable concentration of anesthetic. Certain assumptions may be made to simplify the design.

1. Steady state, i.e., the amount of Halothane into the system equals the amount removed.
2. Total removal in the charcoal.
3. Ideal mixing of the air.

A mass balance on the halothane yields: (number of patients) (%H exhaled) (tidal volume) = (Volume flow rate of air through the charcoal) (%H desired) + (Volume flow rate ventilation) (%H desired).

The first two assumptions are reasonably valid for design purposes, but the third assumption deserves scrutiny.

The positions of the components of the system, i.e., patients, charcoal, and ventilation, can greatly enhance or hinder the system. The best arrangement

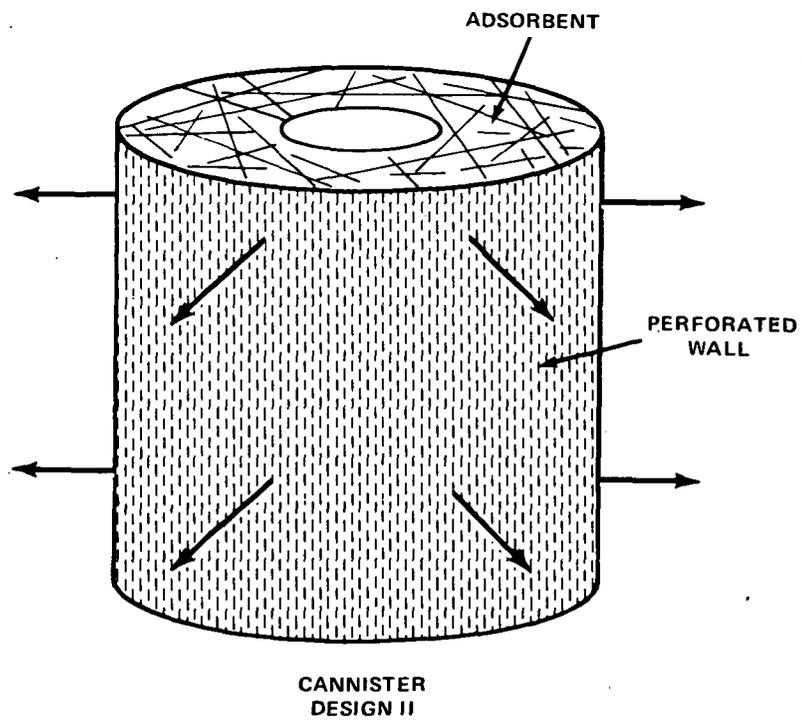
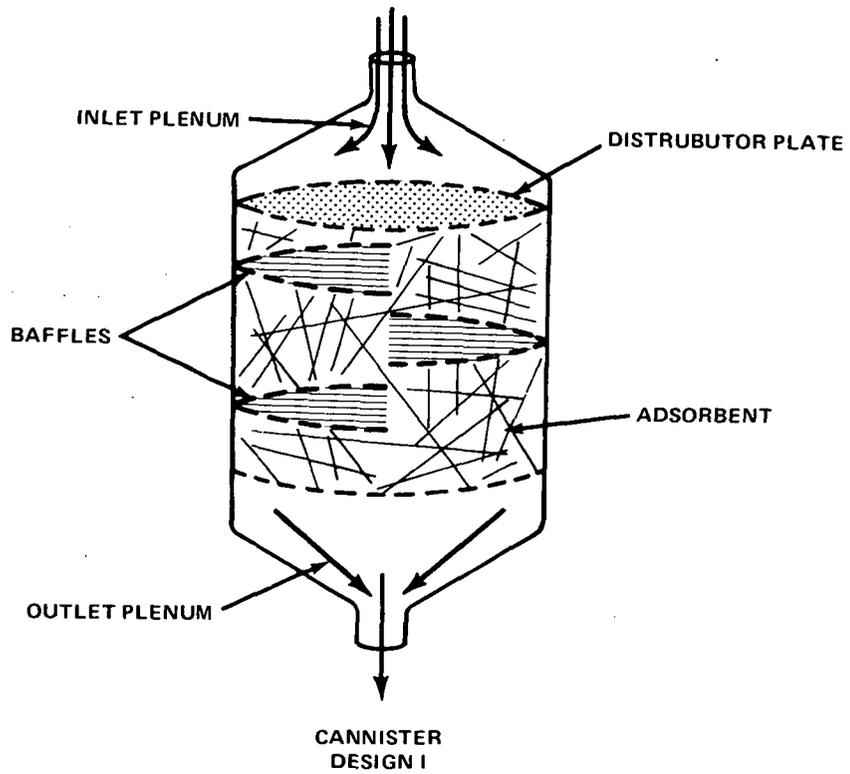


Figure 2-A-7.

would be to have the charcoal near the patients, and far from the incoming air, thereby increasing the concentration going into the charcoal, and decreasing the air flow rate required to achieve the concentration desired.

The amount of charcoal required would be determined solely by its retentivity and the replacement schedule desired.

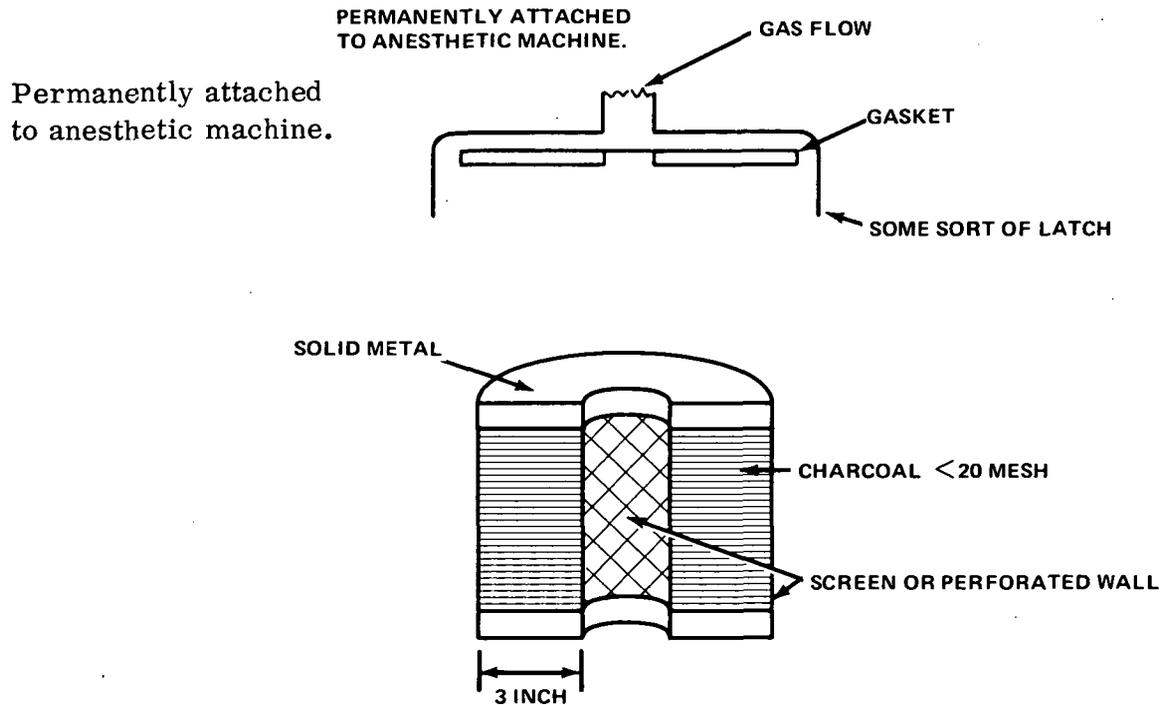
The thickness of the charcoal bed is directly proportional to the pressure drop through it, and would have an effect on the size of the fan required, and therefore the cost of the fan.

The design would depend on an optimization of velocity through the charcoal and the bed thickness (which determines the pressure drop), number and size of the units, replacement schedule and fan capacity.

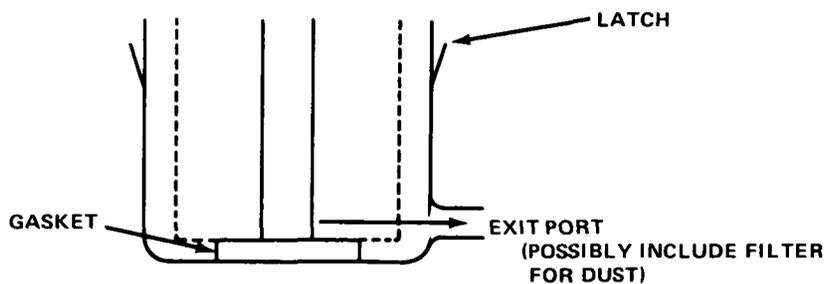
There are a variety of commercial units available and suitable commercial units could probably be purchased immediately.

APPENDIX A

RECOMMENDATIONS FOR CANNISTER DESIGN



Try to locate commercial cannister with about a 3 inch annulus (not less than 2) with a total volume of roughly a liter, with a type of charcoal that works well.



The details of the design should be determined by what's available in pre-made cannisters, and discussions with an experienced machinist or designer (such as Mack Cridlin, Ernie Richie and Larry Fontaine).

APPENDIX B

BROADBAND RECOMMENDATIONS

1. Instill respect in anesthetists for the harmful effects of halothane.
2. Installation of some commercial activated charcoal air purifier in the recovery room.
3. Additional retentivity measurements on different brands and types (wood, coconut shell, petroleum base, etc.) of charcoal, and possibly additional adsorbents.
4. Investigations of acupuncture and hypnosis as a possible alternative to anesthetics.
5. Instill cross-flow laminar flow O.R.'s in the new hospital.

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PART B—A STATE-OF-THE-ART SURVEY OF LAMINAR FLOW OPERATING ROOMS

For well over a decade, laminar-flow clean rooms have been employed for contamination control in industry. The initial patents are held by Dr. Willis Whitfield of Landis Laboratories, but the public popularity of the concept is a result of NASA's extensive application of the technology to the manufacture, assembly, and testing of space-bound vehicles and satellites.

Industry has adapted clean-rooms to increase the reliability of the manufacture of miniature parts where particulate contamination is a major source of product failure. Biological laboratories and pharmaceutical companies employ this same idea to reduce air-borne microbiol. counts and increase their quality control. Laminar flow rooms are even being installed in hospitals for patient-care and surgical applications in an effort to improve aseptic conditions. A 1971 listing^a showed 47 surgery and 20 patient-care facilities in the U.S. using laminar-flow rooms, the majority of which were installed by two firms, Enviroco and Agnew-Higgins, Inc.

Air circulation in a conventional room is turbulent because of the eddies, currents, and large-scale mixing that occur. Figure 2-B-1 is a representation of this type of air flow and shows how particles can be brought into the room, get caught in back-flows, and travel throughout it before being removed at the exit ports. Fox^b quotes Well's report that "the proportion of original room air that remains after one air change is the reciprocal of the Napierian base, e , or $1/2.718$ which is equal to 36.8 percent." Thus, the introduction of up to six room volumes of fresh air might be required to remove an air-borne contaminant (to less than 0.1%). This also means that a particle's chances of settling onto something in this time period are fairly high. The linear velocity of the air is not uniform within the room because of its introduction through diffusers that are spaced along the ceiling and also its removal from isolated ports usually located at the bases of the walls.

Laminar airflow is defined in Federal Standard No. 209 as "...airflow in which the entire body of the air within a confined area moves with uniform velocity along parallel flow lines with a minimum of eddies." Based on information from Daugherty and Ingersoll (1954), laminar or streamline flow is characterized by laminations of an infinitesimal thickness sliding relative to adjacent laminations in a definite and observable path.^b However, the maximum velocity this occurs at is less than that required to maintain uniform directional flow of air subjected to thermal effects, movement of people, and large obstructions. Fox^b prefers the term uni-directional, but the more common designation of laminar will be used in this discussion though it is fully realized that the air velocities are too great to consider the flow truly laminar.

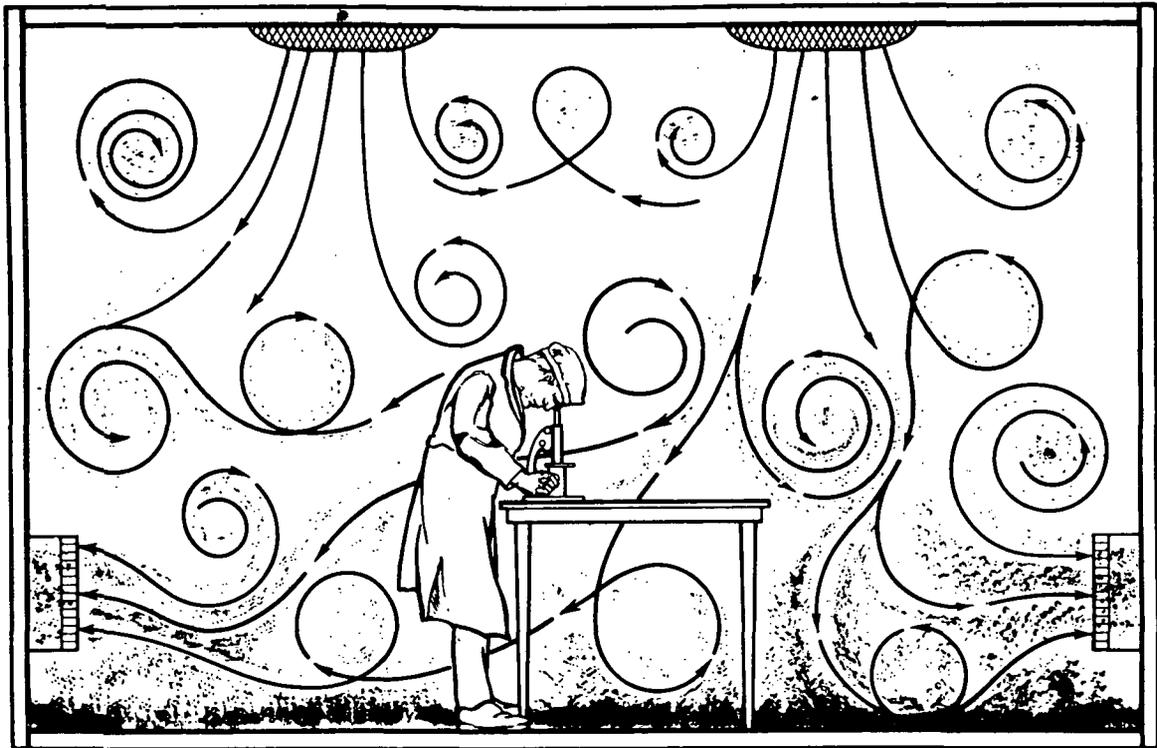


Figure 2-B-1. Turbulent Air-Flow Room

The difference between turbulent- and laminar-flow rooms may be seen by comparing Figure 2-B-1 and 2-B-2. The air in the laminar room is introduced through the entire ceiling and maintains its flow regime until it is removed through gratings in the floor.

Blowers located behind the walls recirculate the air and filters above the inlet gratings remove dust particles. Particles picked up by the air passing through the room are totally removed in a maximum of one air change unless they impinge on an adherent surface. Small-scale turbulence may form behind obstructions, but the mixing effect that results will soon return any trapped particles to the laminar stream.

Two airflow directions are used in laminar clean rooms. When the air is introduced from the ceiling and removed at the floor, the term down-flow is used. The other type, called cross-flow, uses one wall for the entrance and the opposite wall for the exit of the air stream. In the first, recirculation occurs behind one wall, whereas a suspended ceiling is used in the latter.

Air conditions for operating rooms suggested by the American Society of Heating, Refrigeration and Air-Conditioning Engineers are effective temperatures

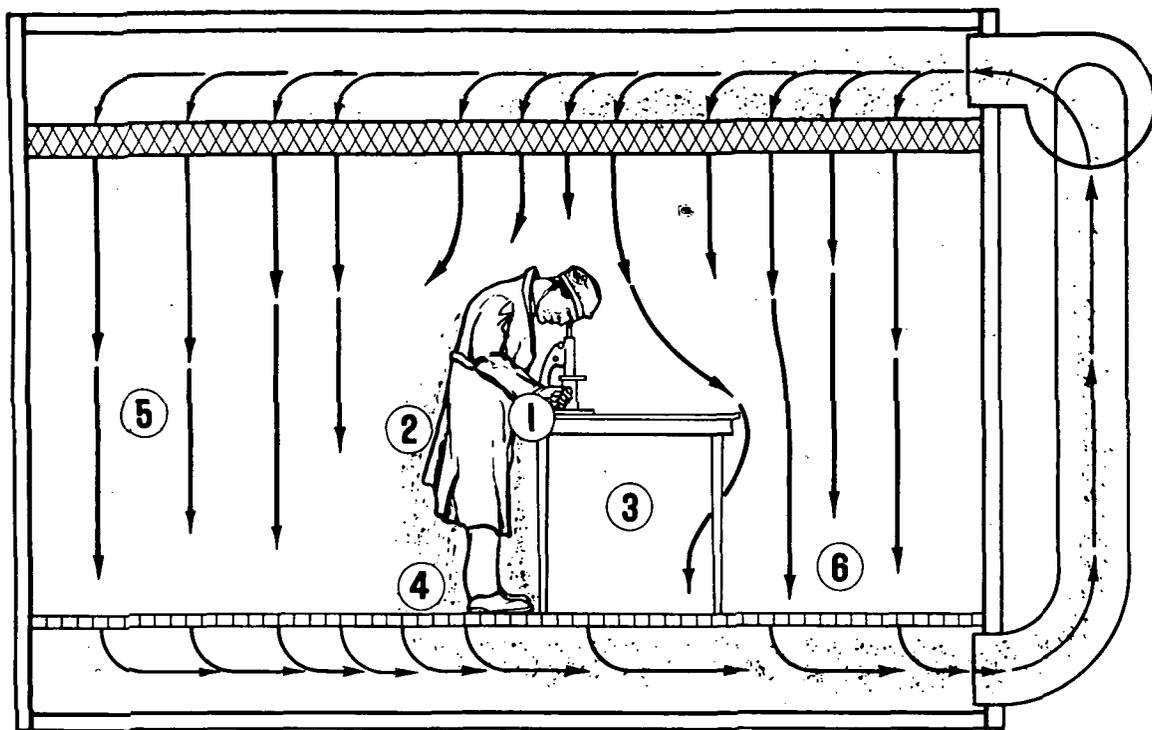


Figure 2-B-2. Uni-Directional Air-Flow Room

of 68°F in winter and 71°F in the summer with a relative humidity (R.H.) of 50%. Freedmen's Hospital, with which this project was conducted, introduces its operating room turbulent air stream through ceiling level vents at conditions 72°-75°F, 50% R.H., and a flow rate of 10,000 cubic feet per minute (CFM). The exit air is removed at a 9,000 CFM rate, hence the room pressure is positive with respect to that of the hallways.

In an interesting experiment suggested by Dr. Whitfield of Sandia, air velocity measurements were recorded for different areas of one of Freedmen's operating rooms. As noted before, the two inlet gratings are located just below the ceiling along the hallway wall. The exit port is a few feet above the floor in the corner on an adjacent wall. The air was observed to descend directly to the center of the room where the operating table is usually located. A 650 ft/min velocity was detected six inches from one inlet port; an average of 80 a range of 50-100 ft/min were observed a foot above the surgery table. Gusts to 200 ft/min occurred just below the overhead lamp. However, along the walls, just above the floor, around the anesthesia machine, and at the exit port, average velocities of less than 10 ft/min were detected. Thus, it appears that wide ranges of air flow can be present in normal operating rooms.

The air velocities presently designed into laminar flow operating rooms vary from 60-90 ft/min for down-flow and from 90-120 ft/min for cross-flow. The air is normally conditioned to 72°F and 50-55% R.H.; but the flexibility of the systems can allow a range of operating conditions with up to 90% of the flow recirculated. In addition, the systems employ High Efficiency Particulate Air (HEPA) filters to remove a guaranteed 99.97% of all particles down to a size of 0.3 microns.

The air flow rate is usually enough to cause a positive pressure in the room, but it can be made equal or negative by varying the relative intake and removal rates.

So that a comparison of the merits of turbulent and laminar rooms may be made here, average values for operating rooms and conditions will be assumed. Inlet air conditions of 72°F and 50% R.H. will be introduced to a 20 ft long by 20 ft wide by 10 ft high room. An expired anesthetic maximum concentration of 1% will be used over an operation time of one hour. The number of operations per room per year for general-use will be estimated at 900 (from the 5300 operations per year for six rooms at Freedmen's). An average of 30 room changes per hour will be used for turbulent flow; velocities of 75 ft/min and 100 ft/min are generally accepted for down-flow and cross-flow, respectively, with 90% recirculation for both.

At the assumed flow rate of 30 room changes/hr, 2,000 CFM total blower capacity would be required for one room. From the discussion presented earlier, based on the assumption of ideal mixing of the inlet air, it can be seen that an instantaneous contamination source might remain in the room for a period of six room changes, approximately 12 minutes. A down-flow room would require 30,000 CFM, equivalent to 450 room changes/hr, or 15 times the blower capacity of a turbulent room. The period of contamination would only be one room change of about eight seconds duration. The cross-flow rate would be less at 20,000 CFM, 300 room changes/hour, or ten times the capacity of the turbulent flow. The contamination removal period would be about 12 seconds.

The air in both turbulent and laminar installations does not differ in the conditions available. Both can achieve the same comfortable temperature and humidity ranges; however, the laminar set-up requires extra conditioning units to handle the increased flow rates and heat loads.

Viable air-borne bacteria levels in laminar flow clean rooms have been demonstrated in numerous reports^{c-2} to be significantly lower than in turbulent flow rooms. Papers presented at a NASA-coordinated symposium^h on clean room operatories gave evidence of decreased infection rates in new surgery

Table 2-B-1

	Air Velocity (ft/min)	Volumetric Rate (CFM)	Room Changes per hr.	Contamination Removal Time
Turbulent	varies	2,000	30	12 min.
Down-flow	75	30,000	450	8 sec.
Cross-flow	100	20,000	300	12 sec.

suites employing the laminar flow concept. The results of the studies cannot be contributed totally to improved air circulation since the aseptic techniques, some quite elaborate, were also introduced during those investigations. However, one might conclude that the decreased bacterial level of laminar flow rooms could indeed be a large factor in producing the decreased infection rates.

No studies have been presented comparing concentrations of anesthetic gases in traditional rooms with those in laminar flow operating rooms. The results of the previously noted recording of air velocities in an operating room at Freedmen's Hospital showed very low flows in the anesthetic machine area. Hence, a removal of the gases from the surroundings of the anesthetist is quite slow; one could easily envision a cloud of halothane enveloping some unsuspecting operator. In a laminar flow room, however, the dilution effect of the increased volumetric flow rate of air will reduce that hazard. At the NASA symposium^h, Dr. John A. Ulrich commented on the level of explosive anesthetic gases in laminar flow operating rooms, "...as far as explosion hazard is concerned: the people most concerned are the underwriters, and they have stated that this is no longer a hazard in surgeries." Dr. Whitfield agreed and added that the results of his tests showed that "there was no buildup at all." These comments applied to explosives such as cyclopropane and ether; but may be equally extended to the toxic gases, halothane, pentane and nitrous oxide.

As laminar flow applications in operating rooms are still being developed and refined, there are several problems that must be overcome before such systems will be thoroughly acceptable. Some frequent objections are (a) a fear that the increased flow rates may cause excessive evaporation of a patient's fluids from the surgical wound, (b) the possibility of bacterial contamination from the exposed skin surfaces of surgeons bending over the wound being swept directly onto the exposed tissue, (c) higher noise levels than those caused by normal air conditioning systems, (d) down flow systems do not provide sufficient lighting facilities, (e) the heat load is greater than that of a turbulent room, and (f) replacement and maintenance of the filters is necessary throughout the year.

The concern about increased evaporation from the exposed tissues during operations was the subject of the three experiments^{i-k} performed in conjunction with this investigation. The results indicate that though the surface of the exposed organs would remain saturated throughout a one-hour operation, over twice as much fluid would be evaporated in a laminar flow room as in stagnant air. This finding might prompt one to regard laminar flow as a potential danger to critically ill patients undergoing surgery. However, this conclusion is not supported by the results of the air flow tests conducted in the operating room at Freedmen's. Velocities equal to and greater than those of both cross flow and down flow systems were recorded directly above the center of the operating table. This suggests that a turbulent flow room may have evaporation rates of the same magnitude as those found in the laminar flow experiments. The fear of excessive evaporation is, therefore, apparently unjustified.

Another objection related to relative air velocities is that of the possibility of contamination being swept from an attending physician's skin directly on to the exposed surgical site by the laminar flow. It is very conceivable that this might happen when someone accidentally enters the upstream area of the air flow past the wound. By again recalling the results of the air velocity tests in a turbulent room, one can see that this condition most likely exists in present facilities and that the introduction of laminar flow would not be a likely cause of increased infection. In fact, increased protection from such contamination could be achieved by restricting the entrance of personnel or any other infection source into the area upstream of the wound. Though this might be unavoidable for the surgeons at critical periods of the operation, it would be an advisable discipline to instill in the other assistants.

The increase in noise level caused by the additional blowers of a laminar installation can cause communication problems between surgeons and their assistants during an operation. The recommended maximum is 45 db., but a discussion with Claude Marsh of Envirco, a leading installer of cross flow rooms, revealed that the minimum level they can presently provide is 55 db. Though this is not excessive, it would be desirable to decrease the noise contributed by the system. Improved duct design and larger blowers revolving at slower rates could solve the problem, but only with a resultant increase in the initial system's cost.

Down flow systems cannot have standard lighting facilities because of the obstructions they present and the inability to support heavy lighting systems from the more delicate overhead supports. The development of fibre-optics may eventually provide a feasible solution to the problem; until that time, however, poor lighting will remain a drawback to down flow systems. As the ceiling in a cross flow system serves only as a recirculation plenum, there is no lighting problem encountered in a horizontal flow room.

The increased heat load in a laminar flow system results from the increase in the number of blowers and from the increased air flow rate. A blower's motor ejects heat to its surroundings; thus, an increase in the amount of blowers present will cause an increase in the temperature, both of the air passing through the blowers and also of the wall separating the blowers from the operating room. This problem is easily solved by either installing a cooling system around the blowers or by increasing the mixture of cooler fresh air with the recirculated flow. Both solutions cause an increase in cost of the total facility.

The filters are the prime maintenance concern of any clean room facility. Industrially, three types are usually used: fiberglass roughing filters, high surface area bag-type pre-filters, and the previously mentioned HEPA filters. Most laminar flow operating facilities employ only the large particle roughing and the small particle HEPA types in a series set-up. Both filter efficiencies improve as the amount of dirt they trap increases. With the fiberglass filters, however, the effectiveness is lost once the trapped-particle layer penetrates the entire filter thickness (usually one inch). This time period for hospitals might be six months. The HEPA filters, on the other hand, might easily last ten years since penetration of the filter media by a layer of small particles is highly unlikely. In any case, experts recommend that both sets of filters be tested for leakages every three months. This means a necessary shut-down period and man-hour investment for each facility in the hospital.

Though the two types of laminar flow rooms have so far been discussed as if they were quite similar, their differences must be investigated before any judgements may be made as to the acceptability of laminar flow. The characteristics examined will be (1) air handling, both discharge into and removal from the room, (2) added construction and equipment required, and (3) contamination control and prevention. Figure 2-B-3 and 2-B-4 are examples of down flow and cross flow clean rooms, respectively.

The velocities and air capacities required have been previously discussed and also listed in Table 2-B-1. Though the down flow has a lower velocity and more room changer per hour, the horizontal room requires less blower capacity. The air removal in down flow could be through a grated floor or a series of exit ports just above the floor. Horizontal flow could be removed either through the wall opposite the entrance or through a grating at the far end of the ceiling. Cross flow with removal through the wall is judged best here.

Vertical flow room ceilings cannot bear large loads because the filters must be airtight, hence, strong supports may not be placed between them. Facilities for lighting can only be located in the system by obstructing the air flowing past it or by not allowing for air to discharge from the section of the ceiling occupied

by the lights. Cross flow rooms pose no lighting problems and provide the ability to have a strongly supported ceiling. Hence, horizontal flow is again more acceptable.

The number of HEPA filters and pre-filters required for a vertical flow room is twice that of the horizontal flow for the room size considered here, since the surface of the down flow entrance is twice that of the cross flow. In addition, the location of the filters in the walls instead of the ceiling allows for easier maintenance in a cross flow system; thus, it is far more preferable than the down flow from an economic viewpoint.

The overall cleanliness of a vertical flow room is better than that of horizontal flow. Dust particles are taken to the floor both by the effect of gravity and by the flow of the air, while they will settle very little in cross flow with gravity exerting the only downward force on them. However, the surgical site, not the entire room, is the matter to be considered for the study of operating rooms. Vertical flow cannot possibly retain a truly unidirectional pattern while flowing past the operating personnel, around the patient, and through the wall of attendants standing around the operating table. Though turbulence would probably not be extreme, the residence time of a particle would be longer than that for unobstructed flow. A danger of vertical flow is that bacteria might be sloughed off from the surgeon's face and carried downward to contaminate the wound site. Since the requirements of an operation necessitate the extension of faces and arms over the incisions, horizontal flow would provide a preferable system that would remove the bacteria without it possibly impinging on any exposed tissue. Only the upfield portion of the patient's body could contribute any infectious particles to the air stream contacting the wound.

The higher velocity of a cross flow room allows for greater dilution of anesthetic gases. The anesthetist would no longer sit in a fairly stagnant cloud of halothane and nitrous oxide. A down flow system, though its dilution effect would not be as great, would carry the gases to the floor and not expose anyone to them. Both systems provide a suitable solution, but treatment of sort to remove the gases would be required, as outlined earlier in this report.

As it would be difficult to make a good estimate of the installation costs of a laminar flow room without specifying its exact characteristics to the company installing the facility, an average price for a 20 ft × 20 ft × 10 ft room will be used here. An entire cross flow room's equipment and installation would cost @ \$21,000: \$18,000 for the actual laminar flow essentials and an additional \$3,000 worth, or about two tons, of air conditioning in excess of that required for a regular turbulent flow room. A ten-year period is estimated before any major overhaul of the system would be necessary. The HEPA filters, with careful

attention to them, should last that long; only the roughing filters would require replacement. Estimating a six-month life for them, nineteen new sets of these fiberglass filters would be required over the ten-year period. As fifty would be needed for one change, the roughing filters could be bought in lots of 200 at \$.40/filter. Thus, one replacement would cost \$20 and the entire nineteen replacement sets would total \$380.

Estimating a 10% replacement of the HEPA filters because of pin-hole leaks and damage during testing, an additional \$500 would be needed for five of those filters at \$100 each. Thus, excluding maintenance, a total ten-year cost of approximately \$22,000 could be anticipated for the cross flow operating room postulated here.

As an average of 900 operations per year is estimated for one operating room, the total number in ten years would be 9,000 patients per room. This amounts to less than \$2.50 expended per patient, a cost the hospital might transfer, should it desire, to the patient as an extra surgical cost.

The future trends in laminar flow facilities may be toward both down flow and cross flow compact, portable units. Costs would decrease and maneuverability would increase; all other things being equal, though, horizontal flow is still the recommended system and will henceforth be the only laminar type considered for this discussion.

Objections to laminar flow operating rooms usually pertain to (1) the necessity for such systems, (2) their actual effectiveness, (3) the inconveniences they present, and (4) the additional cost of the equipment and its installation in view of the already soaring costs of hospital care.

The doctors, though often reluctantly, do in fact report infection rates for operating rooms show the need to improve aseptic surgical techniques. The results of studies have shown that laminar flow rooms have helped decrease post-operation infection; however, none of these have studied laminar flow rooms in isolation, without the addition of either new antibiotics or new procedures such as double-gloving and the wearing of closer woven fabrics. The investigation of the effects of laminar flow alone should provide interesting and conclusive results, and is recommended for one of the existing facilities.

The inconveniences that have been discussed, such as the noise and lighting problems, will have to be solved through better designs. Solutions are possible, but only with concomitant cost increases.

Figure 2-B-5 is a diagram of a proposed operating room set-up in a cross flow system. If the forbidden zone were not entered during the operation, the

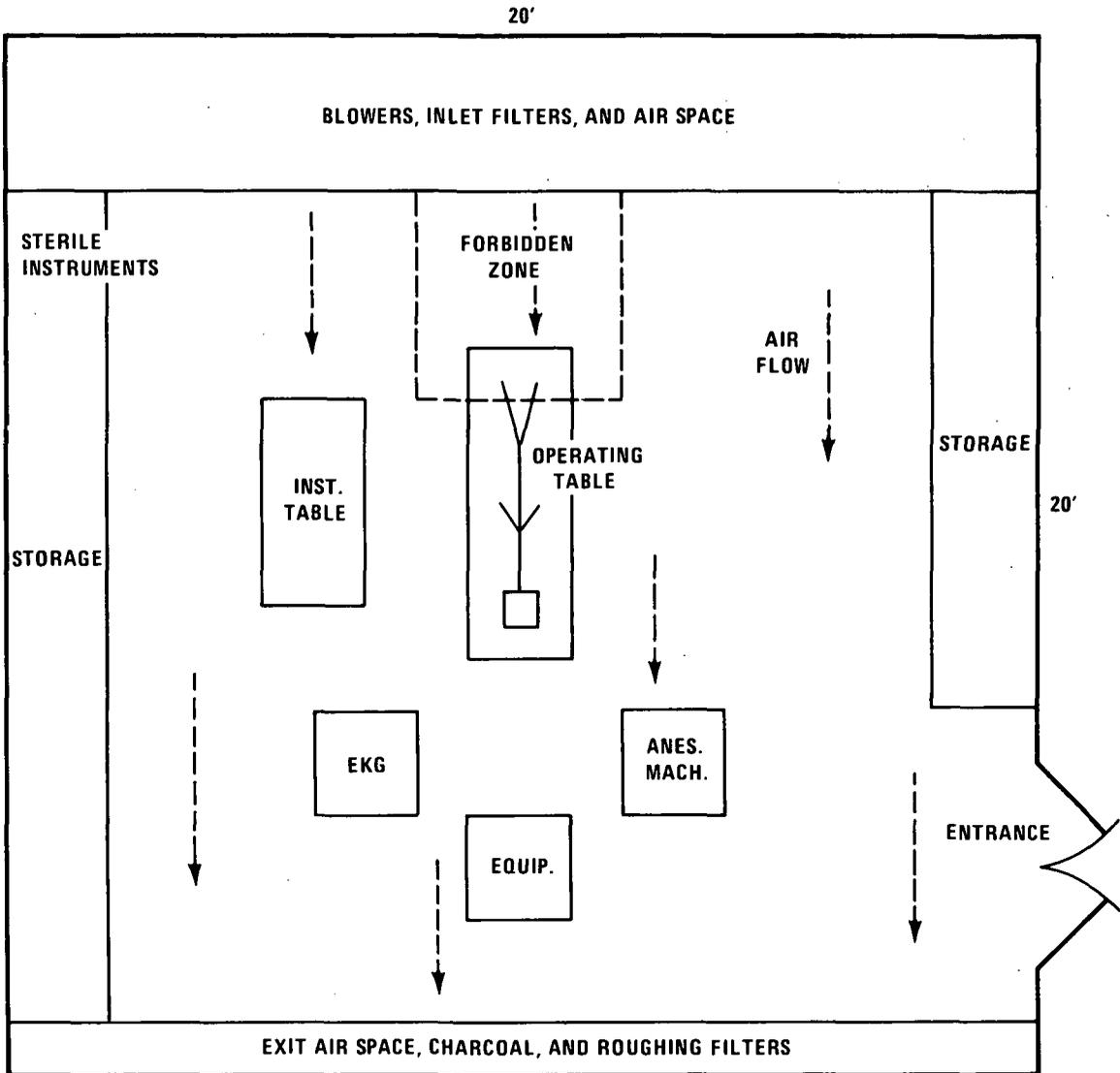


Figure 2-B-5. Suggested Orientation of Cross-Flow Operating Room

only contamination of the wound site would be from the patient's leg drapings. The anesthetist's exposure problem would be solved by having him sit aside of and not down-wind of the air flow. Any gases escaping from the patient's facial area would be diluted immediately.

In light of increasing hospitalization costs, one might hesitate to install a costly air ventilation system for any operating room. However, the \$2.50 cost per operation estimated earlier does not seem too high a price when one considers the inconvenience of an infection to a patient. The extra hospitalization, possibly three months for serious infections at an average of \$80/day would cost \$7,200.

If additional operations and consultations are needed, the price could rise to \$9,000 per infection. By decreasing the infections by one per year, a laminar

flow room would pay for itself in only a few years. Thus, the extra \$2.50/operation seems like a quite reasonable insurance policy against major surgical infections. If one considers also that the costs for the surgical monitoring equipment in Freedmen's new hospital alone will be about \$900,000 then the \$22,000 extra per operating room does not seem exorbitant.

It is therefore recommended that laminar cross flow air systems be installed in the operating rooms of new hospitals. A floor plan such as the one presented here should be adopted, as should an effective treatment method for the anesthetic gases released from the patient's breathing circuit. It is felt, however, that installation of such a system in present operating rooms might be too difficult, cause too many problems, and cost more than its aseptic effectiveness would justify. With new designs and improved aseptic techniques in the operating room, the value of laminar flow would, however, more than justify its cost.

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PART C—LAMINAR FLOW EXPERIMENTS

by

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EXPERIMENT I

DRYING OF CHICKEN INTESTINES

SUMMARY

Drying of chicken intestines under various air conditions has been completed. The laminar flow evaporation rate was shown to be 2.56 that for non-flow conditions. Runs of one hour, the average length of a hospital operation, under laminar flow conditions indicated losses of 17% of the fluids present. Both results warrant serious consideration, even though the apparent condition of the intestines was not alarming after any of the trials. Knowledge of normal surgery conditions is considered necessary to evaluate these results.

INTRODUCTION

Laminar flow ventilation has been used in hospital operating rooms for ten years. The concept has recently gained popularity and also caused controversy in the medical profession. One objection to the continuous air streams of a laminar flow room is that a patient's fluid balance, of concern to both the surgeon and the anesthetist, might be upset by excess evaporation from the operatory wound. As no quantitative reports could be found that dealt with the problem, this investigation was undertaken to determine the evaporative effects of laminar air flow over exposed tissue.

Two types of air flow, vertical and horizontal, are used for laminar flow clean rooms. The equipment available for this experiment limited the investigation to the study of horizontal flow effects. The weight losses of a membrane placed in the air stream at controlled temperature, humidity, and air velocity were observed to determine the evaporation effects. Humidity and time of exposure were varied in different sets of trials.

The membrane used for this experiment consisted of chicken intestines sutured together to form a rectangular sheet. This type material was chosen as a representative tissue exposed during operations and as one that was easily obtainable.

Operating room conditions are usually 72-75°F with a relative humidity (R.H.) of 50%. The R.H. could not be kept that low at those temperatures with the apparatus available. Hence, the constant decided upon for use in these tests was 73°F and 60% R.H.

The limitations of this investigation were (1) only horizontal flow could be investigated and (2) the membrane was not functioning in a living organism and hence had no mechanism to resupply its lost fluids. The first limitation was due to the inability to position the intestines vertically in the air stream without fluids dripping from it. The second, and most important, was experimentally unavoidable, since controlled measurements of weight losses from open wounds would be extremely difficult to obtain. Hence, the conclusions from this experiment must be confined to qualitative comparisons of the results from a non-living and non-human tissue. The similarity between the experimental membrane and a functioning organ will have to be investigated before any quantitative results from this experiment may be quoted.

APPARATUS

Drying was performed in the 2' × 2' mouth of a 6' long wind tunnel (see Figure 2-C-I-1) located on the ground floor of Building 7 at Goddard Space Flight Center. The tunnel exit was extended to a depth of 25' by the addition of aluminum supports and plastic sheeting. One High Efficiency Particulate Air filter was located in the tunnel and formed the inner boundary of the tunnel opening. The entire tunnel was raised 4' above the floor.

Humidity was controlled with a Sears Kenmore Comfort Control Center whose exit air was transferred to the wind tunnel inlet by a galvanized steel duct (see Figure 2-C-I-1). Temperature was controlled with a portable space heater positioned 5' from the humidifier inlet fan.

Wet- and dry-bulb air temperatures were read from a Bendix Psychron battery-operated psychrometer. Air velocities were measured with a David Instrument Co. NBS anemometer suspended from the roof of the tunnel mouth. A 12" × 18" plywood base with a 6" high wire screen cover served as the drying support (see Figure 2-C-I-2 for positions of all three).

9" × 12" aluminum foil sections were used to hold the drying membrane, which consisted of nine 9" lengths of chicken intestines slit up the middle and sutured together with non-capillary, nonabsorbable, silicone-treated, gastrointestinal, 000 silk sutures to form a 9" × 6" sheet (see Figure 2-C-I-2). The intestines were removed from freshly butchered chickens, placed in normal saline (0.9% NaCl) solution, cleaned, and slit on July 21. They remained in

normal saline when not being dried and were stored under refrigeration. The date of the last experiment using the intestines was August 4.

The membrane was placed in 6" × 6" plastic, self-sealing bags for weighing. Tweezers were used to grasp suture loops at the corners for transfer from the foil to the bags. The wooden handle of a cotton applicator was used to flatten and shape the intestines on the aluminum sheet.

Weighings were made on a Christian Becker analytical balance (see Figure 2-C-I-3) when accuracy was desired and on a Mettler P-11 balance for the less accurate values. A General Electric 120-minute alarm timer clocked the drying periods.

EXPERIMENTAL PROCEDURE

Five tests were run for this experiment. Two 15-minute trials were performed, one at 55% R.H. and one at 65% R.H. A 30-minute and a 60-minute run, both at 60% R.H., were also made. All four of these were in a laminar-flow air stream velocity of approximately 108 ft/min and a dry-bulb air temperature of about 73.5°F. The fifth test was at 55% R.H. for 30 minutes with no air flow and a dry-bulb temperature of approximately 75°F. Three determinations were made at each of these conditions. The positions of the platform and the intestines in the air stream were the same for all the trials.

The first step in the daily procedure was to remove the intestines from the refrigerator and let them equilibrate to room temperature. The air velocity, temperature, and R.H. were adjusted to the desired conditions and the weights of the wooden applicator and the aluminum foil were determined. Both were handled by the experimenter, since previous measurements indicated the moisture transfer from his hands to be an order of magnitude less than the experimental error considered for these measurements.

The intestines were placed in a sealed weighing bag, weighed, and transferred to the foil sheet. The applicator was used to quickly (approximately 30 seconds) flatten and form the membrane into a rectangle. The sheet and support were then positioned in the air stream, the timer was started, and the wooden applicator and the plastic bag were weighed to determine the amount of moisture the applicator had absorbed and the weight of the intestines taken from the bag.

During the drying period, the dimensions of the membrane were recorded and several air velocity and humidity readings were made. The weight of a dry weighing bag was determined near the end of the drying interval.

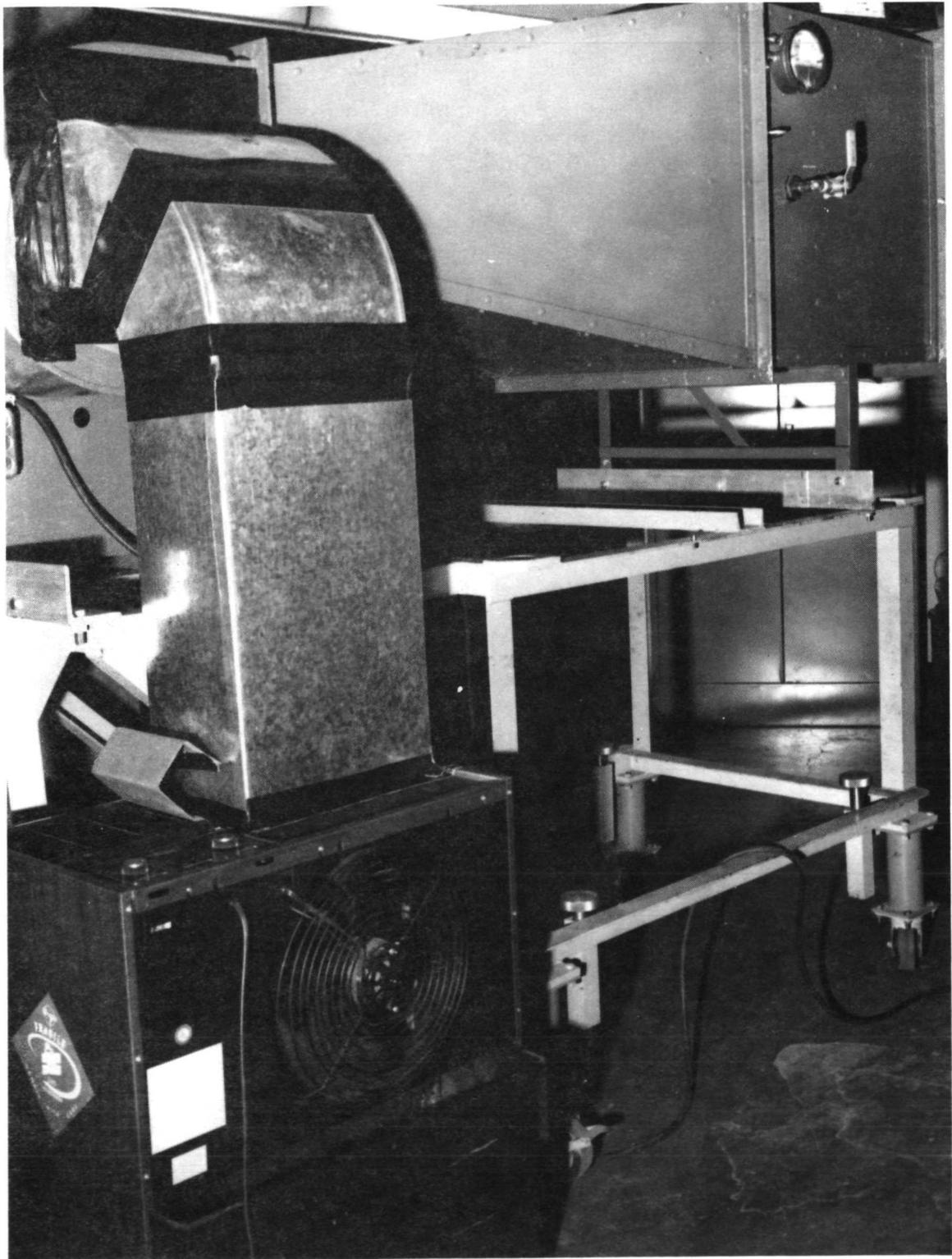


Figure 2-C-I-1. Humidifier and Tunnel

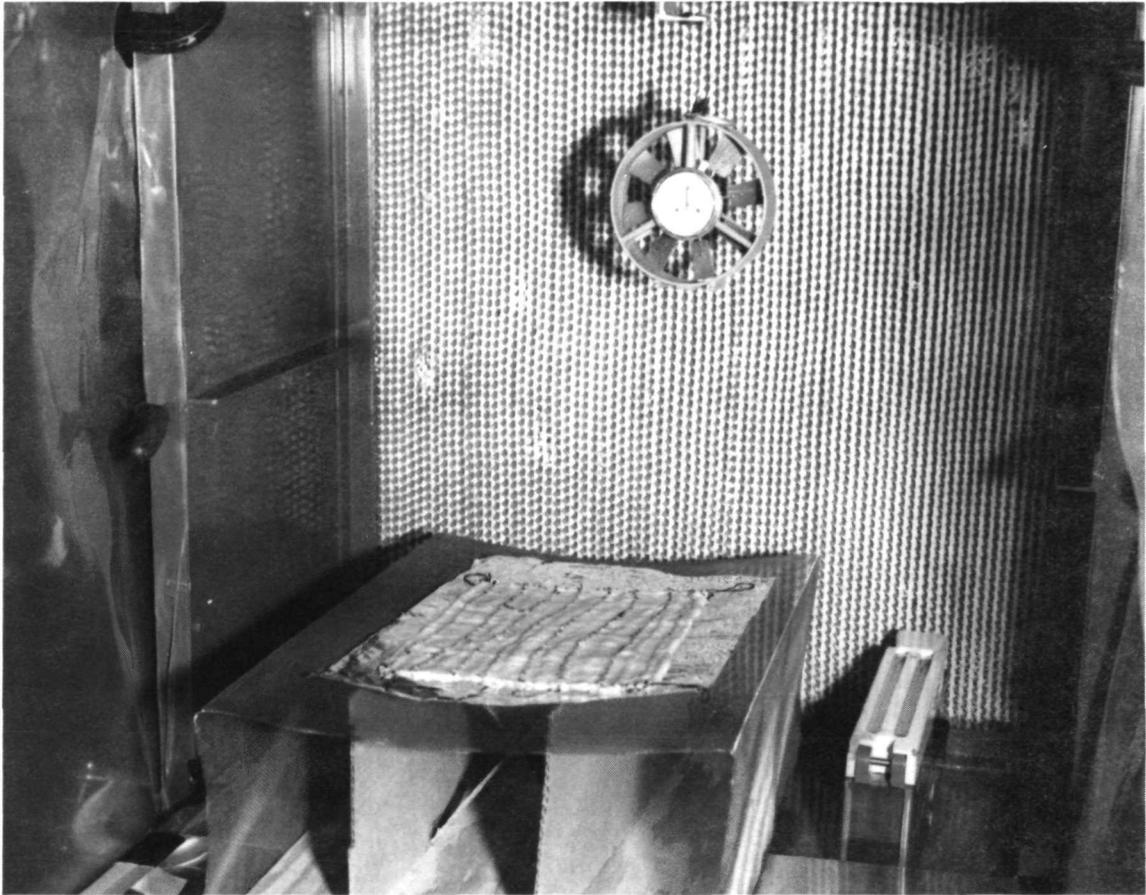


Figure 2-C-I-2. Intestines, Support, Anemometer, Psychrometer, and Tunnel Mouth



Figure 2-C-I-3. Balance, Timer, Support, and Work Bench

When the alarm signalled the end of the drying period, the platform and sheet were removed from the air stream. The intestines were immediately sealed inside the plastic weighing bag, and the foil was folded and quickly weighed to determine the amount of moisture left on it. The intestines and remaining fluids were weighed, then the membrane was placed in the saline solution and allowed to remain there for at least 15 minutes before the next run was started.

The weight of fluids, if any, removed by the tweezers during transfers of the membrane was neglected. It was determined that droplets adhering to the tweezers had masses less than or on the order of the experimental weighing error and hence did not warrant inclusion in the weight loss calculations.

At the completion of the above tests, the membrane was thoroughly dried to obtain approximate drying-rate data and to determine its dry weight. The intestines were placed on a previously weighed sheet of aluminum foil, set in an air flow of 106 ft/min at 42% R.H. and weighed at 15-minute intervals. After all the fluids had evaporated, the dry weight of the membrane was determined.

DISCUSSION

The membrane contained approximately 80% by weight fluids throughout the experiment. The surface area determination was the source of greatest error both for actual length and width measurements and for the uncertainty of the form of the membrane surface (whether stretched, rolled, lumpy, etc.). The weight measurements for the total evaporation of the membrane were with a less accurate balance than for the normal drying runs.

The results of the controlled humidity tests may be observed in runs 1 through 5, run 6 being discarded for an obvious recording error. The average evaporation rates for 1-3 and 4-5 were, respectively, 3.46 and 2.88×10^{-4} gm/cm²-min. This demonstrates a 17% decrease in evaporation rate with a 10% increase in humidity.

The results of time of exposure variation appear in runs 7 through 12. Trials 7-9 and 10-12 lasted 30 and 60 minutes, respectively, and were run at constant R.H. of 60%. The average evaporation rates were 2.68 and 2.65×10^{-4} gm/cm²-min., respectively.

Runs 13-15 were performed at the same air conditions as 1-3, except there was no air flow past the membrane. An average evaporation rate of 138×10^{-4} gm/cm²-min. was obtained; thus the laminar flow in trials 1-3 produced a 150% increase in evaporation rate over that of the nonflow of runs 13-15.

Dry Weight of Solids (± 0.002 gm) - 8.006

Run No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Wt. fluids present (± 0.006 gm)	35.686	35.434	35.419	40.124	33.779	35.301	36.888	36.527	33.853	37.988	33.581	33.215	36.124	35.373	33.957
Wt. evaporated (± 0.008 gm)	2.100	2.022	2.000	1.667	1.834	8.506	2.943	3.208	3.095	5.686	5.738	5.901	1.421	1.598	1.610
Wt. % evaporated (\pm wt. %)	5.89	5.71	5.64	4.16	5.43	24.10	7.99	8.78	9.14	14.99	17.09	17.77	3.94	4.52	4.74
Membrane area (\pm cm ²)	409	373	398	403	408	391	385	382	383	396	354	344	390	379	348
Drying time (min \pm 5 sec)	8	7	8	8	8	8	8	8	8	8	7	7	8	8	7
	15	15	15	15	15	15	30	30	30	60	60	60	30	30	30
Evaporation rate ($\pm 10^{-4}$ wt%/cm ² -min)	3.42	3.62	3.35	2.76	3.00	14.50	2.55	2.80	2.69	2.39	2.70	2.86	1.21	1.40	1.54
	0.10	0.11	0.10	0.08	0.09	0.44	0.08	0.08	0.08	0.05	0.05	0.06	0.03	0.04	0.05
Wt. % evaporation rate ($\pm 10^{-4}$ wt%/cm ² -min)	9.61	10.21	9.45	6.88	8.87	41.12	6.92	7.66	7.96	6.32	8.04	8.60	3.37	3.97	4.54
	0.29	0.31	0.28	0.20	0.27	1.23	0.21	0.23	0.24	0.13	0.16	0.17	0.10	0.12	0.14
Ave. dry bulb (°F)	73.0	73.0	73.1	72.8	73.0	73.0	73.6	73.3	73.5	73.5	73.7	73.4	75.2	75.4	75.4
Ave. R.H. (%)	55	55	55	66	65	65	60	60	59	61	61	60	55	55	55
Ave. air velocity (ft/min)	108	107	107	107	109	110	108	108	108	108	109	109	None	None	None

RESULTS

Air Conditions - 76°F

42% R.H.

110 ft/Min

Wt. Evap. (±0.1gm)	Time Interval (±2 min)	Evap. Rate (10 ⁻⁴ gm/cm ² - min)	Ave. Wt. Fluids (gm)	Ave. Moisture Content (gm fluid/gm solid)
4.4	30	3.7	31.1	3.88
4.0	30	3.3	26.9	3.36
2.4	15	4.0	23.7	2.96
2.3	15	3.8	21.4	2.67
2.2	15	3.7	19.1	2.39
2.3	15	3.8	16.1	2.01
1.8	15	3.0	14.8	1.85
1.8	15	3.0	13.0	1.62
1.9	15	3.2	11.2	1.40
1.7	15	2.8	9.4	1.17
1.5	15	2.5	7.8	0.97
1.3	15	2.2	6.4	0.80
1.3	15	2.2	5.1	0.64
1.1	15	1.8	3.9	0.49
0.8	15	1.3	2.9	0.36
0.7	15	1.2	2.2	0.27
0.5	15	0.8	1.6	0.20
0.3	15	0.5	1.2	0.15
0.2	15	0.3	0.9	0.11

The drying curves for the intestines are presented in Figures 2-C-I-4 and 2-C-I-5. Figure 2-C-I-5 shows the single constant-rate and two falling-rate periods most materials exhibit during drying. The constant rate period signifies saturation at the membrane surface with sufficient diffusion to the surface to resupply any evaporated fluids. The falling-rate zones denote lack of available moisture to resupply fluids to the surface pores. The arrow of Figure 2-C-I-5 indicates the average final moisture content of the intestines for runs 10-12, the trials exhibiting the most total evaporation. As can be seen, they were still well within the constant-rate period and the membrane surface was in no danger of wrinkling or hardening.

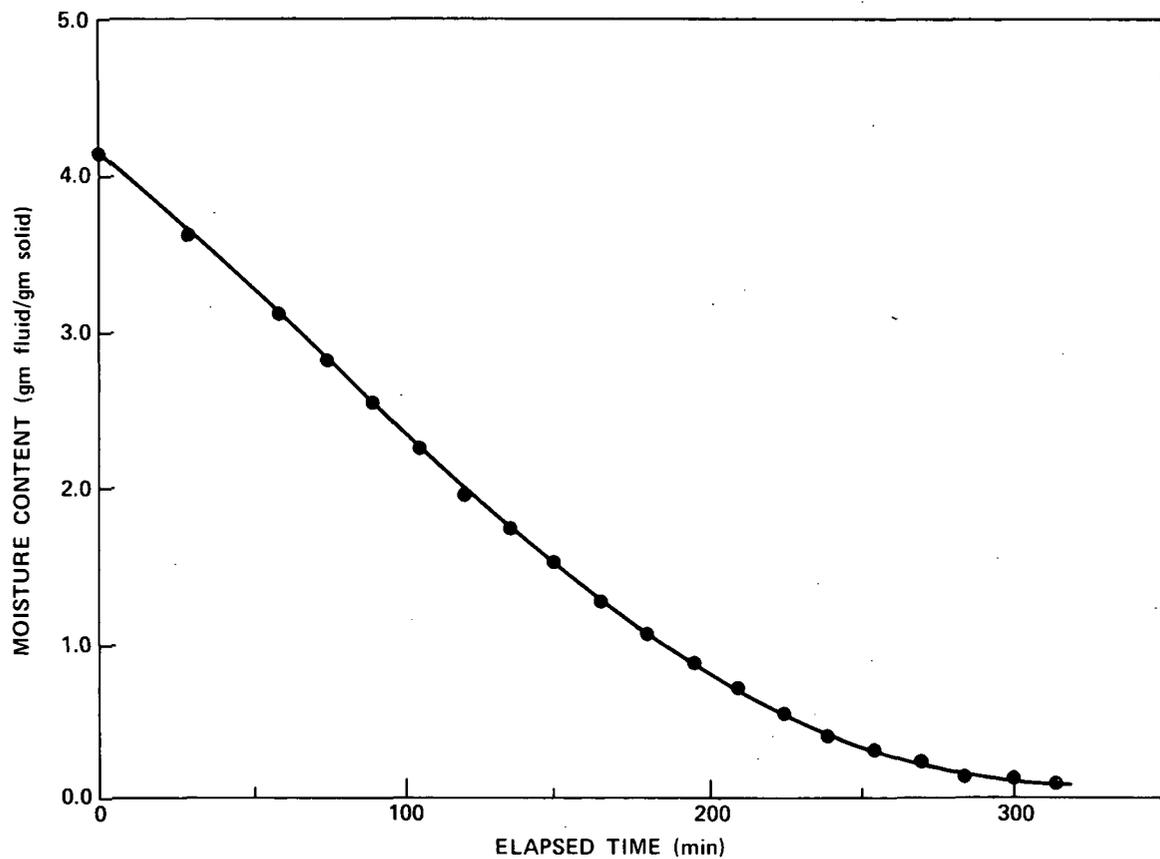


Figure 2-C-I-4. Drying Curve of Chicken Intestines

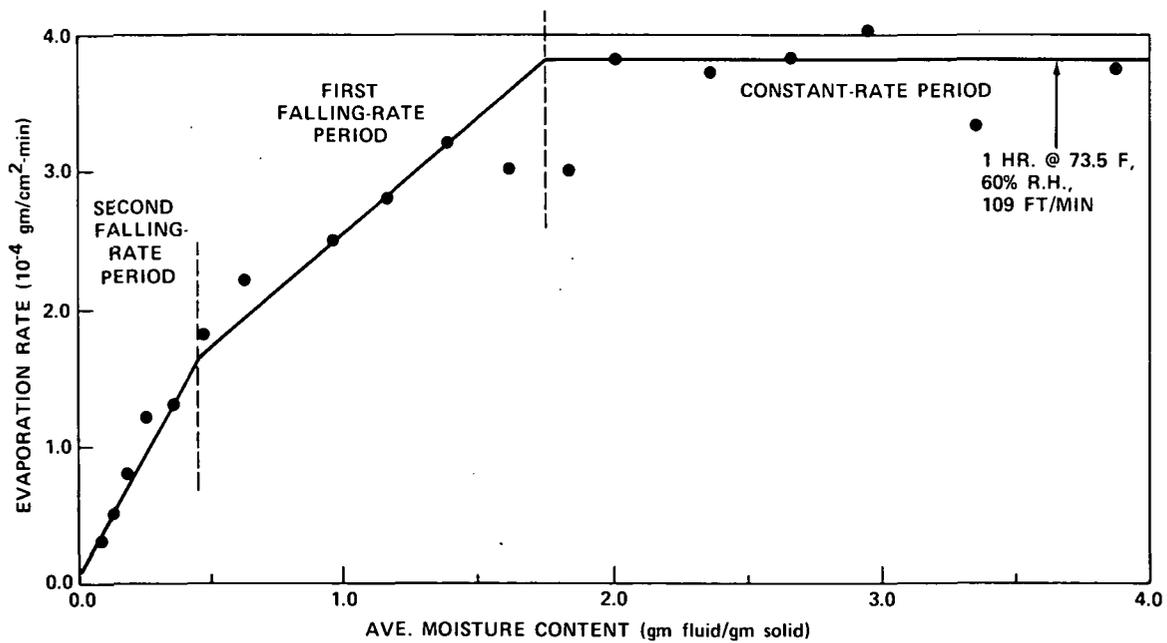


Figure 2-C-I-5. Drying-rate Curve of Chicken Intestines.

DATA

	Run No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Wt. alum. foil (± 0.001 gm)	4.643	4.654	4.657	4.649	4.673	4.678	4.681	4.747	4.753	4.688	4.690	4.692	4.698	4.701	4.703
Wt. applicator (± 0.001 gm)	0.390	0.391	0.394	0.390	0.392	0.392	0.391	0.391	0.391	0.391	0.391	0.392	0.391	0.392	0.392
Wt. wet membrane + bag 5 (± 0.001 gm)	53.672	52.111	51.685	60.056	52.167	51.707	56.163	53.473	50.783	57.140	50.855	50.804	57.715	52.022	50.984
Wt. wet bag 5 (± 0.001 gm)	9.975	8.597	8.253	11.921	10.378	8.395	11.265	8.938	8.919	11.141	9.250	9.573	13.580	8.367	9.013
Wt. wet applicator (± 0.001 gm)	0.395	0.465	0.401	0.395	0.396	0.397	0.395	0.393	0.396	0.396	0.409	0.402	0.396	0.398	0.400
Wt. dry bag 6 (± 0.001 gm)	6.704	6.748	6.776	6.717	6.843	6.844	6.729	6.739	6.766	6.730	6.772	6.738	6.735	6.753	6.755
Wt. wet alum. foil (± 0.001 gm)	5.387	5.385	5.593	6.510	5.301	5.562	5.850	5.921	5.417	5.656	5.091	5.150	5.944	5.742	5.599
Wt. bag 6 + membrane (± 0.001 gm)	47.552	47.435	47.265	51.319	46.166	40.761	47.511	46.890	44.866	46.070	42.220	41.600	48.198	47.493	46.212
Drying time (min ± 5 sec)	15	15	15	15	15	15	30	30	30	60	60	60	30	30	30

DATA

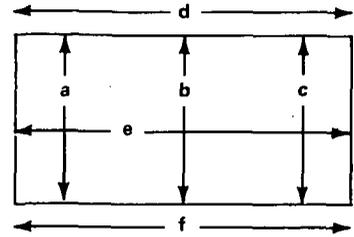
Run No.	Air Vel. (ft/min)	Elapsed Time (min)	Dry Bulb (°F)	Wet Bulb (°F)	R.H. (%)	Elapsed Time (min)
1	108	10	73.0	62.5	55	5
			73.0	62.5	55	9
			73.0	62.5	55	14
2	107	6	73.0	62.4	55	2.5
		14	73.0	62.3	55	10
			73.0	62.2	55	13
3	107	5	73.1	62.2	55	4
		12	73.1	62.2	55	9
4	107	7	72.7	64.9	66	5
			72.9	65.0	66	9
			72.8	64.9	66	12
5	109	9	73.0	65.0	65	6
		13	72.9	65.0	65	9.5
6	110	5	72.9	65.0	65	5
		14	73.0	65.0	65	10
7	108	5	73.5	63.8	60	5
		15	73.5	64.0	60	15
		25	73.8	64.0	60	25
8	109	5	73.2	63.6	60	4.5
		15	73.3	63.6	60	15
		24	73.4	63.6	60	25
9	107	6	73.5	63.5	58	3
		21.5	73.4	63.6	59	10
			73.5	63.8	60	25
10	109	9	73.9	64.0	59	4
		22	73.8	64.5	61	12
		31	73.5	64.5	62	16

DATA (Continued)

Run No.	Air Vel. (ft/min)	Elapsed Time (min)	Dry Bulb (°F)	Wet Bulb (°F)	R.H. (%)	Elapsed Time (min)
10	108	42	74.0	64.5	61	24
	108	53	73.6	64.4	61	30
			73.4	64.2	61	40
			73.2	64.0	61	51
109			9	73.7	64.1	61
11	109	20	73.6	64.1	61	16
	109	34	73.8	64.2	61	33
	109	46	73.7	64.2	61	45
	110	59	73.7	64.0	60	58
	110	6	73.4	63.6	59	5
12	109	20	73.2	63.4	59	18
	109	40	73.3	63.5	59	29
	109	50	73.5	63.6	60	37
	109	50	73.5	63.6	60	49
			73.6	63.6	60	56
75.2			64.7	56	5	
13	None		75.5	64.3	54	10
			75.1	64.1	55	15
			75.0	64.2	56	21
			75.2	64.2	55	26
			75.2	64.0	55	5
14	None		75.5	64.2	55	10
			75.5	64.0	54	16
			75.2	64.3	56	21
			75.5	64.4	55	26
15	None		75.3	64.2	55	5
			75.4	64.3	55	10
			75.2	64.6	56	15
			75.8	64.0	53	20
15	None		75.5	64.2	55	25

DATA

Membrane Dimensions:



Run No.	Length (± 0.1 cm)					
	a	b	c	d	e	f
1	18.0	17.5	17.9	22.5	23.0	23.5
2	16.8	16.5	16.2	22.4	22.6	22.8
3	17.4	17.0	17.4	23.0	23.0	23.0
4	17.2	17.3	17.5	23.3	23.3	23.2
5	17.2	17.5	17.7	22.9	23.9	23.0
6	16.7	16.4	16.4	23.8	23.6	23.7
7	17.3	16.9	17.0	22.9	22.5	22.0
8	17.1	16.8	16.8	22.6	22.5	22.7
9	16.8	17.0	17.4	22.8	22.4	22.1
10	18.0	17.4	17.0	22.4	22.9	22.5
11	16.1	15.6	16.5	21.7	22.3	21.9
12	15.7	15.7	16.0	21.5	22.0	21.9
13	17.8	16.9	17.0	22.9	22.8	22.5
14	17.5	16.5	16.7	22.7	22.2	22.3
15	15.5	15.4	15.3	22.8	22.5	22.4

DATA

Total Evaporation Run:

Wt. alum. foil (± 0.001 gm) - 4.623

Wt. alum. foil + dry membrane (± 0.001 gm) - 12.629

Air conditions - 42% R.H.

76°F

108 ft/min

Wt. Foil + Membrane (± 0.1 gm)	Wt. Membrane (± 0.1 gm)	Elapsed Time (± 1 min)	Wt. Foil + Membrane (± 0.1 gm)	Wt. Membrane (± 0.1 gm)	Elapsed Time (± 1 min)
45.9	41.3	0	21.1	16.5	180
41.5	36.9	30	19.6	15.0	195
37.5	32.9	60	18.3	13.7	210
35.1	30.5	75	17.0	12.4	225
32.8	28.2	90	15.9	11.3	240
30.6	26.0	105	15.1	10.5	255
28.3	23.7	120	14.4	9.8	270
26.5	21.9	135	13.9	9.3	285
24.7	20.1	150	13.6	9.0	300
22.8	18.2	165	13.4	8.8	315

CONCLUSION

The laminar flow drying rate is 2.56 times that for stagnant air. This is significant and warrants consideration should fluid retention be a vital problem. A humidity increase can be used to produce a significant decrease in the evaporation rate, but this is of little value since the humidity would have to be increased beyond a comfortable working level to noticeably affect the evaporation rate.

Increasing the length of the drying period from 30 minutes to one hour did not alter the drying rate. The results indicate that constant-rate drying was always exhibited during these tests. Though 17% of the fluids evaporated in an hour for runs 11 and 12, there was sufficient diffusion of moisture to the membrane surface to sustain saturation and prevent hardening of the exposed tissue. From an engineering standpoint this seems acceptable; however, the loss of any fluids may be undesirable to an extremely ill person, and such a large loss might place undesirable stress on his system. Thus, it may be necessary to prevent any air flow past some patients while they are undergoing surgery.

One must remember, though, that this membrane was not functioning in a living organism and did not resupply itself with any fluids. Judgment of the results presented here should be done by persons with an understanding of operating room conditions and patient tolerances. The safety of a patient cannot be defined in quantitative terms, thus conclusions on the basis of experience are required here.

CALCULATIONS

Run #8 will be used to demonstrate the calculations.

The weight of the wet membrane is the weight difference of bag 5 -

$$\begin{array}{r} 53.473 \\ - 8.938 \\ \hline 44.535 \text{ gm} \end{array}$$

The weight absorbed by the applicator is -

$$\begin{array}{r} 0.393 \\ - 0.391 \\ \hline 0.002 \text{ gm} \end{array}$$

The weight of the membrane on the foil is the difference of the above values -

$$\begin{array}{r} 44.535 \\ - 0.002 \\ \hline 44.533 \text{ gm} \end{array}$$

The moisture left on the foil is the before and after difference of the foil -

$$\begin{array}{r} 5.921 \\ - 4.747 \\ \hline 1.174 \text{ gm} \end{array}$$

The weight of intestines remaining is the weight difference of bag 6 -

$$\begin{array}{r} 46.890 \\ - 6.739 \\ \hline 40.151 \text{ gm} \end{array}$$

The total weight unevaporated is the sum of these two values -

$$\begin{array}{r} 40.151 \\ - 1.174 \\ \hline 41.325 \text{ gm} \end{array}$$

The weight unevaporated is, therefore, the difference of the membrane on the foil and the unevaporated weight -

$$\begin{array}{r} 44.533 \\ - 41.325 \\ \hline 3.208 \text{ gm} \end{array}$$

The weight of fluids present is the difference of the membrane on the foil and the membrane dry weight -

$$\begin{array}{r} 44.533 \\ - 8.006 \\ \hline 36.527 \text{ gm} \end{array}$$

The % fluids evaporated is the quotient of the weight evaporated and the total weight of fluids -

$$\frac{3.208}{36.527} = 8.78 \text{ wt } \%$$

The membrane surface area is found by assuming it to be rectangular with dimensions the averages of the three length and three width measurements -

$$16.9 \times 22.6 = 382 \text{ cm}^2$$

The evaporation rate is the weight loss per surface area per time interval-

$$\frac{3.208}{382 \times 30} = 2.80 \times 10^{-4} \frac{\text{gm}}{\text{cm}^2 \cdot \text{min}}$$

The wt% evaporation rate taken the same way is -

$$\frac{8.78}{382 \times 30} = 7.66 \times 10^{-4} \frac{\text{wt}\%}{\text{cm}^2 \cdot \text{min}}$$

For the drying-rate calculations, the values after 120 minutes elapsed time will be presented.

The moisture content is the quotient of the fluids weight and the dry membrane weight -

$$\frac{15.7}{8.006} = 1.96 \frac{\text{gm fluid}}{\text{gm solid}}$$

The drying rate is the weight lost in the previous time interval per surface area per time interval -

$$\frac{2.3}{400 \times 15} = 3.8 \times 10^{-4} \frac{\text{gm}}{\text{cm}^2 \cdot \text{min}}$$

EXPERIMENT II

LAMINAR FLOW MASS TRANSFER COMPARISON

SUMMARY

Drying of sponges in vertical and horizontal positions in a laminar air flow system has been completed. The evaporation rate of water for vertical flow has been found to be 1.3 times that of horizontal flow. This factor of 1.3 is recommended for use in predicting vertical-flow evaporation rate of fluids from known horizontal-flow values.

INTRODUCTION

The evaporation of fluids from chicken intestines in a horizontal laminar flow air stream has been studied in a previous investigation(1). Limitations in the apparatus prevented the testing of vertical laminar flow using a suspended membrane. The purpose of this experiment is to determine whether there is a difference between the mass transfer coefficients for the evaporation of water in horizontal and vertical laminar air flow.

The direction of laminar flow used in any clean room may be either horizontal or vertical (also called cross-flow and down-flow, respectively). The flow, characterized by parallel air streams when unobstructed, diverts itself around any object in its path and is turbulent to a distance downstream of approximately three obstruction diameters. Air passing around obstructions undergoes a velocity increase until it assumes its former position in the air stream, as is the case in the laminar flow considered here. The velocity increase causes both more turbulent flow and an increased volume of air transported past the surface. As usual, a stagnation layer will be present above the obstruction surface.

Sponges placed vertically and horizontally in the laminar air stream have been chosen for this test of evaporation. Since a vertical sponge will provide a larger obstruction, it might be predicted that more turbulence and a larger volumetric flow rate would occur for it compared to a horizontal sponge. This would cause an increase in the mass transfer of water, hence the weight loss of the vertical sponge should be the greater for identical drying conditions.

The limitations of this investigation are 1) that the sponges used may not be similar, 2) that the same surface saturation must exist for all the sponges at the beginning of evaporation, and 3) that extrapolation of these results to the case of evaporation from human organs must be done with much caution.

The similarity of sponge material must be assumed and can only be supported by repeatable results. The duplication of surface saturation conditions can be achieved through the use of a repeatable moistening procedure, which has been attempted here. The application of the results obtained in this experiment to operating room conditions is difficult. It is felt that through the quantitative results obtained will be of little value, a comparison of evaporation between the cross- and the down-flow systems should still be valid if the results are reliable. Thus, the results be expandable to the vertical flow situation with the use of the ratio of the evaporation values obtained in this experiment.

APPARATUS

The equipment for this experiment was the same as that used for the drying of chicken intestines (see ref. 1 for description).

Six previously unused 3 1/2 in x 4 1/2 in. rectangular Hexagon soldering sponges were dried in this test. The standard metal pans they were contained in had plastic taped around one end to prevent water from dripping out while they were in a vertical position. The sponges had only one surface exposed to the air stream, and the surface area was approximately the same for each sponge.

EXPERIMENTAL PROCEDURE

The six sponges were dried at air conditions of 77.2°F, 42% R.H., and 116 ft/min. Three were positioned horizontally and three vertically in the air stream (see Figure 2-c-II-1). The vertical sponges were placed 4 in. to the right of and 1 1/2 in. behind the horizontals so that neither would interfere with the air flow past the other. The surface dimensions and weights of the dry sponges in their pans were determined prior to the drying runs.

After a known volume of water had been poured onto it in as repeatable a manner as possible, one sponge was weighed and placed horizontally on the support in the air stream. The same was done to another sponge, but it was placed vertically on the support. Both remained in the air stream for 30 minutes and were then weighed to determine the evaporative water losses. This procedure was followed for the two other sets of sponges, with alternation of the order of preparation and placement of the sponges. Humidity and air velocity measurements were made throughout the testing period.

DISCUSSION

The results for the three horizontal sponges show a drying rate range from 5.05 to 5.65 with an average of 5.3×10^{-4} gm/cm² - min. The vertical

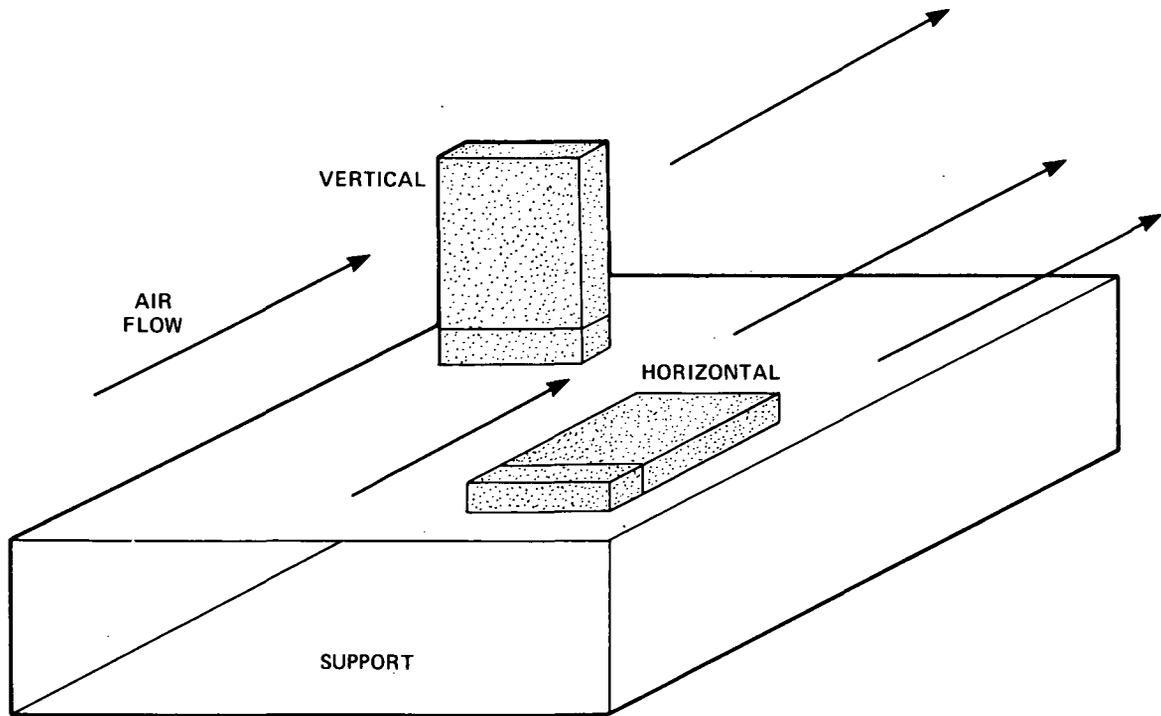


Figure 2-C-II-1. Sponge Positions in Laminar Air Stream

sponge evaporation rates ranged from 6.77 to 6.93 with an average of $6.86 \times 10^{-4} \text{ gm/cm}^2 - \text{min}$. These results indicate a good degree of repeatability.

CONCLUSION

The average evaporation rate for the vertical sponges was 28% greater than that of the horizontal sponges exposed to laminar-flow air conditions of 77.2°F, 42% R.H., and 116 ft/min. It is assumed that this approximate value may be used to compare evaporation of any fluid in a laminar flow system. Hence, the recommended scale factor from horizontal-flow to vertical-flow evaporation is 1.3.

REFERENCE

1. Meyer, J. S., "Drying of Chicken Intestines," 1972.

RESULTS

Average Air Conditions - 77.2°F

42% R.H.

116 ft/min

	Horizontal			Vertical		
Sponge No.	1	4	5	6	2	3
Wt. water added (±0.002 gm)	112.063	116.519	117.226	115.508	114.293	112.665
Wt. water evaporated (± 0.002 gm)	1.333	1.342	1.236	1.671	1.658	1.638
Surface area (± 0.8 cm ²)	82.8	79.2	81.6	81.0	79.7	80.6
Drying rate (± 10 ⁻⁴ gm/cm ² -min)	5.37 0.11	5.65 0.11	5.05 0.10	6.88 0.07	6.93 0.07	6.77 0.07

DATA

	Horizontal			Vertical		
Sponge No.	1	4	5	6	2	3
Length (±0.05 cm)	9.30	9.00	9.16	9.10	9.22	9.15
Width (±0.05 cm)	8.90	8.85	8.80	8.70	8.85	8.85
Wt. dry (±0.001 gm)	54.293	50.083	51.981	53.200	53.742	52.715
Wt. wet before drying (±0.001 gm)	166.356	166.602	169.207	168.708	168.035	165.380
Wt. wet after drying (±0.001 gm)	165.023	165.260	167.971	167.037	166.377	163.742

DATA (Continued)

Air Vel. (ft/min)	Elapsed Time (min)	Dry Bulb (°F)	Wet Bulb (°F)	R.H. (%)	Elapsed Time (min)
115	11	77.2	62.5	42	10
117	24	77.2	62.5	42	25
116	51	77.2	62.4	42	50
116	65	77.2	62.5	42	66
117	90	77.2	62.5	42	92

CALCULATIONS

Values for sponge #5 will be presented for these sample calculations

The weight of water added is the difference in the wet sponge before and the dry sponge values -

$$\begin{array}{r} 169.207 \\ - 51.981 \\ \hline 117.226 \text{ gm} \end{array}$$

The weight evaporated is the before and after difference

$$\begin{array}{r} 169.207 \\ - 162.771 \\ \hline 1.236 \text{ gm} \end{array}$$

The surface area is the product of the length and width of the exposed sponge surface -

$$9.16 \times 8.80 = 91.6 \text{ cm}^2$$

The drying rate is the weight of water evaporated per surface area per time interval -

$$\frac{1.236}{81.6 \times 30} = 5.05 \times \frac{10^{-4} \text{ gm}}{\text{cm}^2 \cdot \text{min.}}$$

EXPERIMENT III

DRYING OF A PIG STOMACH

SUMMARY

A constant-rate drying period evaporation rate of 2.28×10^{-4} gm/cm²-min. has been determined for the drying of a section of pig stomach at slightly less than normal room air conditions. This value compares well with the rate found in an earlier investigation of drying of chicken intestines, thus lending increased validity to the findings of that experiment.

INTRODUCTION

A previous study of the evaporation of fluids from chicken intestines (1) had the unavoidable limitation that the membrane was used for a two-week period and could not be expected to exhibit the same drying characteristics as living tissue. The purpose of this experiment is to study evaporation from a fresh animal organ and compare the results with those of the chicken intestine investigation.

A pig stomach was selected as a representative internal membrane that could be easily obtained and prepared for drying. The evaporation characteristics could be observed from simple weight loss measurements throughout the drying period. If the evaporation rate exhibited by this membrane occurred within the range of those obtained in the chicken intestine experiment, additional confidence could be placed in the results of that study.

The limitation of this experiment is in the constitution of the membrane itself. It was about 1/4 in. thick and both the inner and outer linings were still attached. As the moisture was less than that of the chicken intestines, the comparison of the results of this test and those of the previous investigation must remain qualitative and can only indicate a similarity or a difference in drying characteristics. The constant-rate period values should be comparable since both surfaces would be saturated; however, the falling-rate periods should differ significantly because the diffusion mechanisms of the internal fluids cannot be expected to be identical.

APPARATUS

A Mettler analytical balance was used for all the weighings, a Bendix Psychron battery-operated psychrometer provided wet and dry-bulb temperatures, and a Wakmann stop-watch timed the experiment. A 3 in. × 6 in. irregularly shaped section of a freshly butchered pig's stomach served as the drying membrane, and 4 in × 8 in. sheets of cardboard and aluminum foil were used to support it on the balance pan.

EXPERIMENTAL PROCEDURE

The stomach for this test was recovered from a freshly butchered pig and was placed in a small volume of normal saline (0.9% NaCl) solution during transportation. It was returned to the lab and immediately cut and prepared for drying at slightly harsher room conditions than normal. The dry-bulb air temperature ranged from 69.0 and 70.3°F, the humidity varied between 43 and 52%, and slight air currents were noticeable during the experiment.

The stomach was placed with the inner lining up on the aluminum foil taped to the cardboard sheet, both of which had been previously weighed. This assembly was set on the pan of the analytical balance and allowed to dry with the side doors open. The total weight was recorded every 15 minutes and humidity readings were taken at half-hour intervals. At the completion of the test, the stomach was thoroughly dried in an oven to determine the total solids weight.

Several length and width dimensions of the irregularly shaped membrane were recorded so that a paper replica of its shape could be made. This paper was used to determine the total surface area of the stomach section by weighing it and a piece of paper of known surface area and determining their weight ratio.

DISCUSSION

After 7 1/2 hours of drying, the evaporation rate remained fairly constant. No further data were collected and the stomach was thoroughly dried in an oven. The initial moisture content was 1.92 and the final content was 1.43. Thus most of the fluids were still unevaporated at the termination of the drying.

The solid curve of Figure 2-C-III-2 shows in detail three constant rates observed during the experimental interval. An average rate of 2.28×10^{-4} gm/cm²-min. is represented by the dashed line.

The falling-rate periods were not encountered in this abbreviated run. The surface of the membrane appeared leathery after the 7 1/2 hours, but the inner layers still contained much of the fluids and had a spongy resilience to a finger probe.

CONCLUSION

The three distinct evaporation rates found during the constant-rate period may be explained by uneven drying of the membrane surface. The average rate of 2.28×10^{-4} gm/cm²-min. may be compared with the 1.38×10^{-4} gm/cm²-min. value of the chicken intestines at 55% R.H. in the previously cited study. The

RESULTS

Wt. Dry Stomach (± 0.001 gm) - 23.323

Wt. Stomach (± 0.002 gm)	Wt. Fluids (± 0.003 gm)	Moisture Content (gm fluid/gm solid)	Elapsed Time (min)
68.145	44.822	1.922	0
67.698	44.375	1.903	15
67.306	43.983	1.886	30
66.937	43.614	1.870	45
66.504	43.181	1.851	60
66.112	42.789	1.835	75
65.682	42.359	1.816	90
65.260	41.937	1.798	105
64.834	41.511	1.780	120
64.430	41.107	1.763	135
64.016	40.693	1.745	150
63.616	40.293	1.720	165
63.204	39.881	1.710	180
62.794	39.471	1.692	195
62.395	39.072	1.675	210
62.009	38.686	1.659	225
61.634	38.317	1.643	240
61.272	37.949	1.627	255
60.902	37.579	1.611	270
60.542	37.219	1.596	285
60.180	36.857	1.580	300
59.820	36.497	1.565	315
59.461	36.138	1.550	330
59.108	35.785	1.534	345
58.762	35.439	1.519	360
58.417	35.094	1.505	375
58.071	34.748	1.490	390
57.738	34.415	1.476	405
57.413	34.090	1.462	420
57.085	33.762	1.448	435
56.758	33.435	1.434	450

RESULTS

Area of Stomach ($\pm 5 \text{ cm}^2$) - 111

Wt. lost ($\pm 0.002 \text{ gm}$)	Ave. wt. fluids ($\pm 0.006 \text{ gm}$)	Ave. wt. content ($\text{gm fluid}/$ gm solid)	Drying rate ($10^{-4} \text{ gm}/$ $\text{cm}^2 - \text{min}$)	Interval No.	Interval Time (min $\pm 2 \text{ sec}$)
0.447	44.599	1.912	2.68	1	15
0.392	44.179	1.894	2.35	2	15
0.369	43.798	1.878	2.22	3	15
0.433	43.397	1.861	2.60	4	15
0.392	42.985	1.843	2.35	5	15
0.430	42.574	1.825	2.58	6	15
0.422	42.148	1.807	2.53	7	15
0.426	41.724	1.789	2.56	8	15
0.404	41.309	1.771	2.43	9	15
0.414	40.900	1.754	2.49	10	15
0.400	40.493	1.736	2.40	11	15
0.412	40.087	1.719	2.47	12	15
0.410	39.676	1.701	2.46	13	15
0.399	39.271	1.684	2.40	14	15
0.386	38.879	1.667	2.32	15	15
0.375	38.498	1.651	2.25	16	15
0.362	38.130	1.635	2.17	17	15
0.370	37.764	1.619	2.22	18	15
0.360	37.399	1.604	2.16	19	15
0.362	37.038	1.588	2.17	20	15
0.360	36.677	1.573	2.16	21	15
0.359	36.317	1.557	2.16	22	15
0.353	35.961	1.542	2.12	23	15
0.346	35.612	1.527	2.08	24	15
0.345	35.266	1.512	2.07	25	15
0.346	34.921	1.497	2.08	26	15
0.333	34.581	1.483	2.00	27	15
0.325	34.252	1.469	1.95	28	15
0.328	33.926	1.455	1.97	29	15
0.327	33.598	1.441	1.96	30	15

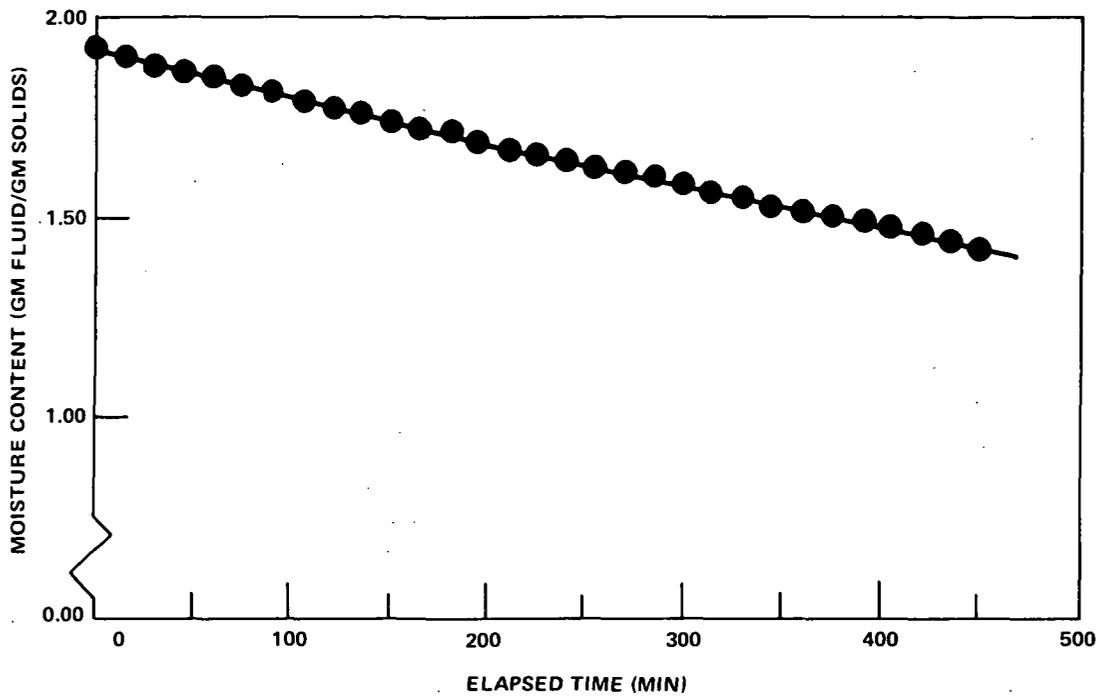


Figure 2-C-III-1. Drying Curve of Pig Stomach

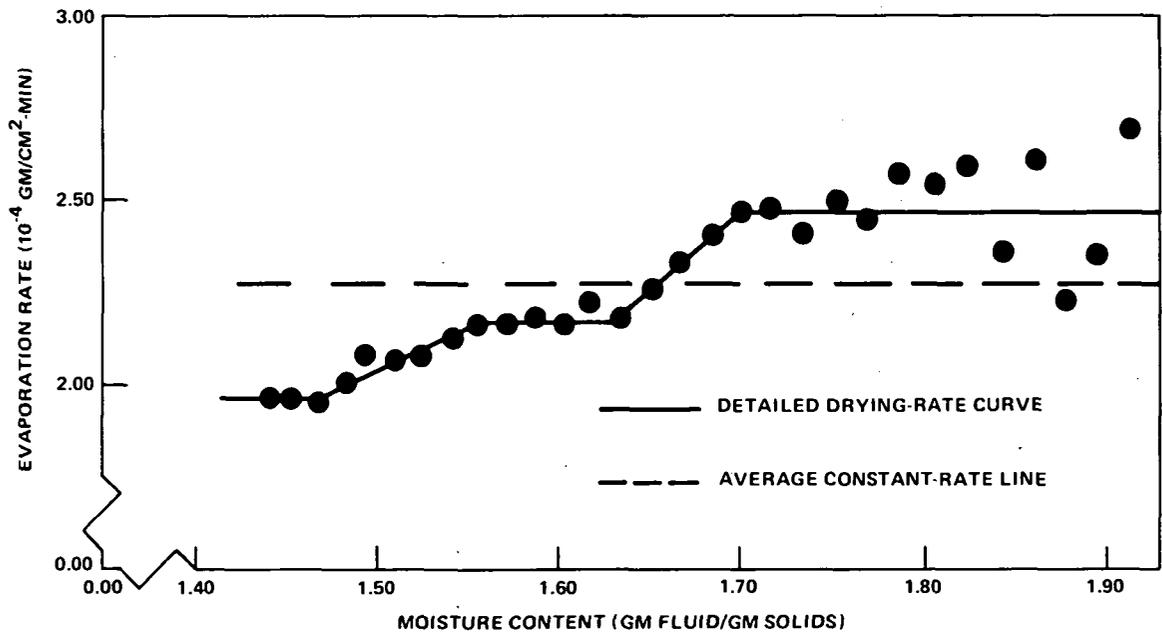


Figure 2-C-III-2. Drying-rate Curve of Pig Stomach

DATA

Wt. Cardboard + Foil + Stomach (± 0.001 gm)	Elapsed Time (min ± 1 sec)	Wt. Cardboard + Foil + Stomach (± 0.001 gm)	Elapsed Time (min ± 1 sec)
78.632	0	72.121	240
78.185	15	71.759	255
77.793	30	71.389	270
77.424	45	71.029	285
76.991	60	70.667	300
76.599	75	70.307	315
76.169	90	69.948	330
75.747	105	69.595	345
75.321	120	69.249	360
74.917	135	68.904	375
74.503	150	68.558	390
74.103	165	68.225	405
73.691	180	67.900	420
73.281	195	67.572	435
72.882	210	67.245	450
72.496	225		

Wt. of paper area of stomach - 0.9679 gm

Wt. of 50 cm² of paper - 0.4341 gm

humidity for the present test was less than that used to dry the chicken intestines, hence one would expect the rate here to be greater than the previous study's. Though for true similarity of drying the difference should not be as large as it is, the rates are of the same order of magnitude and the results of the chicken study may be considered valid enough to extend them to the consideration of the evaporative effects of laminar air flow on functioning tissue.

CALCULATIONS

Values after 210 minutes elapsed time will be presented for these sample calculations.

DATA

Wt. foil + cardboard (± 0.0005 gm) - 10.487

Wt. foil + cardboard + dry stomach (± 0.0005 gm) - 33.810

Dry Bulb (°F)	Wet Bulb (°F)	R.H. (%)	Elapsed Time (min)
69.0	58.0	52	5
69.9	58.2	50	30
70.5	58.2	49	60
70.5	57.3	45	90
70.2	57.3	46	120
70.3	57.0	44	150
70.8	57.0	43	180
70.7	57.0	43	210
70.6	57.5	44	240
70.6	57.5	44	270
70.3	57.3	45	300
70.2	57.1	45	330
70.2	57.3	46	360
70.2	57.6	47	390
70.4	57.9	47	420
70.3	58.0	46	450

The weight of the wet stomach is the difference of the total weight and the cardboard and foil weights -

$$\begin{array}{r} 72.882 \\ - 10.487 \\ \hline 62.395 \text{ gm} \end{array}$$

The fluids weight is the difference of the wet stomach and the dry stomach values -

$$\begin{array}{r} 62.395 \\ - 23.323 \\ \hline 39.072 \text{ gm} \end{array}$$

The moisture content is the ratio of the fluids weight and the dry membrane weight -

$$\frac{39.072}{23.323} = 1.675 \frac{\text{gm fluid}}{\text{gm solid}}$$

The drying rate is the weight lost in the interval per surface area per time interval -

$$\frac{0.399}{111 \times 15} = 2.40 \times 10^{-4} \frac{\text{gm}}{\text{cm}^2 \cdot \text{min.}}$$

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SECTION 3
HEMATOLOGICAL VISCOMETRY

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ORIGINAL PROBLEM STATEMENT

As paraphrased from:

Written by:

Dr. Roland Scott
Dr. Robert Hudson

Eugene M. DeLoatch, Ph.D.

TITLE: RAPID DISPLAY OF RELATIVE VISCOSITY OF BLOOD SAMPLES

STATEMENT OF THE PROBLEM

Blood disorders are often detectable from measurements made of the viscosity of blood. It is thought that this might be the case in patients with sickle cell anemia. Early detection of this phenomena may result in prescribing treatments to minimize suffering in those effected. Thus, a quick method which presents a viscosity reading of the patient would present a clinical tool useful as a continual baseline check mechanism.

SCOPE OF WORK

Presently available ultrasonic techniques used in viscosity measurements seem readily adaptable to quick digital display readouts. The missing link appears to be the proper transduction and signal conditioning between presently available ultra-sound apparatus and those devices which would permit easy interpretation by medical personnel. Blood samples inclusive of several pathologies would need to be coded.

HEMATOLOGICAL VISCOMETRY

PROBLEM STATEMENT

Originally, our problem was the development of a rapid display system for blood viscosity measurements. The display system was to be developed in conjunction with ultrasonic techniques employed to make the measurement. A literature search, however, told us that the ultrasonic method was no longer available. Even if it were, the information that it would provide would not suit our purposes. With this in mind, and a better insight into the nature of blood viscosity, our problem was restated as follows: to find or develop instrumentation for improving the measurement of blood viscosity. An improved method is needed so that a base line measurement can possibly be established for normal blood. Present methods for making the measurement were studied and analyzed for their advantages, disadvantages and possible adaptation or use, as an initial step.

INTRODUCTION

Many blood disorders can often be detected by measurement of the viscosity of blood. This may be the case with Sickle Cell Anemia. It is known that a person undergoing cardiac arrest experiences drastic changes in his blood viscosity. One disease exists for which the measurement of blood viscosity is the only diagnostic tool. If early detection of any of the aforementioned phenomena were possible, it could possibly lead to treatment to relieve the associated suffering.

What is viscosity and, in particular, blood viscosity? Viscosity is a fluid's resistance to flow or its stickiness or cohesiveness. The phenomenon may be physically depicted if we consider two flat parallel plates separated by some small distance, h , with a liquid between the two. If a force, F , is tangentially applied to one of the plates, a velocity distribution will be established in the fluid. The velocity of the fluid can be determined from one plate with respect to the other and with this determination, a shear rate can be found. The shear rate is defined as the velocity across the plates divided by the separation. This is expressed as

$$\dot{\gamma} = V/h \text{ (sec}^{-1}\text{)}$$

The tangential force establishes a shear stress across the fluid defined as the force divided by the area of the fluid, expressed as

$$\sigma = F/A \text{ (dyne/cm}^2\text{)}$$

Shear stress is also expressed as

$$F/A = \mu V/h$$

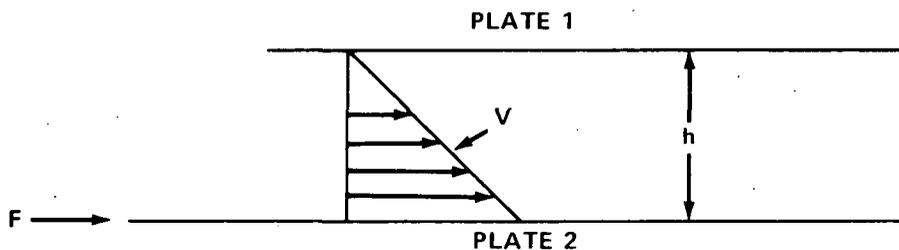
where μ is the viscosity of the fluid, which links shear stress to rate of shear. From the above expression, viscosity is given as

$$\mu = \frac{F/A}{V/h} = \frac{\text{Shear Stress}}{\text{Shear Rate}}$$

The commonly used dimension for viscosity is

$$\frac{\text{dynes/cm}^2}{\text{sec}^{-1}} = \frac{\text{dyne-sec}}{\text{cm}^2} = \text{poise}$$

Multiplication by 100 converts the unit to centipoises. Thus, by determining the shear stress and rate of shear in a fluid, its viscosity can be determined. Figure 3-1 depicts the above explanation. For our purposes, we must be aware of the fact that fluids fall into one of two categories when considering viscosity. The first of these is Newtonian, for which viscosity is a constant for all rates of shear. That is, the viscosity is independent of shear rate. The second category is non-Newtonian, for which viscosity is a non-constant function of shear rate.



$$F/A = \mu \frac{V}{h}$$

$$\mu = \frac{F/A}{V/h}$$

$$\frac{V}{h} = \text{SHEAR RATE}$$

$$\mu = \text{VISCOSITY}$$

$$F/A = \text{SHEAR STRESS}$$

FLOW BETWEEN PARALLEL PLATES

Figure 3-1. Flow Between Parallel Plates

Whole blood characteristically falls into the non-Newtonian category. The primary reason for this is that whole blood contains small suspended particles (red blood cells) which significantly affect its flow properties at low rates of shear. At high shear rates, the effect is much lessened and blood approaches Newtonian fluid behavior. Figure 3-2 depicts the difference between the two fluids.

TECHNIQUES OF VISCOSITY MEASUREMENT

Three different techniques of viscometry were studied to find the latest advances in the field. These techniques include capillary tube flow, rotational viscometers, and the determination by employing ultrasound. Of the first two,

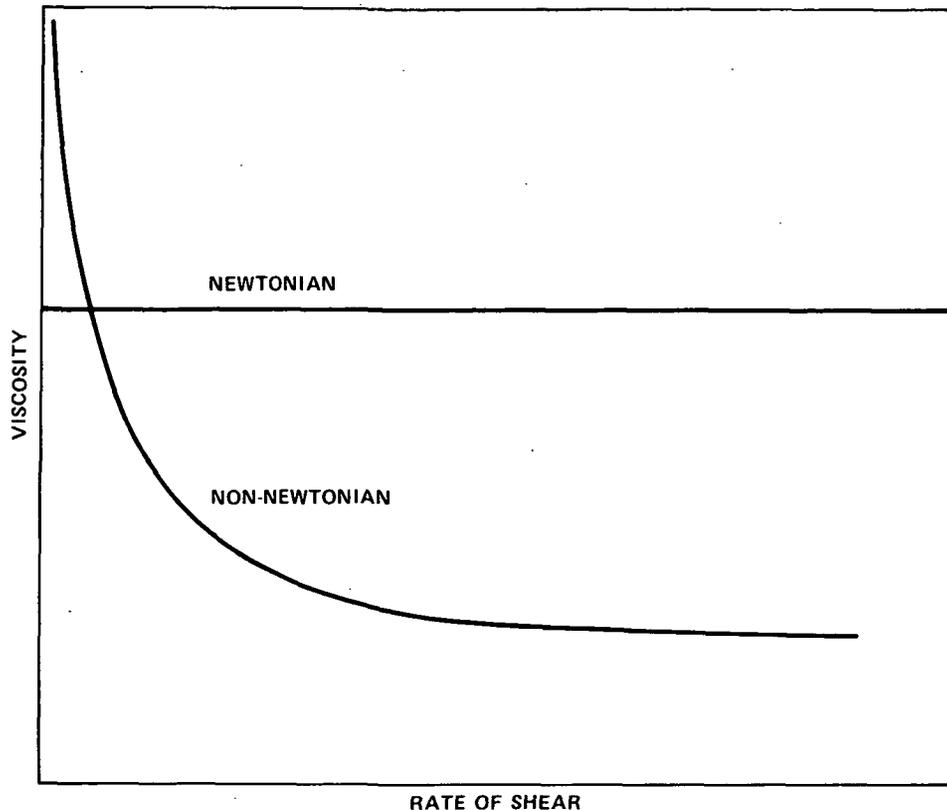


Figure 3-2. Viscosity vs Rate of Shear

rotational viscometers approximate the parallel plate concept and appear to be more readily adaptable to blood viscosity measurements. This section takes a look at some of the technical aspects of these techniques.

CAPILLARY FLOW

In capillary flow a fluid is forced through a fine bore capillary tube and the viscosity of the liquid is determined by measurements of volumetric flow rate, applied pressure and tube dimensions. A complete capillary viscometer consists of (1) a fluid reservoir; (2) a capillary of known dimensions; (3) a unit for measuring and controlling applied pressure; (4) a unit for determining flow rate and (5) a unit for controlling temperature.

The following conditions are postulated in describing capillary flow: (1) steady flow; (2) no radial and tangential velocity components; (3) axial velocity must be a function of radial distance from the axis; (4) $V = 0$ at $r = R$; (5) tube is of sufficient length so that edge effects are negligible; (6) the fluid is incompressible; (7) no external forces; (8) isothermal conditions prevail, and (9) viscosity is not appreciably affected by the pressure change down the tube.

Consider a column of fluid moving in laminar flow in a tube of radius, R , and length, L , with a pressure difference, p , across the tube. The situation can be described mathematically as follows:

$\Delta P (\pi r^2)$ is force tending to move column

$S \cdot A = S(2\pi rL)$ force tending to resist fluid flow

Because of steady flow the forces must be equal so that:

$$S = \Delta p r / 2L \quad (\text{shear stress})$$

if $\sigma = f(s)$

$$\frac{1}{r} [d(rs_{rz}) dr] = [\rho g c - (dp/dz)]$$

so that:

$$s_{rz} = r/2 [\rho g c - dp/dz] + C_1 \quad (\text{shear stress in rz direction})$$

where

$$C_1 = 0 \quad \text{since } s = 0 \text{ at } r = 0$$

The velocity of the fluid in the z direction is given as:

$$V_z = r^2/4n [\rho g c - dp/dz] + C_2$$

The velocity gradient or rate of shear is expressed as:

$$\frac{-dv}{dr}$$

This is normally given as a function of stress where

$$\frac{-dv}{dr} = f(s)$$

Solution of

$$\frac{-dv}{dr} = f(s)$$

Measurements of pressure and volumetric flow rate are used to determine an apparent viscosity

Volumetric flow rate,

$$Q = \int_0^R 2\pi r v(r) dr$$

Integration yields

$$Q = \pi r^2 v \Big|_0^R - \pi \int_0^R r^2 (dv/dr) dr$$

from

$$r/R = s_r/s_R$$

we get

$$4Q/\pi R^3 = 4/s_R^3 \int_0^R f(s) s^2 ds$$

differentiation yields:

$$(-dv/dr)_w = f(s_R) = 3/4(4Q/\pi R^3) + 1/4s_R [d(4Q/\pi R^3)] / ds_R$$

by transformation:

$$(-dv/dr)_w = 3/4(4Q/\pi R^3) + 1/4(4Q/\pi R^3) [d\log(4Q/\pi R^3)] / d\log (\Delta p R/2L)$$

letting:

$$b = d\log 4Q/\pi R^3 / d\log \Delta p R/2L$$

yields:

$$(-dv/dr)_w = [(3 + b) / 4] (4Q/\pi R^3)$$

$(-dv/dr)_w$ indicates rate of shear at the wall. For Newtonian fluids the above is fine. However, for non-Newtonian fluids the viscosity measurement covers a wide range of shear rates for which allowances must be made. Also, if the tube is too narrow, wall effects may influence the results. For blood the diameter is less than 100. Advantages of capillary tubes are (1) low cost (2) use of small samples (3) easy to use, and (4) good reproducibility.

In order for flow to be steady and laminar a Reynolds number, Re , is used to impose restrictions on the tube dimensions. This number is expressed as

$$Re = 2Qe/n\pi R$$

where e is density, n is viscosity and the other quantities have been given. Values of Re have significance as follows:

$$Re > 2000 - \text{turbulent flow}$$

With this number and quantities such as Q , e , and n , the range of values for R are determined.

Based on the advantages of capillary viscometers, we attempted to use it. The idea was to use a tapered tube, induce a volumetric flow through it and measure the pressure gradient. By doing this the viscosity of blood could be determined at various mean shear rates. Because blood is a non-Newtonian fluid, the velocity profile is not parabolic as for Newtonian fluids and correction factors appear in the flow equations. Corrections must be made for (1) edge effects; (2) kinetic energy losses and (3) drainage. Kinetic energy losses are due to effective pressure losses because of kinetic energy in the issuing stream. Edge or end effects are energy losses due to viscous or elastic behavior when a fluid converges or diverges at the capillary ends. Drainage is used to describe wall effects such as liquid adhering to the reservoir walls.

Due to a shortage of time, this approach was dropped after initial mathematical analysis.

Error Corrections for Capillary Tubes

Kinetic energy corrections:

$$\Delta p = \Delta P_{\text{obs}} - \rho V^2 a_v / a = \Delta P_{\text{obs}} - \rho Q^2 / a \pi^2 R^4$$

where

Δp = corrected pressure

ΔP_{obs} = observed pressure

a = kinetic energy correction factor
=

ρ = fluid density

bore and end effects important!

$$a = 2 / (2 - \Psi / s_w) \text{ for Bingham-body fluid}$$

$$a = (4\eta + 2)(5\eta + 3) / [3 \cdot (3\eta + 1)^2] \text{ Power-law fluids}$$

Effective kinetic energy corrections:

$$\eta = \pi R^4 h \rho g \theta / 8LV - \xi V / 8\pi L \theta$$

or

$$V = \eta / \rho = \pi R^4 h g \theta / 8LV - \xi V / 8\pi L \theta$$

where

h = mean hydrostatic head

V = fixed volume of bulb

θ = is efflux time

ξ = correction factor

$$\text{let } V = k\theta - k_1\theta \quad \text{and} \quad k_1 = \xi V / 8\pi L \quad k = \pi R^4 h g / 8LV$$

(ξ increases with Reynolds number)

Trumpet-shaped requires less ξ for particular Re than square.

$$\text{for Bell } \xi \sim 1/\theta \rightarrow V = K\theta - K_2/\theta^2$$

the latter equation can be rewritten

$$V = K\theta - K_2/\theta^m \rightarrow \text{by increasing efflux time the kinetic energy correction is made negligible.}$$

$m = 2$ for trumpet-shaped

$m = -2/3$ for square

trumpet-shaped (rounded) ends have less frictional loss.

Long tube or large length to radius ratio reduces end effects. 200 is a considerable value for the ratio.

ROTATIONAL VISCOMETRY

In rotational viscometry, two members are used having a common axis and separated by the test fluid. One member is made to rotate while the other is left free. Because of the fluid a viscous drag is produced on the free element which tends to rotate it with the rotated member. By measuring the angular velocity of the rotated member and the torque produced on the free member and using the geometry of the system, the viscosity of the fluid can be determined.

Two types of rotational viscometers are now commercially available and used. One is the co-axial cylinder arrangement and the second the cone-plate type.

The co-axial cylinder viscometer consists of a bob and a cylindrical pot in which the test fluid is placed. The bob and pot are concentrically mounted. The pot is rotated at a constant angular velocity and a viscous drag transmitted through the fluid is measured by the angular deflection of the bob or the force needed to return it to its original position. The process can be rearranged so that the bob is rotated and the viscous drag transmitted to the pot and recorded. Both arrangements approximate the ideal parallel plate concept by the geometry and the approximation gets better as the radii increase and the annular gap decreases.

With its good points the co-axial cylinder viscometer has its drawbacks. As just stated, the radii have to be large and the annulus width small so that a large radii to gap ratio is needed for a good measurement. Corrections must be made for the drag transmitted between the bases of the pot and bob and sometimes for the presence of a free surface to the test fluid. The latter may lend the transmission of forces brought about by denaturing of the plasma, for example. If it is desired to work with small samples at low shear rates (as we do) the expense of a good instrument is everything but conservative. It has been shown by Whitmore that if the fluid consisted of a suspension of dispersed particles in a liquid any redistribution of the particles across the annulus should not alter the torque, provided the flow closely approximated that between flat parallel planes and the suspension obeyed the equation:

$$\eta_r = \frac{1}{1 - k_1 c}$$

Unfortunately, blood probably does not obey the equation so that radial movement of the cells from the viscometer walls will probably influence the measured viscosity.

For Newtonian fluids under laminar flow the transmitted torque is expressed as

$$T = \frac{4 R_1^2 R_2^2 h w}{R_2^2 - R_1^2}$$

where R_1 = radius of inner cylinder, R_2 = radius of outer cylinder, h = height of the fluid, and w = angular velocity of outer cylinder. The mean shear stress is given as

$$\bar{T} = T \left(\frac{R_1^2 + R_2^2}{4 h R_1^2 R_2^2} \right)$$

and the mean shear rate by

$$\bar{D} = w \frac{R_1^2 + R_2^2}{R_2^2 - R_1^2}$$

For non-Newtonian fluids the shearing stress is no longer directly proportional to the rate of shear and the measured torque is no longer directly proportional to the angular velocity.

In the cone-plate viscometer, illustrated in Figure 3-1, the test fluid is contained between a cone of very apex angle and a flat surface normal to its axis. In available units the cone is rotated and the torque required to turn it against the viscous drag of the fluid in the stationary plate is measured. The angular velocity of the cone is also measured. The angular velocity of the cone is also measured. It is apparent from the geometry of the system that the perpendicular distance between cone and plate increases with the radius. The linear relative velocity of the cone is also linearly proportional to the radius. The distance h is given by

$$h = r \sin \theta$$

where θ is the angle between cone and plate. The peripheral velocity of the cone is expressed by

$$V_p = 2\pi r N/60$$

where N is the number of revolutions per minute, 60 is the conversion factor to change minutes to seconds. The rate of shear is given by

$$\gamma = \frac{V_p}{h} = \frac{2\pi r N/60}{r \sin \theta} = \frac{2\pi N/60}{\sin \theta}$$

Before the viscosity of the fluid can be determined the shear stress must be determined. Using the geometry of the cone-plate system and a torque measurement, the shear stress can be calculated. The torque, T, is given by

$$T = 2\pi\sigma \int_0^{r_e} r^2 dr = 2/3\pi\sigma r_e^3$$

from which the shear stress is expressed as

$$= \frac{T}{2/3\pi r_e^3}$$

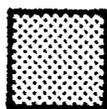
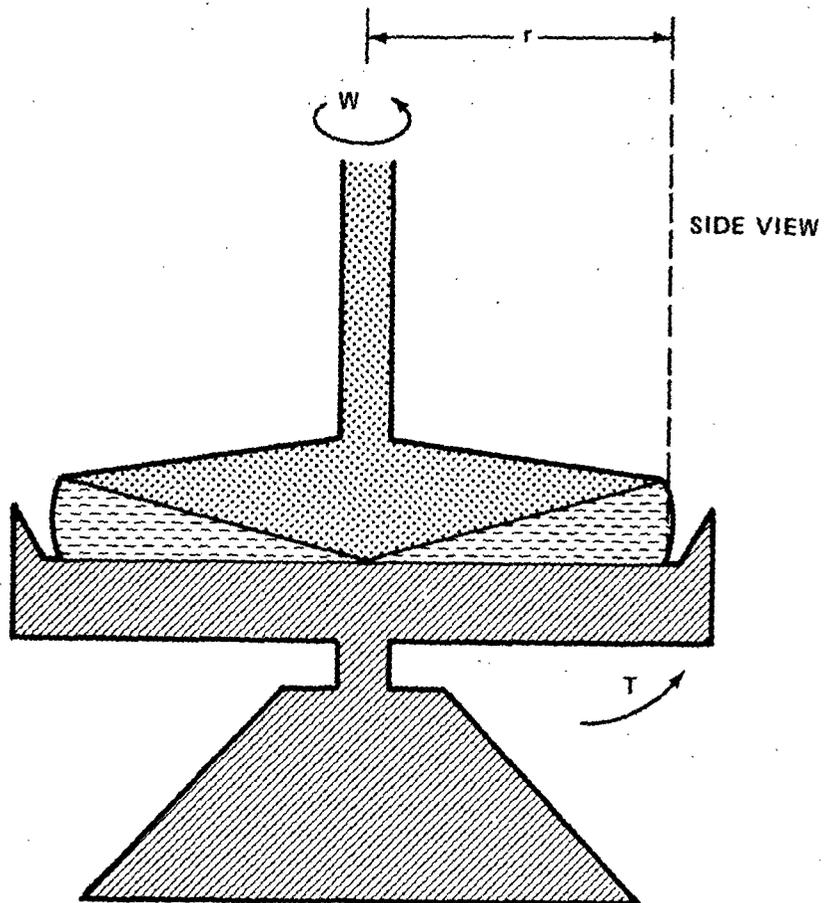
Using the definition of viscosity as being shear stress divided by shear rate, the viscosity is given by

$$= \frac{T/2/3\pi r_e^3}{2\pi N/60 \sin \theta}$$

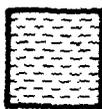
Letting $w = 2 N/60$ the viscosity is given as

$$= \frac{3T \sin \theta}{2\pi r_e^3 w}$$

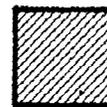
In the commercial viscometer, the torque measuring device is a copper-beryllium calibrated spring. The spring connects the drive mechanism to a vertical spindle



DRIVEN
CONE



TEST FLUID



FIXED
PLATE

$$\mu \text{ (poise)} = \frac{\text{SHEAR STRESS}}{\text{SHEAR RATE}}$$

$$\text{SHEAR RATE} = v = \frac{V}{h} = \frac{W}{\sin \theta} \text{ (sec}^{-1}\text{)}$$

$$\text{SHEAR STRESS} = \frac{T}{\frac{2}{3} \pi r^3} \text{ (dyne/cm}^2\text{)}$$

$$\mu = \frac{T \sin \theta}{\frac{2}{3} \pi r^3 W} \text{ (poise)}$$

Figure 3-3. Cone-Plate Viscometer

from which the cone is suspended. The torque applied to the cone has a linear relationship to the deflection of the spring. A pointer connected to the spring overrides a dial which rotates in phase with the drive shaft. The drive mechanism is a synchronous motor with a speed reducing gear transmission system. Only a discrete number of speeds can be obtained with this system, limiting the available shear rates to a discrete number.

The clearance between the cone and plate is adjustable and critical in obtaining good results. The desired gap is 0.001 inch.

ULTRASONIC TECHNIQUES

Ultrasonic viscometry was based on the principle that losses would appear in a fluid when motion occurred. The losses are due to the viscosity of the fluid. In the instrument once used, an electrical pulse was applied to an ultrasonic probe placed in a fluid. The pulse caused the probe to vibrate. The pulse was later removed and the vibration decayed in direct proportion to the viscosity of the fluid. For Newtonian fluids absolute viscosity values were given. However, only apparent values could be given for non-Newtonian fluids.

PROPOSED INSTRUMENT

Of the available viscosity measurement devices, the cone-plate viscometer appears to be most readily adaptable to blood viscosity determinations. Its primary advantages over other methods include:

1. constant shear rates
2. small sample size < 3ml
3. relative simplicity for a rotational viscometer

While they show some promise, commercially available cone-plate viscometers possess many characteristics which make them undesirable for the laboratory determination of blood viscosity. Among these are:

1. On present devices viscosity is determined indirectly via a rotating torquemeter. This torque sensing element is dependent on a calibrated spring for range and accuracy. Thus the device is range limited and subject to environmental effects on the spring.
2. Current interest in blood measurements centers on viscosity determination at low shear rates 1 sec^{-1} . Reasonably priced devices only yield reliable data at shear rates in excess of 5 sec^{-1} .

3. Only discrete shear rates may be obtained, pre set by gear transmission.
4. So-called "edge effects" caused by the air-fluid interface are ignored.
5. Operator technique is critical. Common mistakes lead to misleading and erroneous information.
6. There are difficulties involved in reading the meter which is rotating with the indicator.

In the attempt to alleviate some of the current shortcomings while returning the positive features of presently available devices, we have devised a comparison viscometer based on the cone and plate principle. Our device consists of two cone and plate chambers, a variable speed drive, and rotational velocity detectors.

The design makes use of two cone-plate systems directly coupled with one system driving the other by viscous drag transmitted through a test (standard) fluid. With this design, direct drive can be employed eliminating the intervening mechanical parts and a wider and lower range of shear rates can be obtained. The torque measurement has also been eliminated and replaced by an angular velocity measurement, as a means of determining the viscosity. The angular velocity measurements can be processed electronically.

Since blood is a non-Newtonian fluid, viscosity values must be specified at a known rate of shear. We will show our device that the relation:

$$\text{SHEAR RATE} = \frac{W_a - W_b}{\text{Sin}_A}$$

and the resulting viscosity at that shear rate will be

$$\mu \text{ blood} = \frac{\mu(\text{STD}) \text{ WA}}{\text{WA} - \text{WB}}$$

The following pages display the concept and present the defining equations. The central idea is to use viscosity standards, for which the viscosity is known and calibrated, to determine the unknown viscosity of the specific blood sample.

DEVICE REQUIREMENTS

Based on our studies and analysis, the following requirements have been cited as having importance in the development of new instrumentation depicted in Figure 3-4.

1. Small sample size < 3 ml
2. Accuracy
3. Easy and simple operation
4. Reproducibility
5. As error proof as possible

In addition, it is desirable that the system afford the following improvements over available cone and plate:

1. Lower shear rates should be attainable. A rate of $.1 \text{ sec}^{-1}$ is a conservative estimate for blood.
2. Wider range of viscosity measurements can be made simply by changing the viscosity oil STANDARD rather than selecting another torque sensing device.
3. The device can be used in a comparison mode to track small changes in viscosity over time.
4. Some degree of internal compensation is obtained for edge effects.
5. A direct digital readout is proposed so that operation is simplified.

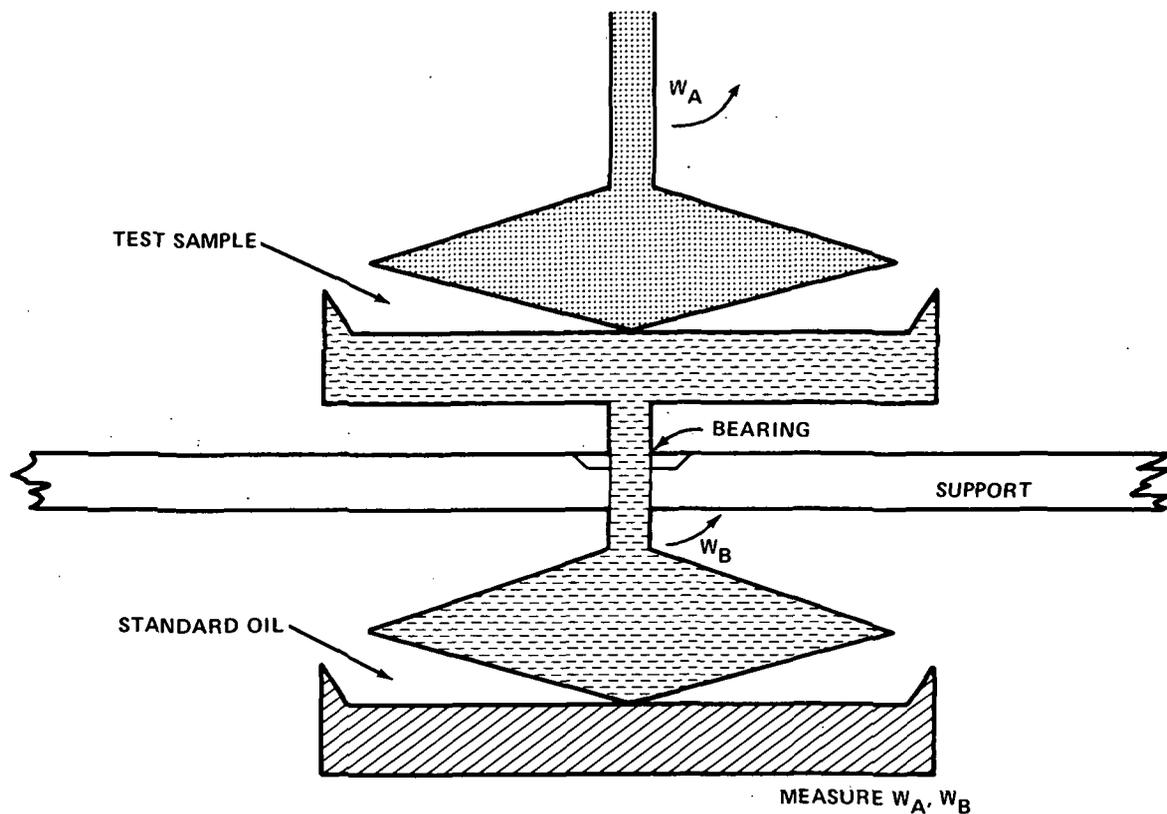
SUMMARY OF REPRESENTATIVE FORMULAE

Standard Cell

$$\text{I. SHEAR STRESS} = \frac{T}{2/3\pi r_b^3}$$

$$\text{II. BUT (STD)} = \frac{\text{SHEAR STRESS} \times 100}{\text{SHEAR RATE}}$$

$$\text{III. SHEAR RATE} = \frac{W_b}{\text{sine } \theta}$$




 FREE CONE
 PLATE


 DRIVEN
 CONE


 FIXED
 PLATE

$$\text{SHEAR RATE} = \frac{\omega_A - \omega_B}{\sin \theta_A}$$

$$\mu (\text{sample}) = \frac{\mu(\text{STD}) r_b^3 \omega_b \sin \theta_A}{\sin \theta_B r_a^3 (\omega_A - \omega_B)} \quad (\text{c.p})$$

$$\theta_A = \theta_B \quad r_a = r_b$$

$$\mu (\text{sample}) = \frac{\mu(\text{STD}) \omega_b}{(\omega_A - \omega_B)}$$

Figure 3-4. Sketch of Proposed Instrument

IV. Solving for T(dyne cm) in eg. I

$$T(\text{dyne cm}) = \frac{\mu \text{ STD } W_b \ 2/3\pi r_b^3}{100 \text{ sine } \theta_b}$$

Sample Cell

$$\text{V. SHEAR RATE} = \frac{W_a - W_b}{\text{sine } \theta_A}$$

$$\text{VI. SHEAR STRESS} = \frac{T}{2/3\pi r_a^3}$$

Substituting IV into VI for T and solving viscosity eg. for sample

$$\mu \text{ blood} \frac{W_a - W_B}{\text{sine } \theta_A} = \frac{\mu(\text{STD}) W_b r_b^3 \text{ sine } \theta_A}{(W_a - W_b) r_a^3 \text{ sine } \theta_B}$$

$$r_a = r_b, \theta_A = \theta_B \quad \mu(\text{Blood}) = \frac{\mu(\text{STD}) W_b}{(W_a - W_b)}$$

Derivation of Equations

We know that for the cone and plate

$$\text{SHEAR STRESS} = \frac{T}{2/3\pi r^3}$$

$$\text{SHEAR RATE} = \frac{W}{\text{sine } \theta}$$

$$\text{VISCOSITY,} = \frac{\text{SHEAR STRESS} \times 100}{\text{SHEAR RATE}}$$

For the lower chamber containing a Newtonian fluid with viscosity μ STD, the torque required to turn the cone at W_b is

$$T = \text{SHEAR STRESS} \times 2/3\pi r_b^3$$

BUT

$$\text{SHEAR STRESS} = \frac{\mu \text{STD} \times \text{S.R.}}{100}$$

ALSO

$$\text{S.R.} = \frac{W_b}{\text{sine } \theta_b}$$

THEREFORE

$$T = \frac{\mu_{\text{STD}} W_b \frac{2}{3}\pi r_b^3}{100 \text{ sine } \theta}$$

If the system is in equilibrium

$$\frac{\partial W_a}{\partial t} = \frac{\partial W_b}{\partial t} = 0$$

By writing a torque balance for the sample we see that the rate is proportional to the relative velocity between the cone and plate.

HENCE

$$\text{SHEAR RATE} = \frac{W_A - W_B}{\text{sine } \theta_a}$$

AND

$$\text{SHEAR STRESS} = \frac{T}{\frac{2}{3}\pi r_a^3}$$

If equilibrium persists, the torques can be equated and by mathematical manipulation:

$$\mu_{\text{blood}} \text{ S.R.} = \frac{W_a - W_b}{\text{sine } \theta_a} = \frac{\mu_{\text{STD}} W_b r_b^3 \text{ sine } \theta_a}{W_a - W_b r_a^3 \text{ sine } \theta_b}$$

IF

$$\theta_a = \theta_b \text{ and } r_a = r_b$$

THEN

$$\mu_{\text{blood}} \text{ S.R.} = \frac{W_a - W_b}{\text{sine } \theta_a} = \frac{\mu_{\text{STD}} W_b}{W_a - W_b}$$

The following calculations are done to illustrate the magnitude of the parameters required to be measured.

At a shear rate of 0.1 sec^{-1} blood with a hematocrit of 35% has a viscosity of approximately 50 centipoise. With these numbers and assuming an angle of 1.5° we have

$$10^{-1} \text{ sec}^{-1} = \frac{W_a - W_b}{\sin 1.5} = \frac{W_a - W_b}{0.025}$$

$$10^{-1} (2.5 \times 10^{-2}) = 2.5 \times 10^{-3} \text{ rad/sec} = W_a - W_b$$

Assuming the use of a viscosity oil of 1 centipoise we have

$$\mu \text{ blood} = \frac{\mu_{\text{STD}} W_b}{W_a - W_b}$$

By substitution

$$5 \times 10^1 = \frac{W_b}{W_a - W_b}$$

$$5 \times 10^1 = \frac{W_b}{2.5 \times 10^{-3}}$$

$$W_b \ 12.5 \times 10^{-2} = 1.25 \times 10^{-1} \text{ rad/sec}$$

By conversion

$$1.25 \times 10^{-1} \text{ rad/sec} = 1.25 \times 10^{-1} \text{ rad/sec} \frac{60 \text{ sec}}{1 \text{ min}} \frac{1 \text{ rev}}{2\pi \text{ rad}} = 1.20 \text{ rpm}$$

$$W_b = 1.20 \text{ rpm} \quad W_a = 1.22 \text{ rpm}$$

We devise an adjustable speed so that the shear rate can be continuously varied, and easily determined and controlled. A D.C. shunt motor best serves our purposes. With the D.C. shunt the speed can be controlled by the armature voltage and the output torque will remain constant at rated current regardless of the speed. With a properly designed motor direct drive can be employed eliminating the need for gearing thus providing greater speed variability and hence a wider choice of shear rates.

Motor Specifications

D.C. Shunt

Adjustable Speed ($0.3 \approx 20$ rpm)

Armature - Voltage Control

Varying Duty Cycle

Motors are commercially available that go to 1/64 rmp.

Price information is needed.

Other specifications such as input must be determined.

In order to maintain the advantage of small sample size, the dimensions used for commercial instruments were chosen for our device.

Viscometer Specifications

Cones - diameter - 50 mm
= 1.5°

Plates - diameter - 54 mm
depth - 20 mm

Sample Size

The bearing should be the Roller Element. They have the following features:

1. smooth running or limited vibration
2. low or uniform operating torque
3. control of contact angle and/or preload levels

4. extreme cleanliness

Bearings were available with diameters ranging from 1/8 inch and up.

DIGITAL MEASUREMENT TECHNIQUES

The relative velocity viscometer requires the measurement of two angular velocities. We hope to accomplish these measurements photoelectrically using slotted stroboscope-like discs with a light and two photo detectors (photo diodes or solar cells). This enables us to put no additional torques on the shaft, due to measurements. The strobe-like arrangement would give an output of pulses whose repetition rate is proportional to the angular velocity of the shaft. (Figure 3-5).

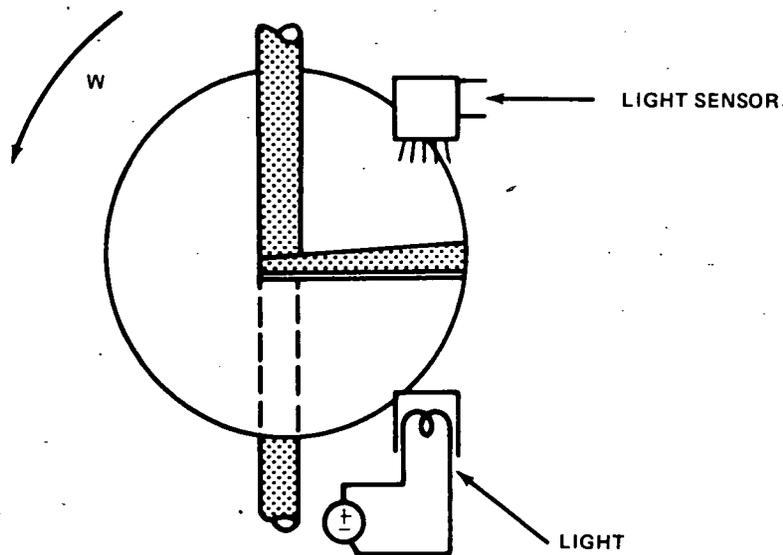


Figure 3-5

Two systems have been considered for handling this pulse train. The first system is a simple one using d.e. meters for the output. The output of the two detectors A, and B, are fed into a one-shot multivibrator. The on time for the multivibrators should equal each other with a period of about 50% of the lowest photo detectors repetition period expected. The signal from A is inverted using an Op amp. Then both signals are fed into a summing Op amp circuit. (Figure 3-6.) The average d.e. level is obtained from low pass filters or from the averaging done by the meter itself.

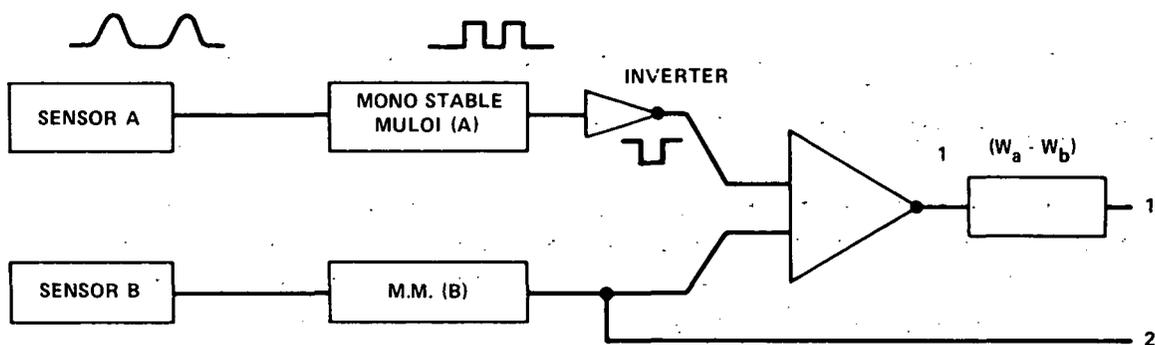


Figure 3-6

This system has the advantages of relative low cost and direct read out. However, it suffers from lack of fine resolution from the reading of the meters. It is also critical that the tone of the multivibrators be matched, as errors in matching cannot be resolved by a simple calibration constant (due to additional processing). It could be corrected however, by making one of the multivibrators with a variable on time. The two shafts could be turned in unison and ton would be adjusted to null the output of the summing circuit. With the addition of suitable filtering and an analog quotient circuit direct viscosity read outs may be obtained.

The other system considered could have better resolution and digital read out at the cost of added complexity and expense. The outputs of the photo detectors are again fed into mono-stable multivibrators. This time ton is small in comparison with the pulse repetition rate of the detectors. The outputs of these pulse shaping circuits are used to operate flip-flops in the toggle mode. The flip flops are used to gate an oscillator-counter combination. Addition digital processing might be considered. The output could either be digital display or punched tape. (Figure 3-7).

Scope of Work

We have spent some time becoming familiar with some basic elements of digital techniques and have produced devices suitable for use as mono-stables, flip-flops and gates.

ALTERNATE CONSIDERATIONS

Several methods were considered as approaches to this problem. They are presented in the following.

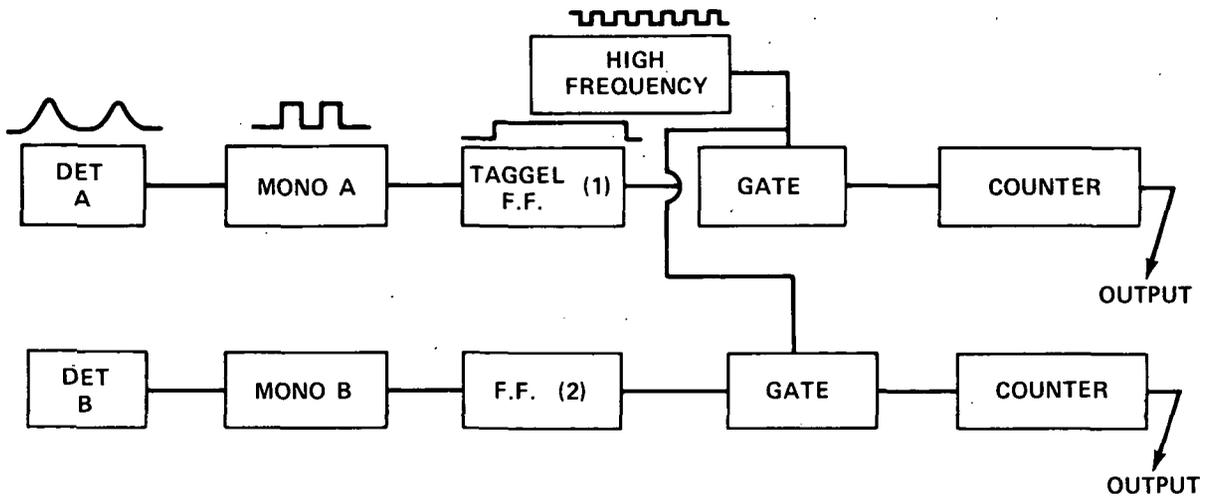
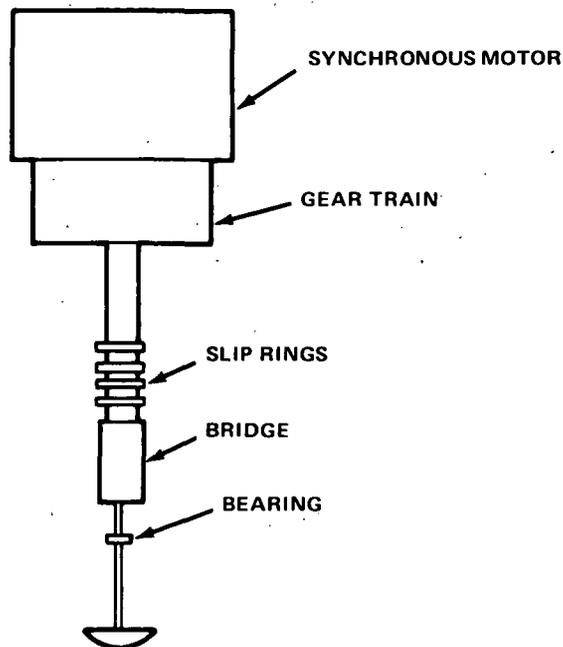


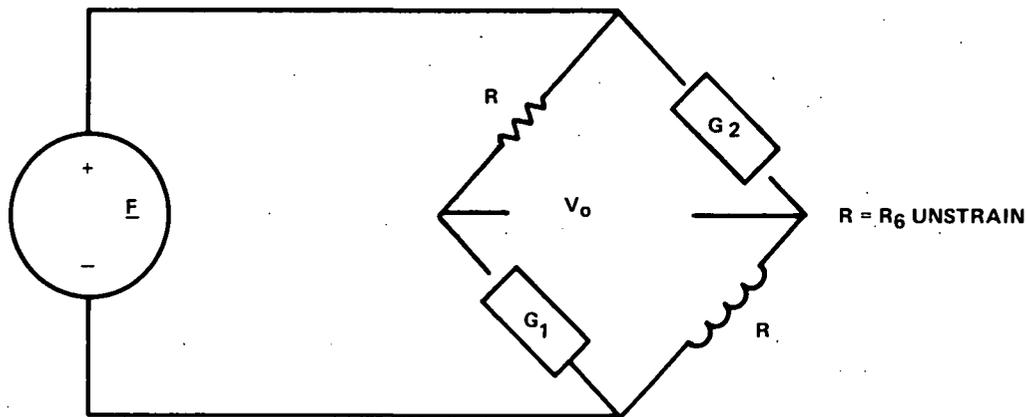
Figure 3-7

IMPROVED CONE AND PLATE (Strain Gauge T Torque Meter)

A different conversion using torquemeter of shaft modification of the Wells.



Strain gauges on opposite sides of spring shaft encircling 45° from the shaft axis and 90° in the angle between them. The gauges are connected on opposite bridge resistance gauges.



Advantages

1. Direct reading is facilitated (no moving gauge)
2. Output signals can be readily recorded or processed electronically

Disadvantages

1. Four slip rings are required which makes manufacturing and maintenance difficult. Poor slip rings would affect output.
2. Range and accuracy are limited by spring considerations.

Only more readable output would likely be gained. Cost would be increased by required electronics.

Note: The slip ring problem could be eliminated if the torque measurement were made on the plate. However, this increases system complexity.

SYNCHRONOUS MOTOR PHASE ANGLE TORQUE SENSOR SYSTEM

A synchronous machine consisting of an A.C. excited stator and a D.C. excited armature rotates at a speed of

$$n_{syn} = \frac{120f}{p} \text{ RPM}$$

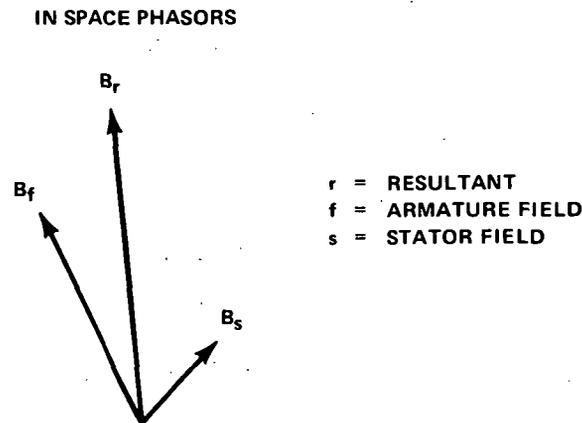
f = frequency in hertz

p = # polyphase poles

The machine is locked at this speed and as the rotor will stop if below W_{syn} because zero net torque will be supplied by interacting magnetic fields (assume no damper windings).

While the rotator must turn at W_{syn} the angle at which the shaft lags the rotating magnetic field changes as the torque is increased.

The phase of the stator B-field can be determined from the phase of the applied a.c. excitor current. If we measure the phase of the resultant B-field in the gap, we can determine the phase difference or spacial lag of the armature B-field with respect to the stator. This is a measure of the torque produced on the shaft.



By changing the intensity of the armature field, manipulating the D.C. armature current, we can change the sensitivity of phase lag to shaft torque.

Sensing of the gap B-field might be accomplished by a small coil.

Alternately a device which physically determines the shaft position could be employed.

If electrical phase, L , is employed Schmitt Triggers could be employed to produce a square wave output which could then be fed into a phase detector similar to the one shown in Figures 3-8 and 3-9.

Possible Advantages

1. Torque measurement and drive unit could be combined in one device
2. Direct readout for easy processing possible.

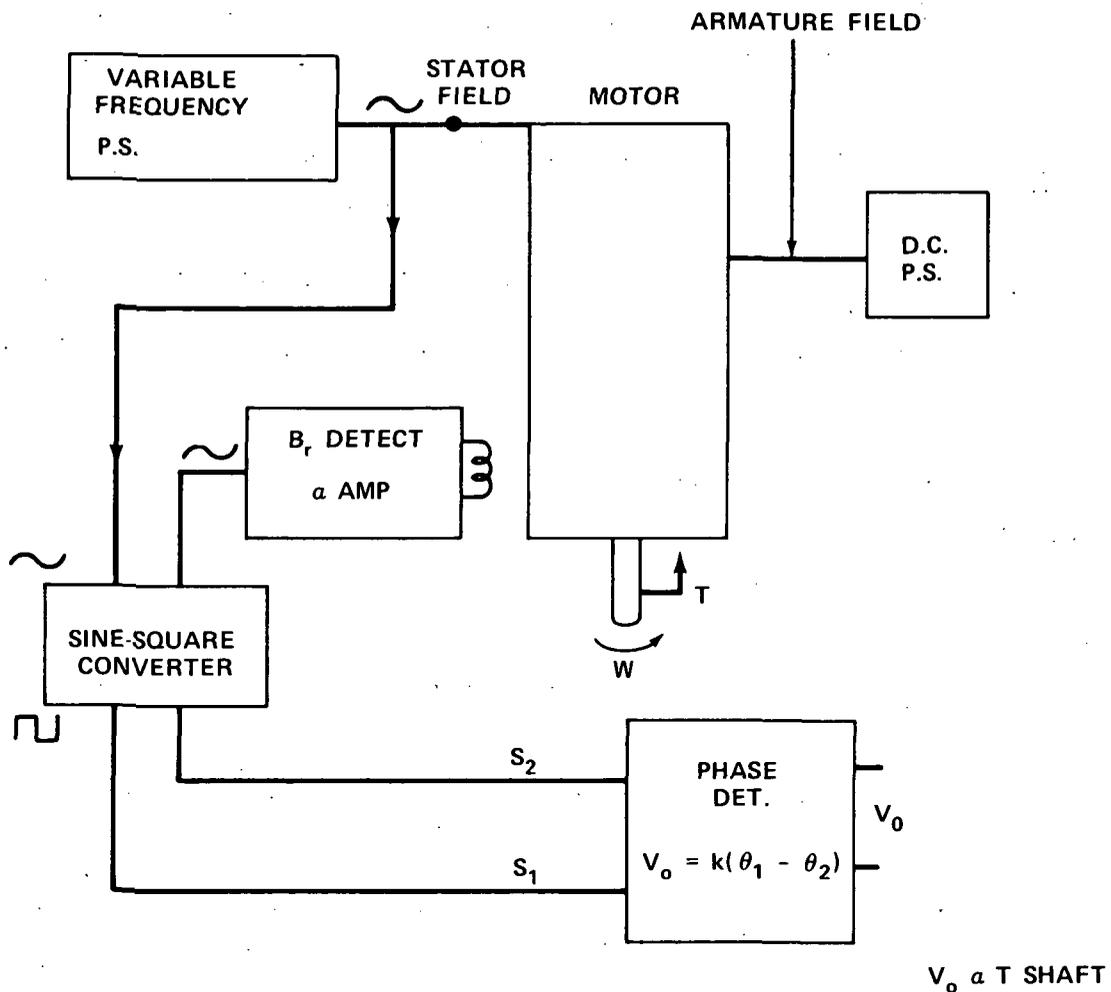


Figure 3-8. System Block Diagram

3. Does not rely on vulnerable calibrated spring.
4. Phase difference techniques allow for good resolution.

Disadvantages

1. If direct drive is used, in order to obtain 1 RPM output a twelve pole device would have to be driven at 0.1 Hz.

$$\text{syn} = \frac{120f}{p}$$

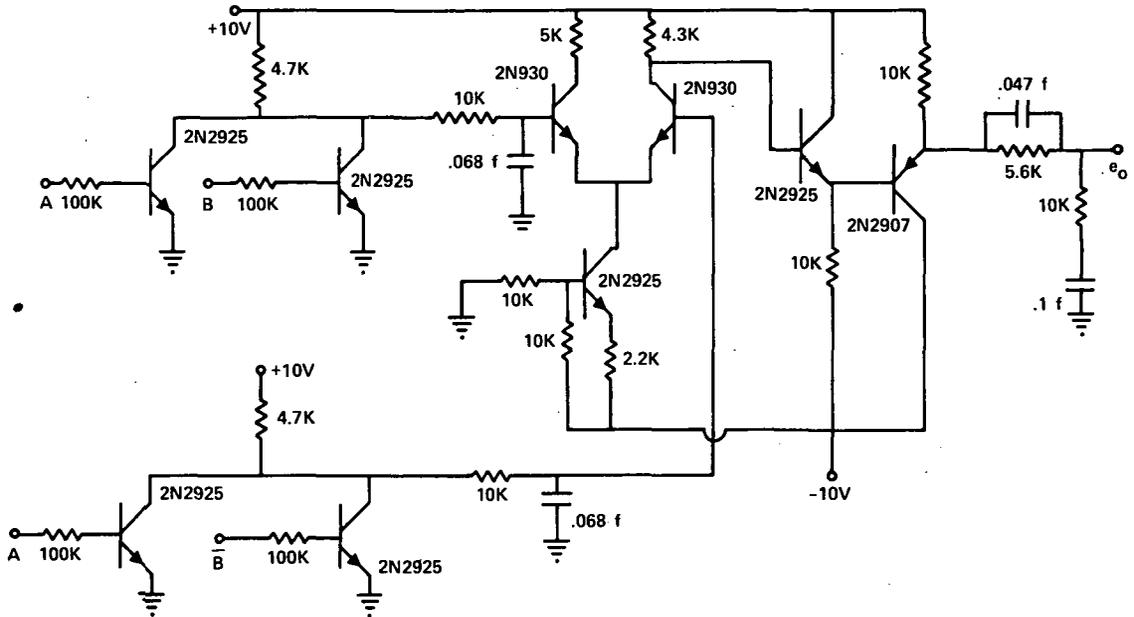


Figure 3-9. Phase Detector II

If a gear train were added so that a two pole motor could be used at reasonable driving frequencies, 20-400 Hz, additional torques produced by gear friction would eliminate any advantages that phase differences afforded. In addition, saturation of magnetic materials in the stator becomes a concern as the frequencies are varied. Due to the speeds at which measurements are to be taken this technique is inapplicable to this problem.

TAPERED TUBE CAPILLARY VISCOMETER

If a capillary viscometer of length, l , is connected to a device which forces fluid through the tube at a constant rate, a pressure difference P is developed between the ends of the tube which is proportional to the viscosity of the fluid. If the same mass flux of a Newtonian fluid is placed through a smaller tube of the same length, the same phenomena occur. However, the mean velocity within the tube increases over that of the larger tube.

It can then be seen that since the velocity at the sides of the tubes is zero, the velocity gradient in the smaller tube is larger than in the larger one.

If a mass flux, Q , is now induced in a tapered tube and the pressure gradient is measured, the viscosities of the fluid at various mean shear rates can be determined.

In practice, the pressure gradient could not be measured but would be approximated by a number of salient pressure sensors.

As blood is a non-Newtonian fluid, the radial velocity profile would not be the parabola found in a Newtonian fluid so that correction factors would be required.

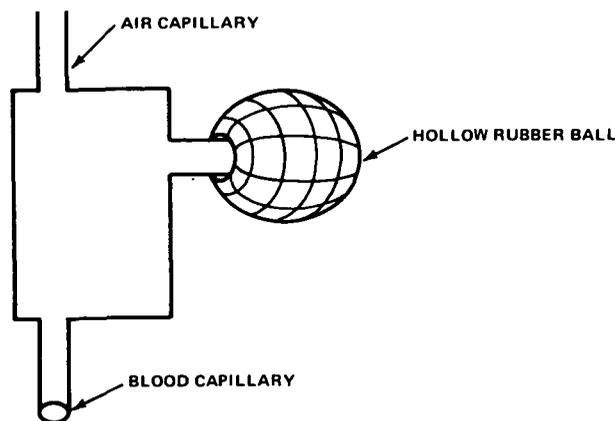
After initial attempts at more extensive mathematical analysis, this attempt was dropped due to time constraints in favor of a less radical approach.

CURRENT DEVELOPMENTS

Presently under development at Purdue University is a random signal sonar system for the detection of blood flow in deep lying vessels operating at a center frequency of about 5 MHz. Using doppler shift for flow detection, this device might be applied to in vivo viscosity measurements as follows: As it is expected that the minimum range resolution of the system is in the range of $\lambda/4$ it would be possible to obtain a velocity profile inside a capillary tube or blood vessel. If a blood vessel of suitable geometry is found to approximate a capillary tube, and a pressure drop along a segment is measured, it would then be possible to obtain viscosity measurements at various shear rates. While such a system might provide an ultimate solution to the blood viscosity measurement problem, the realization of this performance is still a way in the future. It is expected that development of the random signal sonar system will require at least two or more years.

A QUICK SMALL SAMPLE VISCOMETER FOR HIGH SHEAR RATE VISCOSITY DETERMINATIONS

Kawai has developed a simple viscometer for making rapid blood viscosity determinations using only 0.3 ml of blood sample. It consists of two capillary tubes, a small chamber and a rubber squeeze ball.



The device is operated by squeezing air out of the ball through the capillaries. The lower capillary is immersed in the blood sample and the ball released. The height to which the blood rises within the chamber is a measure of viscosity. Complete mathematical treatment is found in the references mentioned below.

This device does not give valid results equivalent to determinations of viscosity made at shear rates lower than $\approx 100 \text{ sec}^{-1}$. Any use of this type instrument should be made with the awareness that the non-Newtonian character of blood is ignored.

REFERENCE

Kawai, Helni, et al.; "A New Capillary Viscometer for Measuring the Viscosity of Small Amounts of Blood." Nasa Document, N67 14584.

COMPONENT ANALYSIS

$$J = 1/2 \cdot \frac{W}{g} R^2 = \frac{\rho\pi}{2g} LR^4, \text{ for rod}$$

W = weight

g = acceleration due to gravity

ρ = density

L = length

R = radius

J = moment of inertia

J for the following materials is:

Brass or steel - $0.0012LR^4$ lb-in-sec²

Aluminum - $0.0004LR^4$ lb-in-sec²

$$J = \frac{\rho\pi}{2g} L(R_2^4 - R_1^4), \text{ for annular ring}$$

$$J = J_{cg} + \frac{W}{g} R^2, \text{ for general object}$$

R = distance from center of gravity of object to axis of rotation

J_{cg} = inertia of object about parallel axis through its center of gravity

Based on the equation of J for the three materials above and an inside diameter of 1/8 inch for the bearing, R is chosen as 1/16 inch for the shaft. The coneplate gap is chosen as 0.002 inch in lieu of the one used on commercial machines. L is chosen as two inches.

CONVERSION FACTORS

2.54 cm = 1 inch = 25.4 mm

1 cm = 10 mm

SPECIFICATIONS

D.C. SHUNT WOUND VARIABLE SPEED MOTOR

Maximum Speed - 20 RPM

Maximum Torque - 673.7 dyne-cm

Direct Drive

VISCOMETER

Cones:

Diameter - 50 mm (1.57 inches)

Depth - 20 mm (0.788 inches)

1' 2

Plates:

Diameters - 54 mm (2.12 inches)

Depth - 20 mm (0.788 inches)

BEARINGS

Type:

Roller element or air

Diameter - 1/8 inch (inside)

Cone-Plate Gap Clearance - 0.002 inches

DEVICE SPECIFICATIONS (Figure 3-10)

Cone Diameter - $1.97(2) = 3.94$ inches

Plate Diameter - $2.12(2) = 4.24$ inches

Plate Depth - $0.788(2) = 1.576$ inches

Bearing I.D. - $1/8(2) = 1/4$ inches

Shaft Radius - $1/16(2) = 1/8$ inches

Shaft Length - $1(2) = 2$ inches

Cone-Plate Clearance - $2/1000(2) = 4/1000 = 1/250$ inches

Housing Diameter - $2.18(2) = 4.36$ inches

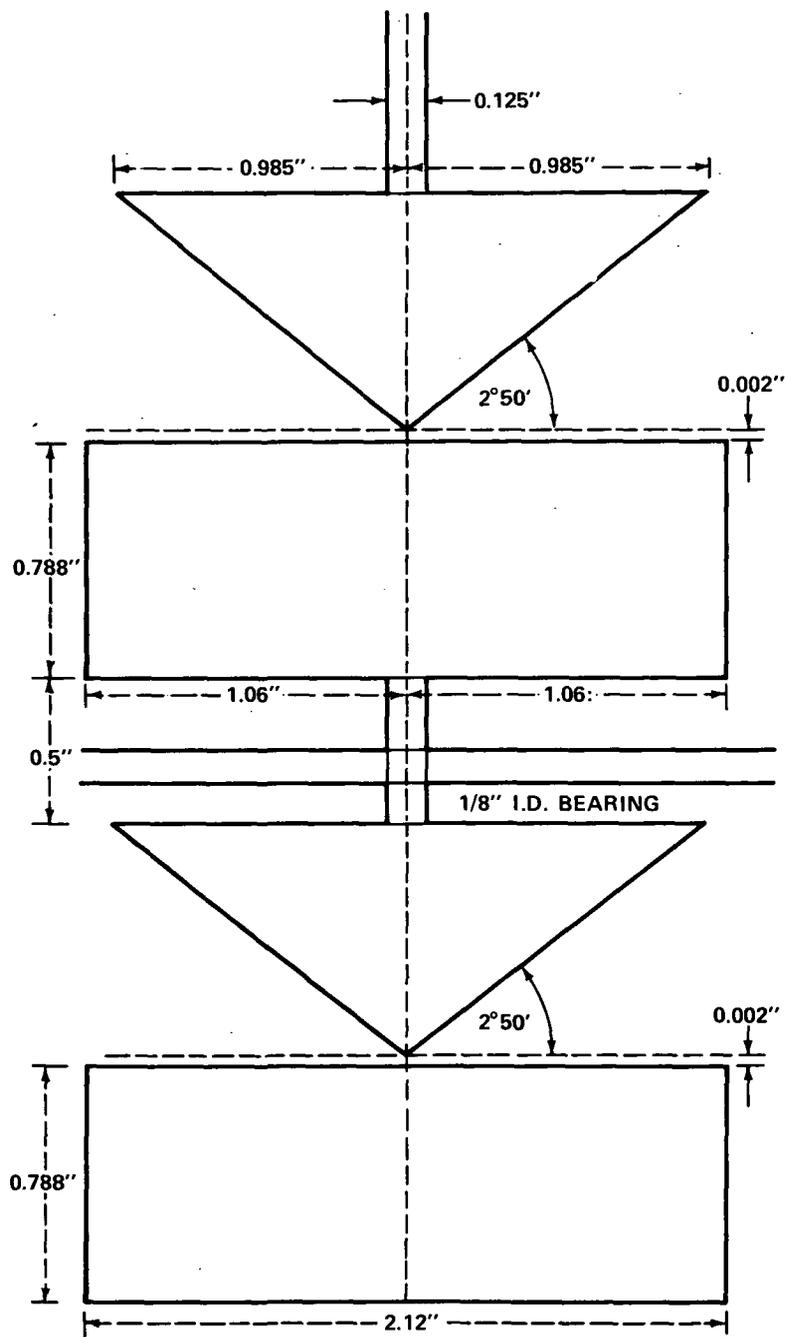


Figure 3-10. Device Specifications

SECTION 4

HANDLING SYSTEM FOR IRIDIUM-192 SEEDS

Submitted by:

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Medical Advisors:

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HANDLING SYSTEM FOR IRIIDIUM-192 SEEDS

STATEMENT OF THE PROBLEM

The problem is to design a system for safe and easy handling of Iridium-192 seeds used to internally irradiate malignant growths. The overall design of the system should cover all aspects of the problem from calibration and sorting to administration of the seeds.

DESIGN CRITERIA

1. The device should afford maximum safety to the operator.
2. The seeds should enter the system directly from the container in which they were shipped from the reactor.
3. The calibration should be correct to within $\pm 5\%$.
4. The calibration should be displayed in such a way as to make reading of it quick and simple. This possibly could be a digital display.
5. The seeds should be sorted into three or four categories determined by their activity.
6. The seeds should be loaded into the magazine which will be adaptive to both permanent and temporary implanting techniques.
7. The system should be kept as simple as possible to reduce the need for maintenance.

The seeds which are to be handled are made of a fine iridium core which is encapsulated in two thin layers of stainless steel.

They are produced by inserting an iridium wire in a stainless steel tube; this tube is in turn inserted in a second. The tubes with the iridium core are then cut to the desired length. The optimal dimensions are shown in Figure 4-1.

The actual dimensions when measured with a micrometer were: length 0.124 in, diameter 0.020 in, with the length varying \pm a couple thousandths from seed to seed (Figure 4-2).

The handling of the seeds must be looked at in a few general procedures which must take place. First, the seeds must be introduced to the system.

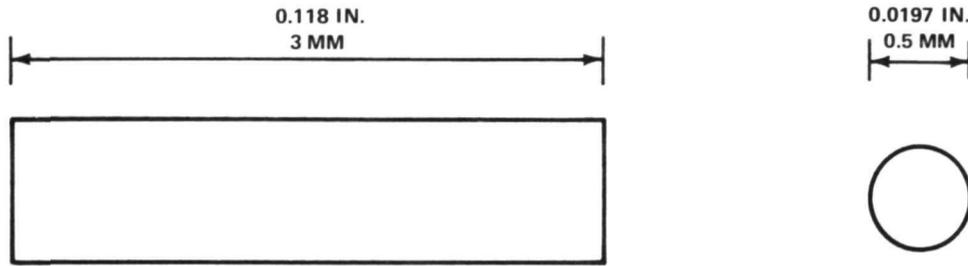


Figure 4-1

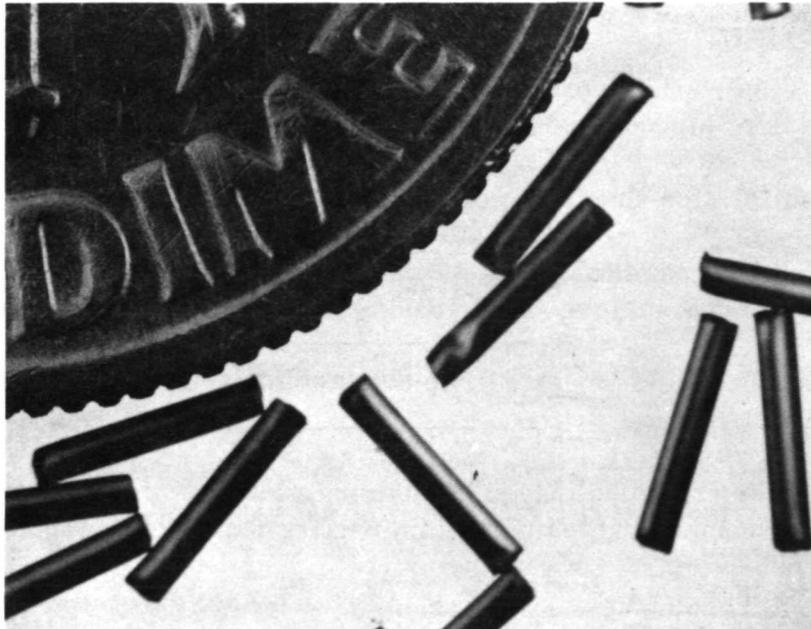


Figure 4-2. Perceptual Illustration of Seed Size

Then the seeds must be calibrated and sorted into groups by their activity. Then they must be placed in a magazine on some other type of storage arrangement which will facilitate easy usage of the seeds. Last, the seeds must be administered to the patient (Figure 4-3).

The first problem we encounter is getting the seed from the container as it arrives into the sorting system. The container is an aluminum cylinder of 1 inch diameter which is surrounded by approximately 1 1/2 inches of lead. (Figure 4-4).

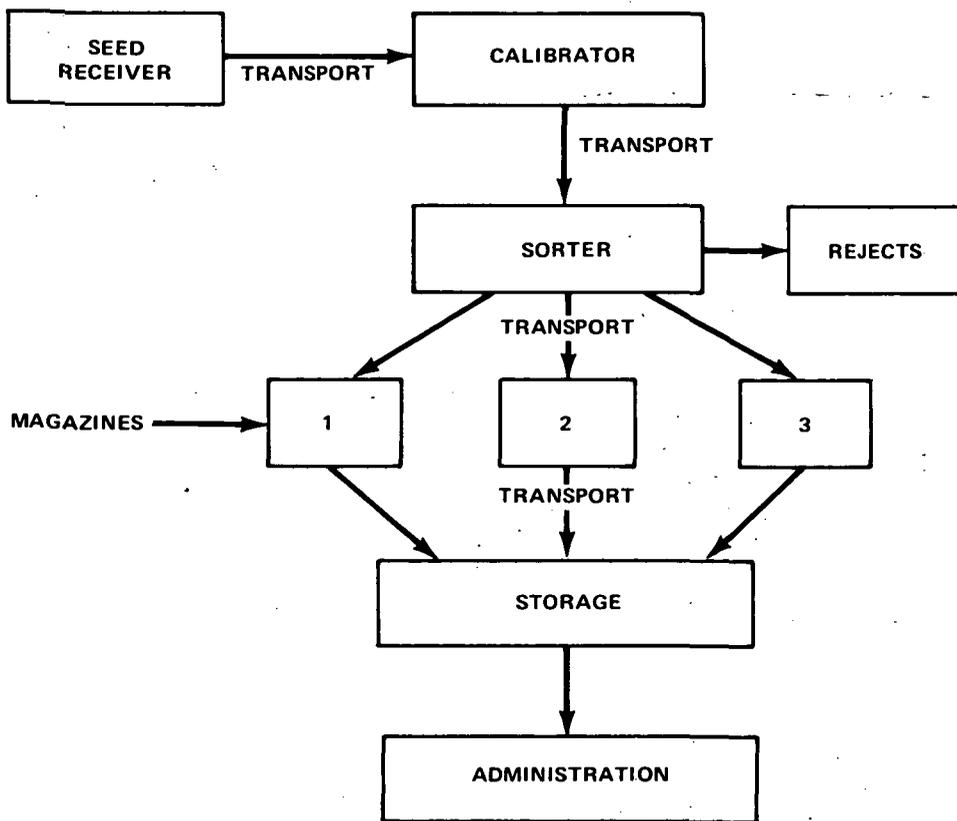


Figure 4-3. Flow Chart of Seed Handling

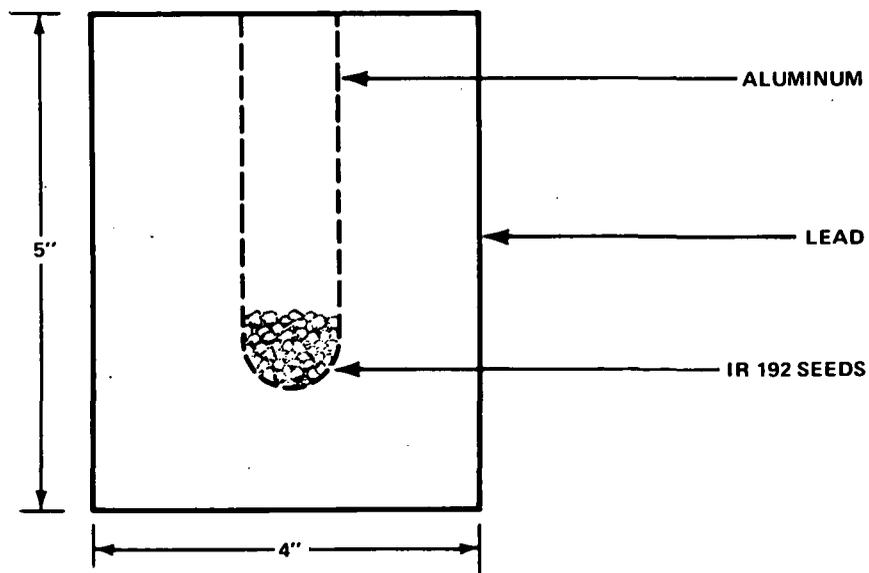


Figure 4-4

The seeds are activated in lots of 2,500. They must be fed one at a time into the calibrating device. This can be done by:

1. Taking the seeds individually from the container
2. Dumping all the seeds into a hopper from which they will be fed individually.

Taking the seeds individually from the container requires a probe to enter the container to the desired depth, pick up one seed, remove it from the container, and place it in or on some other transfer mechanism. One modification would be to have probe act as the transfer system—that is, it would hold on to the seed and transport it to the calibration and sorting stations before releasing it.

The motion of this probe may be achieved a number of ways:

1. Pneumatic or hydraulic cylinders
2. Solenoid
3. Gearing

The pick up of the seeds might be accomplished by:

1. Magnetism
2. Suction

Having the probe transport the seed after removing it from the container might be done by rotating it to the different locations.

This approach seems to have some major drawbacks. Magnetism is undesirable as a pickup mechanism because it leaves the seeds slightly magnetized and therefore causes problems with the stainless steel equipment which is used to implant them. Suction would work but would be hard to implement in a system which must move back and forth and possibly also rotate. Finally the movements required will be difficult to implement, the back and forth motion of the probe will have to change as the level of the seeds in the container recedes, and if the probe is to rotate to the different stations it will have to stop and locate the seed very accurately for calibration and sorting.

For these reasons we feel that it would be more advantageous to use a hopper to feed the seeds into a transporting system. This solution however is also not without problems. The minute size of the seeds will cause them to

jam in a regular gravity type feeder. Some provision must also be made to take the seeds one at a time as they are fed out. We hope to use a vibratory hopper to eliminate the jamming problem. A rotating wheel may be employed to control feeding the seeds individually—the seed will drop from the hopper into a hole large enough to accomodate only one seed, this hole will then rotate to place the seed in the transporting mechanism and then return for the next seed from the hopper.

Consideration is being made for transport of the seeds from one point to the other involving the following:

1. Pneumatic
2. Conveyor
3. Gravity
4. Wheel (disc)

The pneumatic system would probably consist of small air lines or pipes which would serve as a conduit for the seeds to be blown down. Small stops could be placed along the line to stop the seed at the desired spot so that the calibration say could be conducted. Also, a bi-stable fluid amplifier could be used to sort the seeds after calibrations. (Figure 4-5).

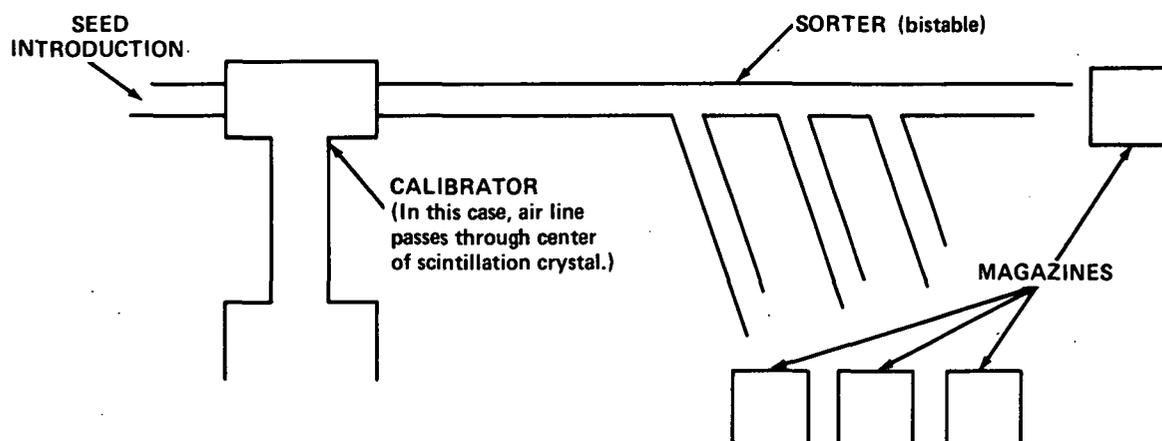


Figure 4-5

The conveyor system would consist of a small conveyor belt with niches or grooves to hold the seeds as the belt moved from one location to another. The

belt would index so that one seed would be placed under the crystal one at a time, and the belt would empty the seeds into different hoppers depending on activity. Some means of placing the seeds on the belt would have to be devised (Figure 4-6). Some way would also have to be devised to index the belt to the correct spot near the calibrator every time.

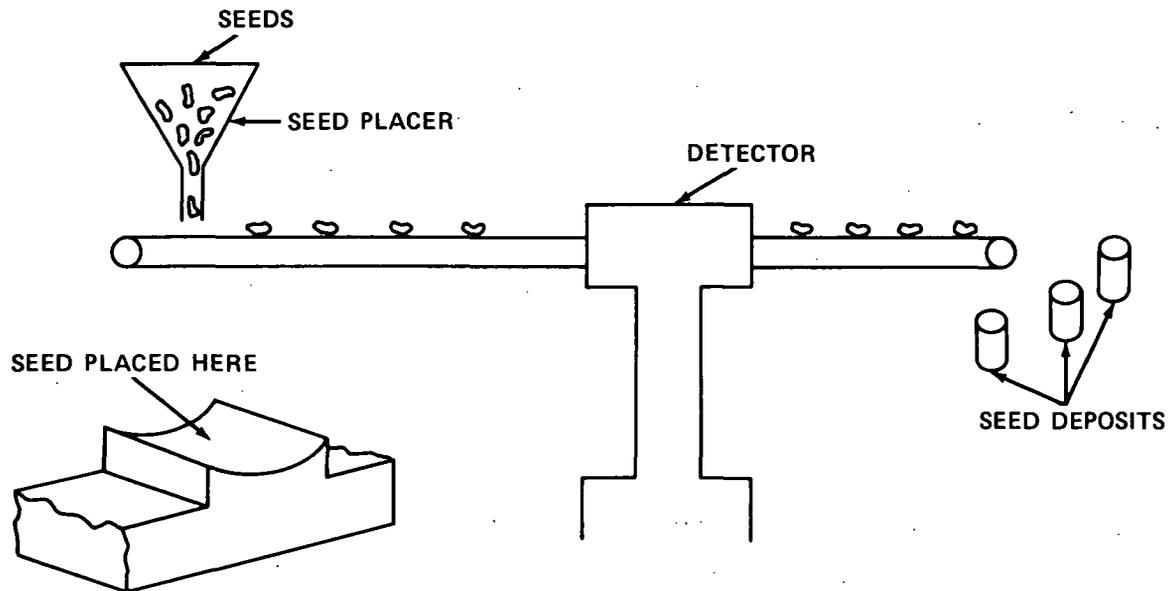


Figure 4-6. Idea of Belt

The gravity feed transport system would be a system of shoots and funnels which would at approximate levels stop the seed and perform the necessary function. The stops could be small traps released by solenoids which would be controlled by personnel. One trouble might be in getting one seed to fall at a time. The system might also be prone to jamming which would require a great deal of troubleshooting the device while active seeds are still contained in it. The sorting process might also be a problem in that trying to get the seed to fall into a selected hole might prove to take a great deal of precision both in building and aligning the system (Figure 4-7).

The wheel transport system would employ a large diameter disc with a hole about the same size as the seed, drilled through it. The seed would then be placed in this hole and the wheel would index to the next station which would be the calibrator. After calibration the wheel would move on to the location at which the seed will be placed into the magazines or storage area. The hole would then return and another seed would be placed in it. This mechanism would facilitate quick and easy handling of the seed. Problems might arise in the indexing

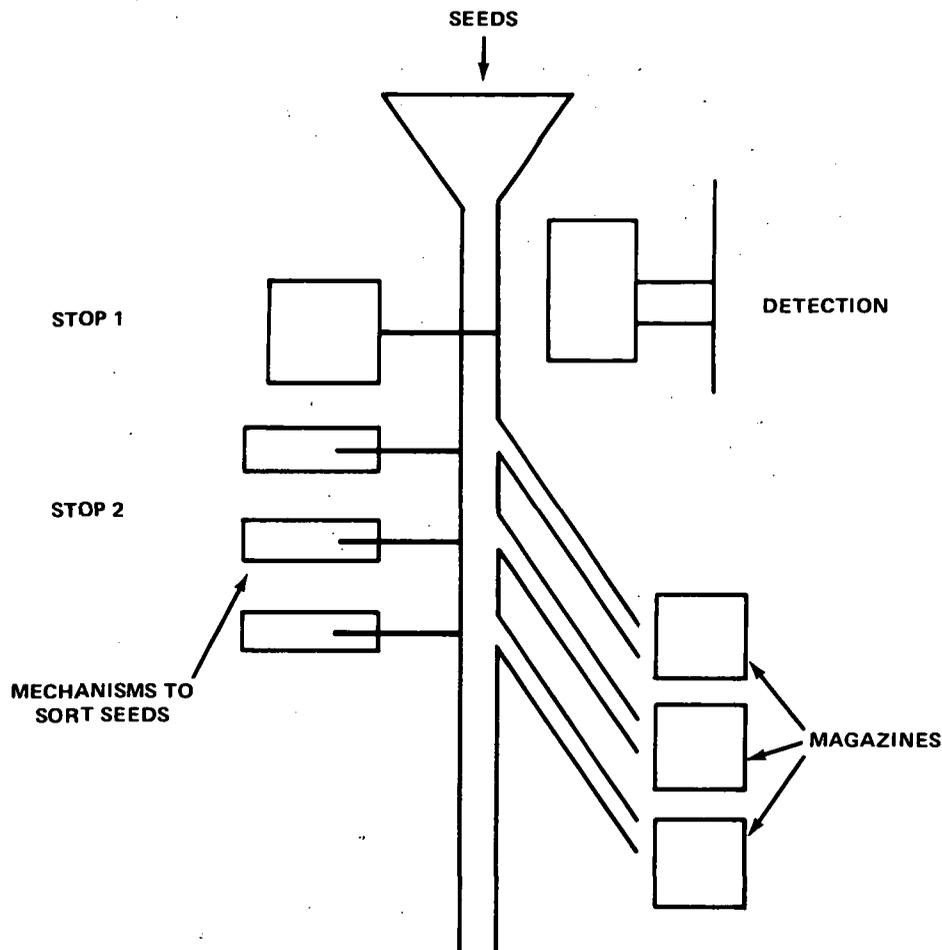


Figure 4-7

of the wheel, and the placement of the seed into the hole. A driving force and gearing would have to be considered. The receptacle for the seed after it leaves the wheel will have to be indexed or changed in position. This indexing of the magazines must be done only when a seed has been placed in it and therefore it must be a selective process (Figure 4-8).

Many designs are being considered for the magazine. It is our intention at this point to make the magazine out of some type of radioactive resistant plastic, because of the easy handling, cheap cost, and flexible properties. Three ideas have come up at this time (Figure 4-9).

This design would facilitate a mechanism which would require a flexible magazine. The seeds would be held in place by the tight fit of the tubes. The

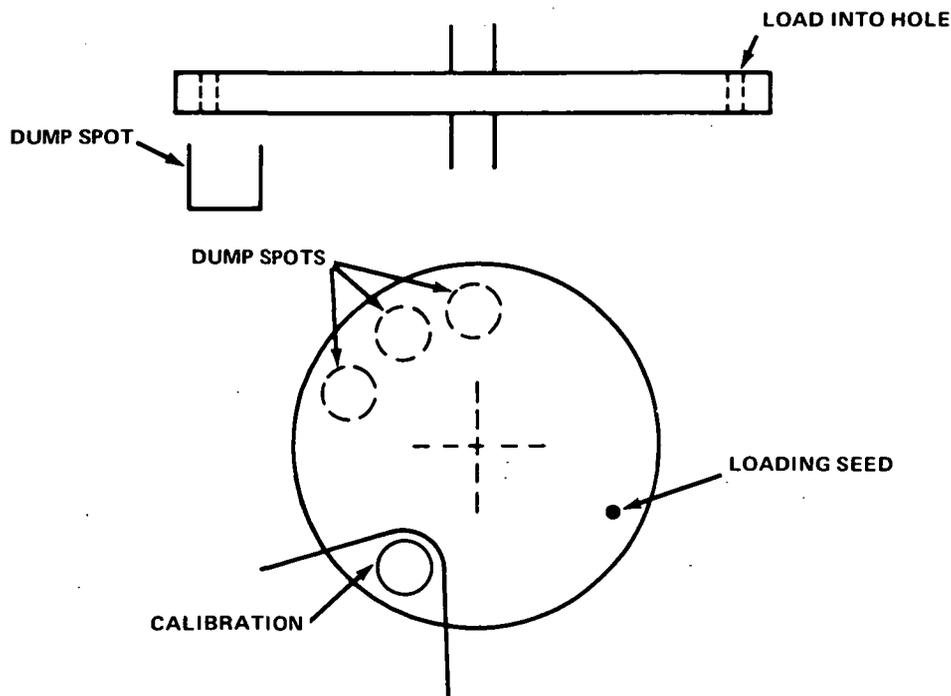


Figure 4-8

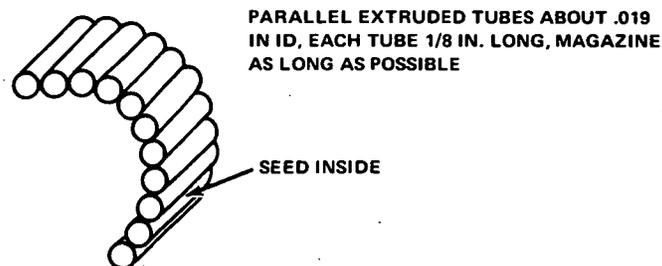


Figure 4-9

magazine could be indexed by some type of gear or sprocket whose track would conform to the shape of the outside of the magazine and which would be indexable by the required distance. The magazine could also be coiled up for easy storage inside a suitable lead container (Figure 4-10). This magazine would consist of a rigid bar of plastic (polypropylene or polyethylene or some other reduction resistant material) with holes drilled into it for the seeds to be placed in. This again would be a force fit. A track would be machined below the holes to serve as an indexing guide. The bar would be as long as possible. (Figure 4-11).

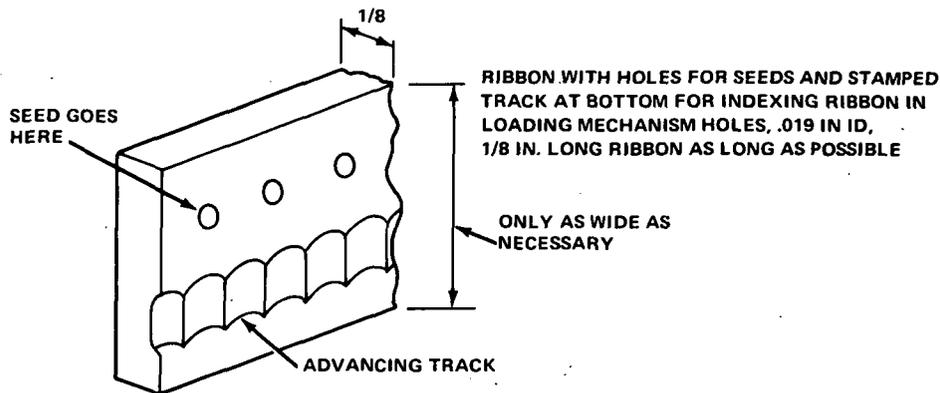
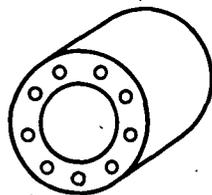


Figure 4-10



A SMALL SEGMENT OF TUBING WITH THE HOLES EXTRUDED IN THE PERIMETER OF THE TUBE. HOLES ARE .019 IN ID. TUBES ARE 1" ID 1/8 IN. LONG.

Figure 4-11

The design of this magazine consists of a segment of plastic tubing with holes in the walls to accommodate the seeds. This possibly could be accomplished by extrusion. The design will insure a small sized magazine for easy storage. Indexing could be accomplished by using a ratchet mechanism to index a shaft on which the tube is placed. The design might call for an expensive die as would the others.

CALIBRATION

The calibration of the seeds will probably be done with a scintillation crystal. It is necessary to place the source in the same point with respect to the crystal everytime so as to get an accurate reading. Therefore, the crystal will have a limiting factor on the transport system which will be used. One idea is to have the seed pass through the center of the crystal by means of a hole bored through it. This would make the effect of position have less effect on the outcome. Problems would be encountered in passing the seed through this hole. As long as the seed is near the crystal, and they all are approximately in the same position when they are counted, then a pretty accurate count can be taken.

We have checked the reliability of various counter systems using the scintillation crystal photo-tube scalar counter scheme. One crystal was obtained from Howard University; the other from GSFC. Different scalars were used for counting. The optimum voltage on the photo-tube contained in the crystal from Howard was also determined by finding that voltage at which the most counts can be obtained. The experiments (data) follow as well as the calculations.

Test #1

Determination of Optimum Voltage for Crystal
From Howard University

VOLTS	COUNTS/MINUTE
1350	301
1400	3882
1450	10398
1500	21919
1550	104461
1600	103504
1650	105635
1700	104648
1750	102645
1800	99283
1850	95992

From this data it was determined that the crystal from Howard worked most efficiently at about 1650 volts. Therefore all tests with that crystal will be run at 1650 volts.

The next test will be to check different systems of crystal and counter and then proceed with that system which is the most consistent.

Test #2

Using GSFC crystal with Ludlum scalar.

Using a .741 BA - 133 source and a lead collimator (6 sec. count)

<u>Trial #</u>	<u>Count</u>	<u>Trial #</u>	<u>Count</u>	<u>Trial #</u>	<u>Count</u>
1	392	4	413	7	427
2	423	5	426	8	404
3	434	6	422	9	417

Test #2 (Continued)

<u>Trial #</u>	<u>Count</u>	<u>Trial #</u>	<u>Count</u>	<u>Trial #</u>	<u>Count</u>
10	423	21	420	31	401
11	414	22	412	32	390
12	396	23	438	33	393
13	405	24	375	34	410
14	445	25	404	35	390
15	423	26	422	36	437
16	394	27	425	37	403
17	394	28	398	38	391
18	401	29	426	39	434
19	420	30	395	40	399
20	398				

$$\bar{X} = \frac{\sum X_i}{n} = 410.8$$

$$S^2 = \frac{n \cdot \sum X_i^2 - (\sum X_i)^2}{n(n-1)}$$

$$S^2 = 272.028$$

$$S = 16.493$$

1 deviation from the X $\frac{16.493}{410.8} = .04$ or 4% error

Therefore this configuration was at an error of about 4%.

Test #3

Using the crystal from Howard with a nuclear electronic scalar 1650 Volts, 6 sec. count. Source - Ir-192 seed.

<u>Trial</u>	<u>Count</u>	<u>Trial</u>	<u>Count</u>	<u>Trial</u>	<u>Count</u>
1	14807	13	14663	25	14713
2	14728	14	14936	26	14964
3	14729	15	14876	27	15121
4	14926	16	15019	28	15049
5	14719	17	14814	29	14945
6	14808	18	14646	30	14724
7	14889	19	14756	31	14881
8	14697	20	14796	32	14614
9	14855	21	14806	33	14672
10	14746	22	14919	34	14835
11	14737	23	14018	35	14919
12	14912	24	14034		

$$\bar{X} = 14836.3$$

$$S^2 = \frac{n \cdot \sum X_i^2 - (X \sum_i^2)}{n(n-1)} = 15596.6$$

$$S = 124.8$$

$$1 \text{ deviation from } \bar{X} = \frac{124.8}{14806} = /--84 = .84\%$$

Therefore this set up has an accuracy of about .84%

It will probably be necessary for this set up to have a standard source run through it, so that the accuracy of the instrument can be checked periodically. Slight changes in voltage and other factors might vary the readings. The standard would therefore be used to readjust intervals of counts corresponding to the 3 categories of seed activity.

The scintillation crystal is probably the best detector mainly for its sensitivity. Dozigation chambers and devices that work on that principle are not

nearly as accurate as the crystal and would probably not afford the $\pm 5\%$ tolerance specified in the design criteria.

Another way in which a magazine could be made is with a heat seeding process. If the seeds could be sandwiched one by one between two strips of a thermoplastic material, then a hot cog can be used to heat-seal the seed into place and make a machine-gun-belt affair. (Figure 4-12).

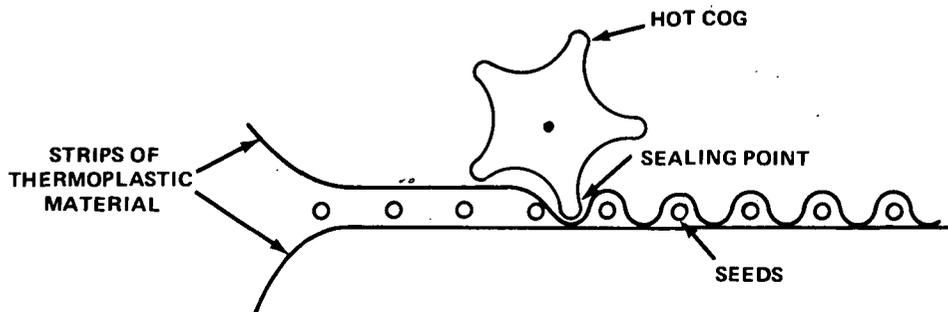


Figure 4-12

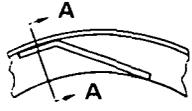
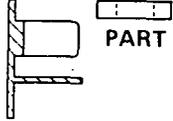
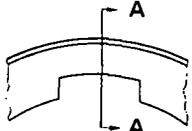
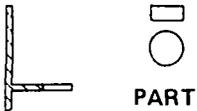
The method might not be exact as far as placement of the seeds, but it would be simple and cheap. It might be difficult to place the seeds between the strips and to pick them out again when they are needed.

VIBRATORY FEEDER FOR SEEDS

A bowl type vibratory parts feeder is the accepted way of handling small parts. It consists of a bowl which has an inclined track on its inner wall. The bowl is caused to vibrate in a helical motion by an electromagnetically powered drive unit. A rectifying element in the control circuit changes 60 cycle AC into pulsating DC which energizes the electromagnetic drive. A rheostat is used to control the rate of parts movement up the spiral track.

Control devices are also implemented along the track itself for orienting the parts properly. To feed parts oriented single file lengthwise a wiper and a cut out can be used (Figure 4-13).

Making the cut out the appropriate size is difficult because of the small diameter of the seeds (.020"). The area remaining between the wall and the cut out should be only slightly wider than this diameter.

ORIENTING DEVICE	PLAN VIEW	CROSS-SECTION A-A	FUNCTION OF DEVICE
1. WIPER			Wipes off excess parts and those perpendicular to the spiral track.
5. CUTOUT			Produces the effect of a narrower track.

From Syntron Parts Feeders Catalog
 Bulletin 400571
 Syntron, FMC Corporation

Figure 4-13

After several attempts we have managed to produce a desirable cut out. The cut out works well enough that we realize the wiper will not be needed in our case to achieve the single file feeding order which we desire.

Our next problem is to feed the seeds into a tube for introduction into the rest of the system. This can be done by having the seeds enter a trough in the track which gradually deepens and finally becomes a subsurface tunnel large enough in diameter for just one seed at a time. A cut out would be placed at the opening of the tunnel so that seeds which tended to pile up here would fall back into the bowl. (Figures 4-14, 4-15, and 4-16).

Upon discussing our design with Frabrication, we were informed that making the tunnel and trench in the small diameter which we desired would not be practical.

As an alternate approach we decided to imbed a piece of tubing in the bowl to replace the tunnel which would have been machined into the bowl itself. (Figure 4-17).

Of all our ideas for transporting the seed in the sorting system, the rotating disk seems to be the most practical approach. The Pheumatic and conveyor belt systems are both more complicated to implement than the more basic wheel or gravity ideas. Since the wheel is to be used in any case to take the seeds individually from the feeding tube and since it affords accurate positioning of the seed for calibration and sorting it appears to be more favorable than gravity.

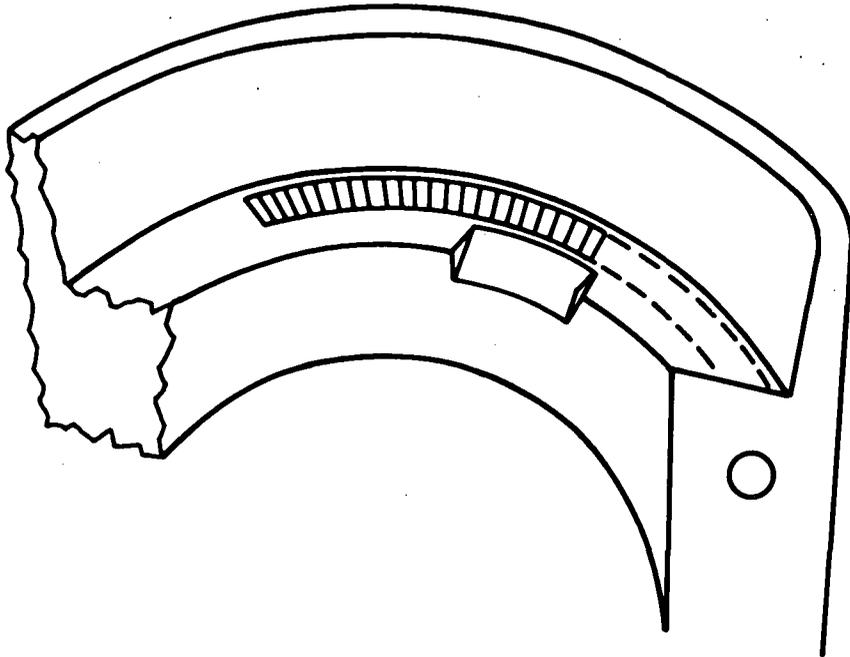


Figure 4-14. Tunnel For Feeding Seeds Out of Vibrator

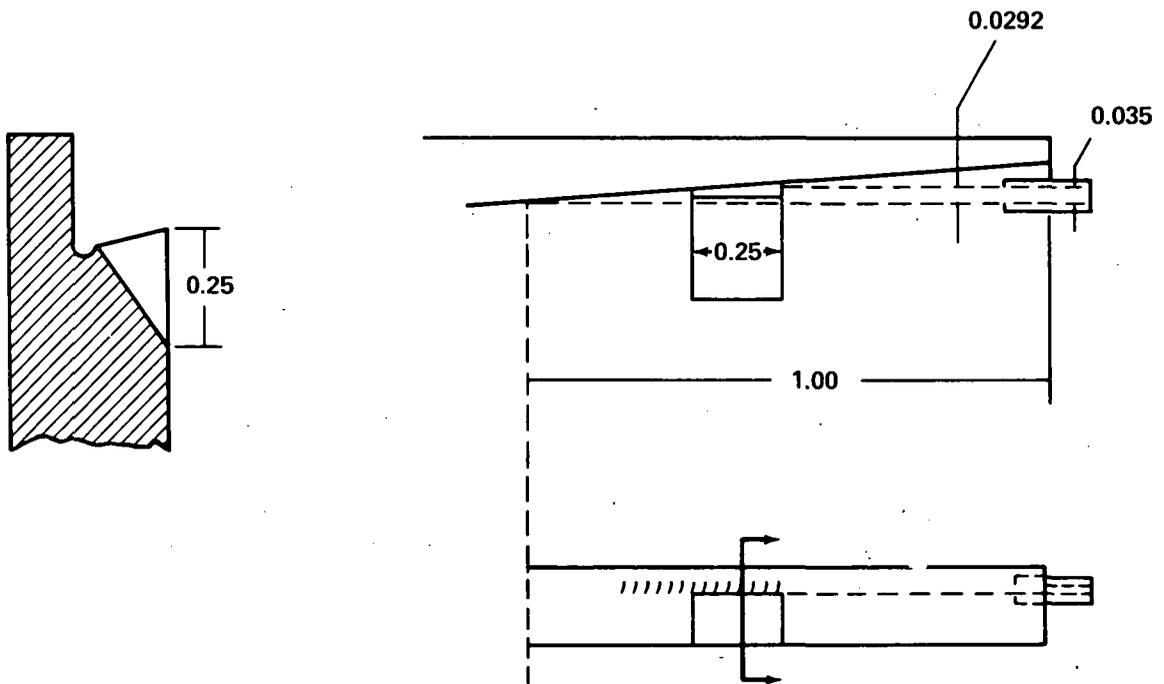


Figure 4-15. Tunnel Design

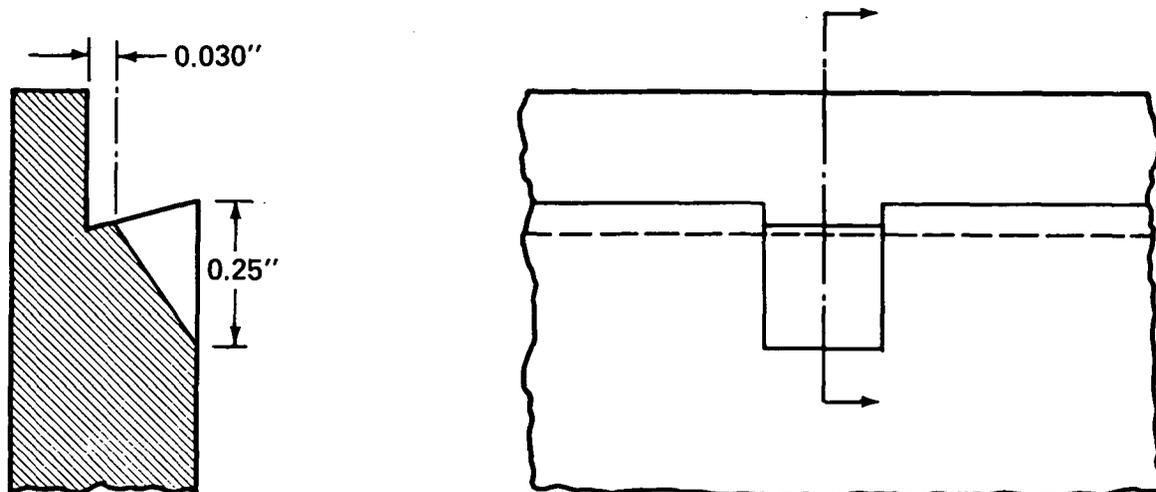


Figure 4-16. Cut Out of Tunnel

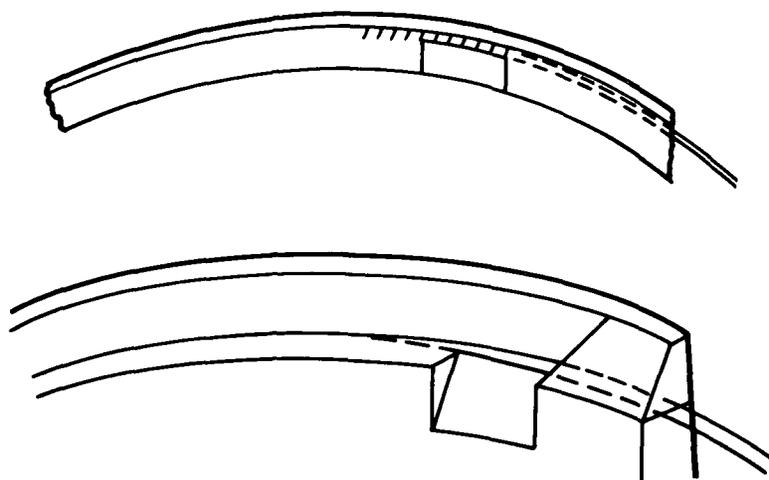


Figure 4-17. Vibratory Bowl With Imbedded Tube

The wheel may be turned manually through the use of a crank and some gearing, or it may be driven mechanically with the use of a small electric motor.

The movement off the wheel does not seem to be nearly as critical a problem as the stopping of the wheel at its different stages. The introduction of the seed from the tube, the placement of the seed under the scintilation crystal and the positioning of the seed for placement in a magazine, all require accuracy with tolerances of only a few thousandths.

For the cases of feeding the seed in the stopping under the scintillation crystal, we felt that a solenoid activated plunger could be used to stop the wheel at these locations every time the hole for the seed comes to these locations. The plunger would have a tapered end which would be forced into a wedge-shaped slot in the disk. Thus once the plunger enters the slot it will be pushed by the spring to the bottom of the slot, accurately positioning the wheel. It would then be released by activating the solenoid to withdraw the plunger from the slot.

There will be from two to four different locations at which the wheel should stop for sorting, but for each revolution it should stop at only one of these locations (as determined by its activity) and bypass the others. (Figure 4-18).

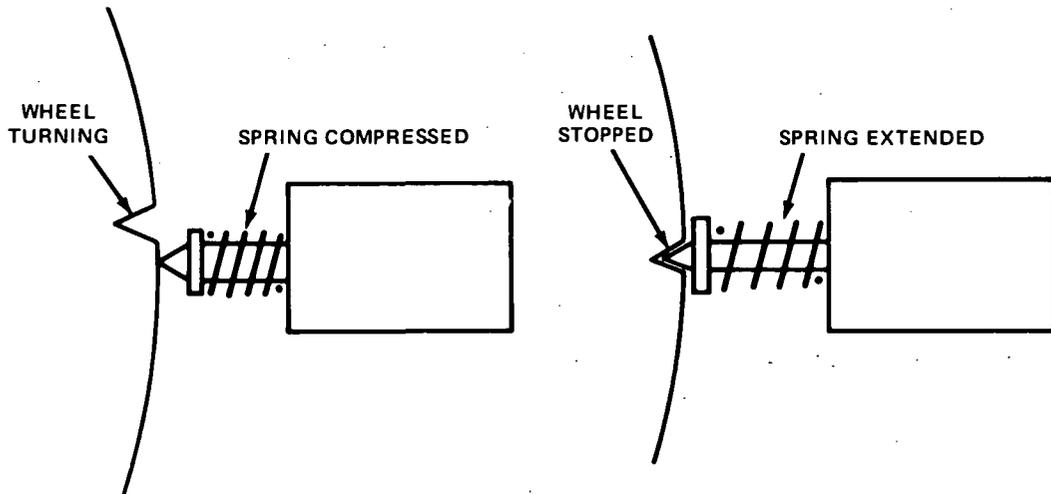


Figure 4-18

For this operation we have proposed a gear chain mechanism for each unloading location which will both index the magazine into which the seed is to be placed and stop the disk at the appropriate position. The gears at a particular location will be engaged only when the location is selected by the operator as the stopping point.

The engaging mechanism would consist of a pinion shaft which is controlled by a solenoid. With the solenoid on, the pinion would be pushed into contact with the driving gear which is concentric to and rotating at the same speed as the disk.

Stopping of the disk is accomplished when arms which protrude from the pinion shaft are pulled into slots in the shaft collar. These slots will be placed for accurate positioning of wheel and magazine (Figures 4-19 and 4-20).

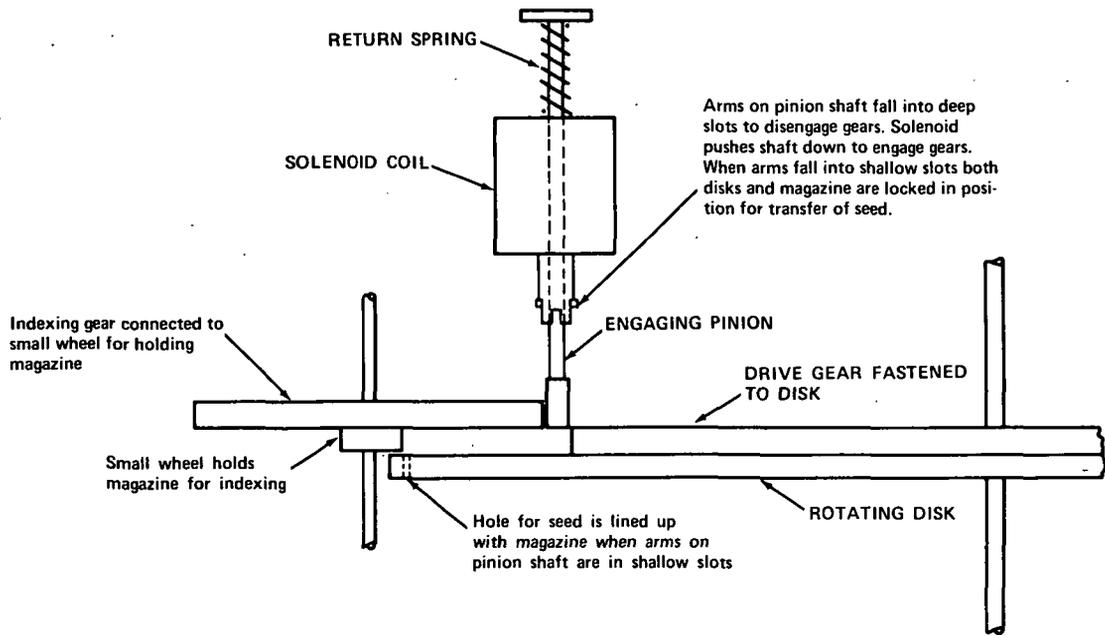


Figure 4-19. Mechanism for Indexing and Stopping at Appropriate Magazine

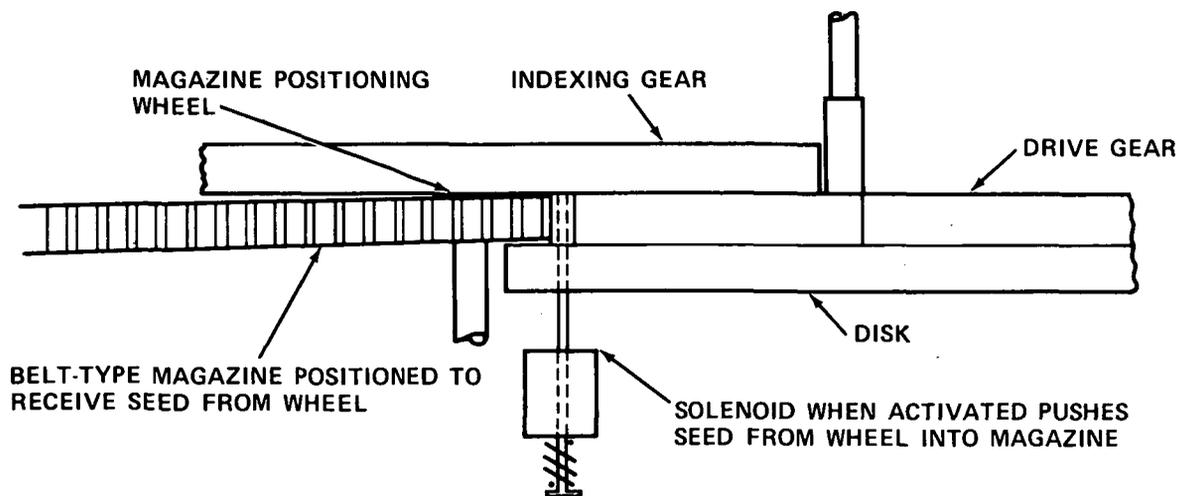
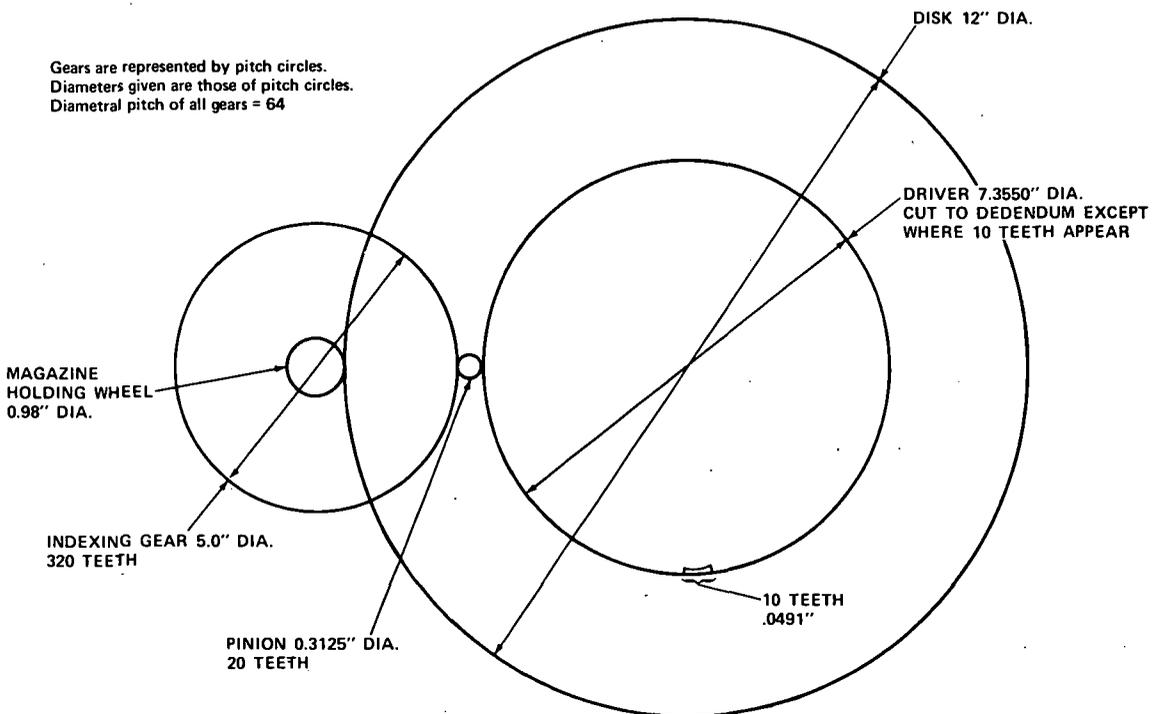


Figure 4-20. Use of Solenoid to Push Seed From Disk to Magazine

The gears should be made of nylon or a similar plastic material that will allow slippage along the faces of the gear teeth as the pinion is moved up and down without generating excessive heat or wear on the gear tooth faces.

The driving gear will have teeth on only a small portion of its circumference. At the appropriate location, the pinion will be pushed downward by activating the solenoid as the teeth on the driver approach. Engagement will occur and the driver will turn through half the distance which the teeth occupy, during which time the power to the solenoid is shut off. This will correspond to a 90° rotation of the pinion. At this point the return spring will draw the arms on the pinion shaft into the shallow slots in the collar locking the magazine and wheel in place for the transfer of the seed. After the seed is transferred to the magazine by a solenoid activated pushrod mounted below the disk, the pinion solenoid is activated to release the arms from the slot; the solenoid is deactivated after the driver begins to rotate again. The driver turns through the second half of its active portion corresponding to another 90° turn of the pinion. At this time the arms of the pinion shaft are drawn into the deep slots on the collar clearing it from the driving gear (Figure 4-21).



THIS MECHANISM IS DESIGNED TO INDEX SEED BELT 0.10" IN CIRCUMFERENCE AT MAGAZINE HOLDING WHEEL

Figure 4-21. Indexing Gear Chain

As we expected the gears which we desire to use are not available commercially as stock items. We have also been informed that Goddard's Fabrication Shop has no gear cutting facilities. For these reasons we sought an alternate approach for indexing of the magazine.

The indexing might be accomplished with the use of a geneva mechanism, or stepping motors.

The geneva provides for indexing the magazine, but has no provision for stopping the wheel to discharge the seed.

Stepping motors would provide both accurate indexing and stopping but are extremely expensive (Figures 4-22 and 4-23).

Since there will be several thousand seeds in the proximity of the scintillation crystal, shielding will have to be provided so that their activity will not affect the measurement of the strength of the seed which is in the disk.

Lead may be used to surround the crystal except for a small opening through which the activity of the individual seeds may be measured. Since the activity will be concentrated in the vibratory bowl, a lead shield around it would also be advisable.

The half value layer of iridium 192 in lead is 2.5 mm (.0985 in). For 1 inch of shielding approximately 1/1000 of the radiation escapes or for Z inches approximately 1/1,000,000 of the energy will permeate the shielding.

A wall 2" thick around the crystal and another 1" thick between the vibratory bowl and crystal should alleviate the problem of undesirably high background counts.

Another 2 to 3 inches of lead should surround the entire system in order to afford protection to the operator (Figure 4-24 and 4-25).

Consideration of the fabrication of a magazine to be loaded by our present design has ended in the realization that the precision needed to make such a piece is not attainable. It seems that in machining and drilling plastic pieces it is impossible to be as accurate as is needed for our design. Therefore, all previous ideas for a plastic magazine are now out of the picture except for the heat sealed process. But that idea is also going to be hard to adapt because of very inaccurate spacing of the seeds in the magazine.

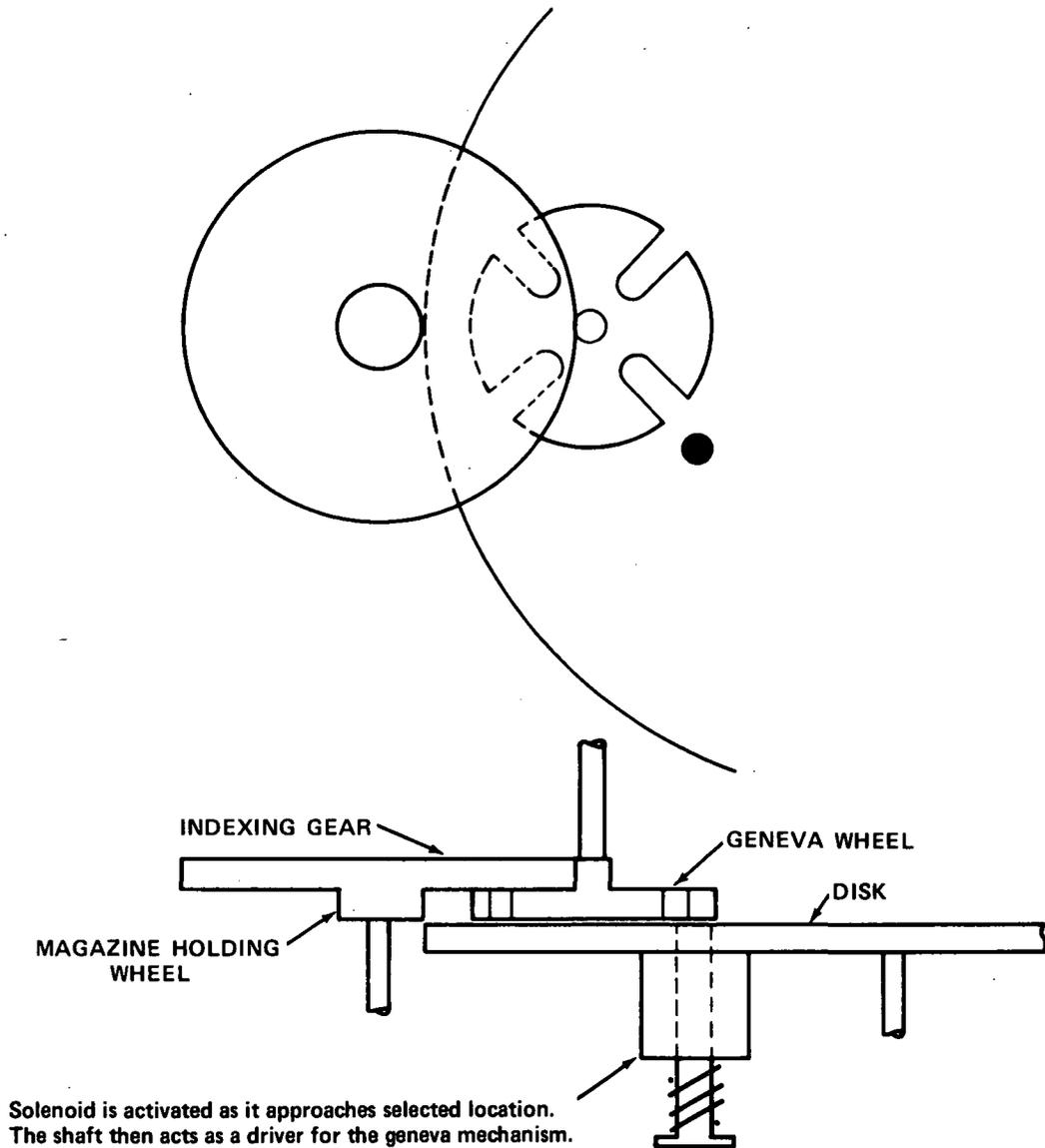


Figure 4-22. Geneva Mechanism for Indexing Magazine

In our present design a plastic (or at least flexible) magazine with very accurate machinery is necessary. Since this seems to be impossible to obtain, a great deal of modification seems necessary.

Modification to the design will be to delete all parts of the mechanism which have to do with loading a magazine. Therefore it will still consist of a vibrating bowl feeding a seed into a hole on the perimeter of a disc which will then rotate, placing the seed in the correct spot at the correct time. Instead of their pushing the seed into a magazine it will drop the seed into a container.

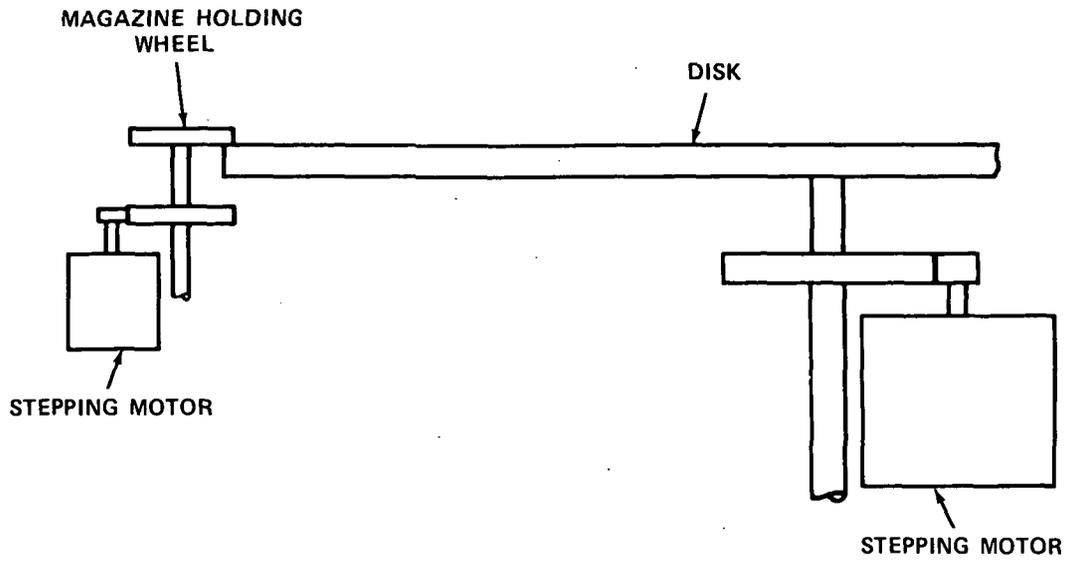


Figure 4-23. Stepping Motor Indexing and Stopping

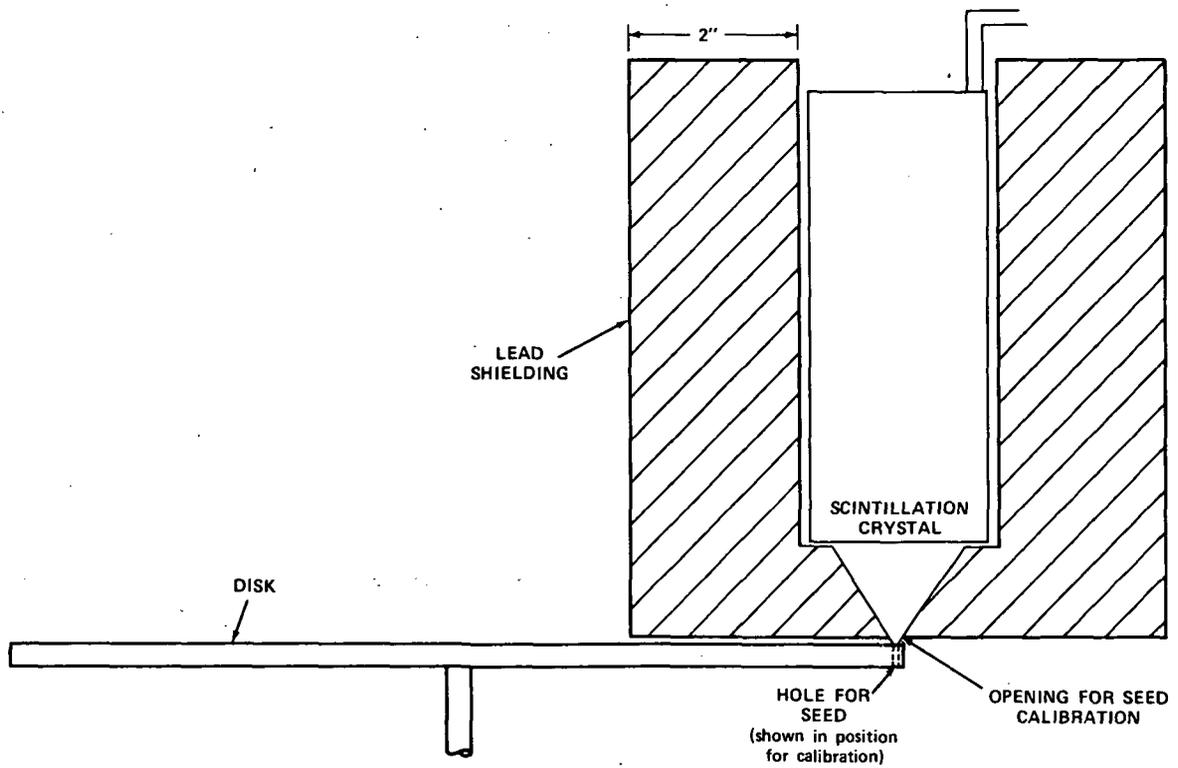


Figure 4-24. Shielding of Scintillation Crystal

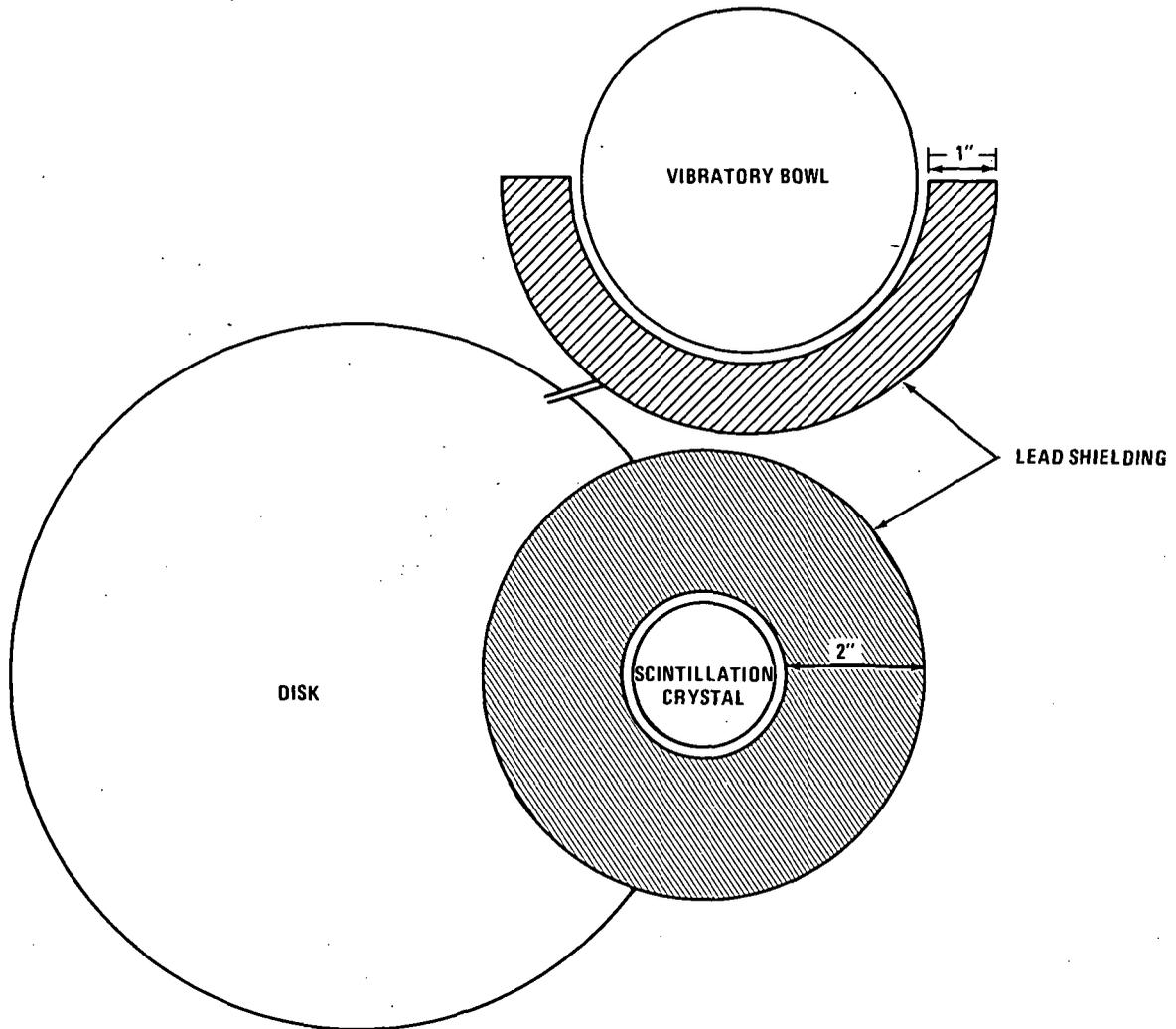


Figure 4-25. Shielding of Scintillation Crystal

Therefore this device uses the same basic ideas but leaves open the chance for adaption of a new way to load a magazine. The wheel would probably be driven by a synchronous motor which would have constant speed. The stops of the wheel would be controlled by contacts and micro switches located on the control ring. At a suitable point, a normally on-normally off, double pole micro switch will be located. A cam or raised area on the disc will activate the switch at the right time. The switch will cause a clutch to disengage the motor and will activate a solenoid whose plunger will penetrate a hole on the disc. The hole and plunger will be tapered to insure accurate placement. Thus the wheel will be stopped and placed accurately for placing a seed into the hole. After the seed is in place the operator will push a botton which will engage the motor and deactivate the solenoid so as to continue the motion of the disc once again. The

same process will again take place at the calibration spot so as to get an accurate placement of the seed under the crystal.

The solenoid which releases the seed into the receptacles is activated by contacts being made between a set of strips and brushes located at the correct spot around the wheel. When the operator has selected his choice of category for the seed, he presses a button down and the wheel continues on until it has reached the point at which the seed is to be dropped. The contacts and brushes touch, and the solenoid is activated releasing the seed into the container. The wheel continues to move on around until the loading point is again reached and the cycle repeats itself. The contacts and brushes will normally not be connected to the power supply. Only when that category for which they are used is picked will they be hot. In that way the solenoid will only be activated by the contacts and brushes pertaining to the category in which the seed belongs. The length of the contacts and the speed of the wheel will determine the duty cycle for the solenoid. Therefore it is necessary to adjust these perimeters so as to get the most accurate dropping of the seed (Figures 4-26, 4-27, 4-28, and 4-29).

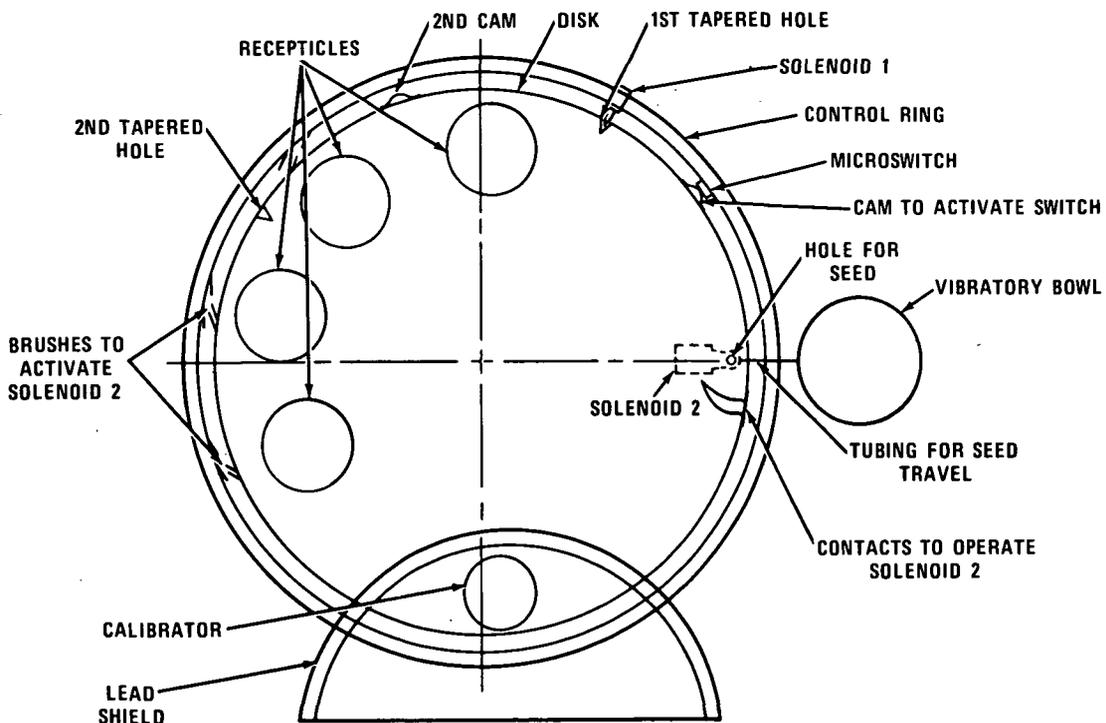


Figure 4-26. Basic Set-up for Control of Disk Movement

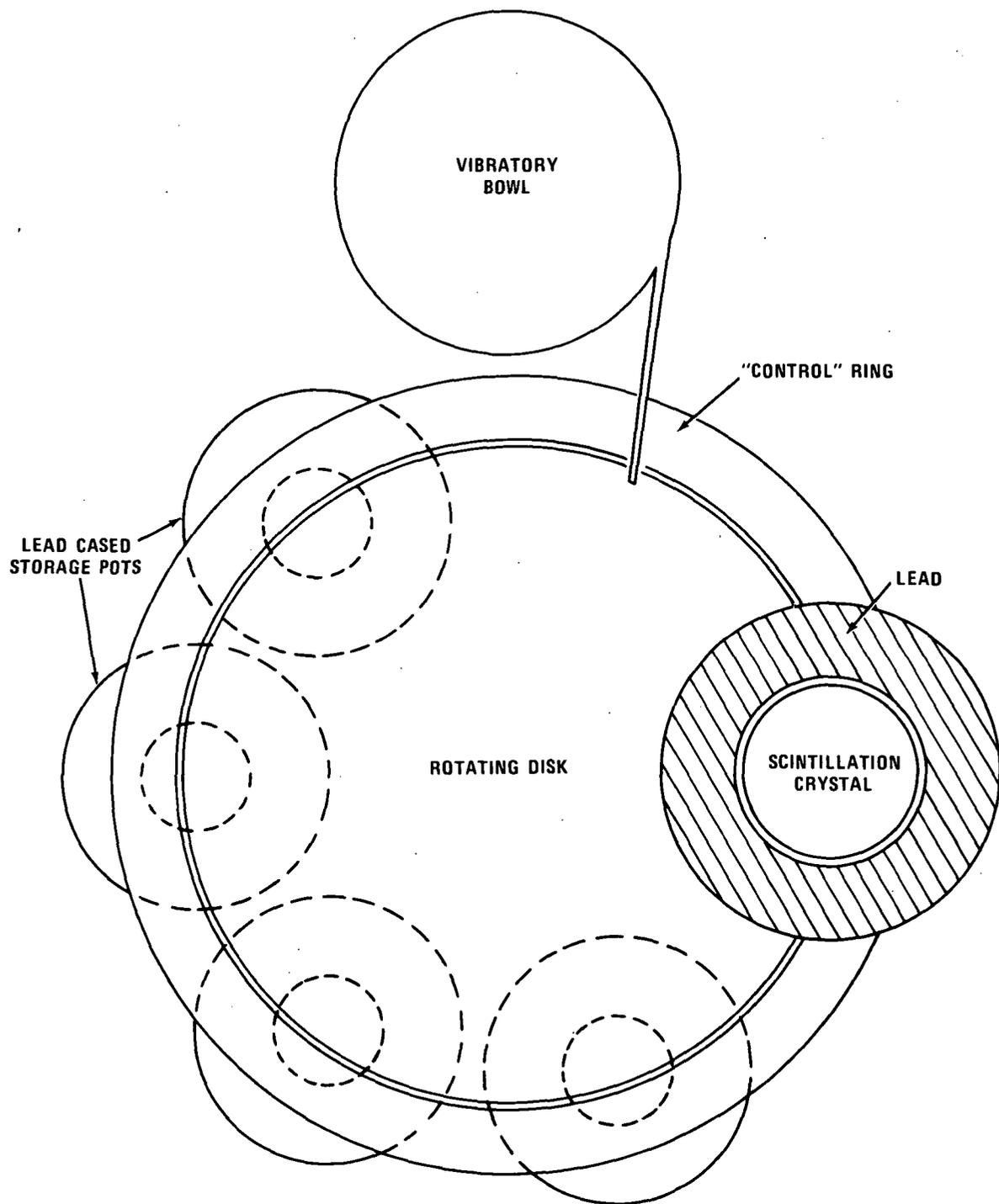


Figure 4-27. Iridium 192 Sorter

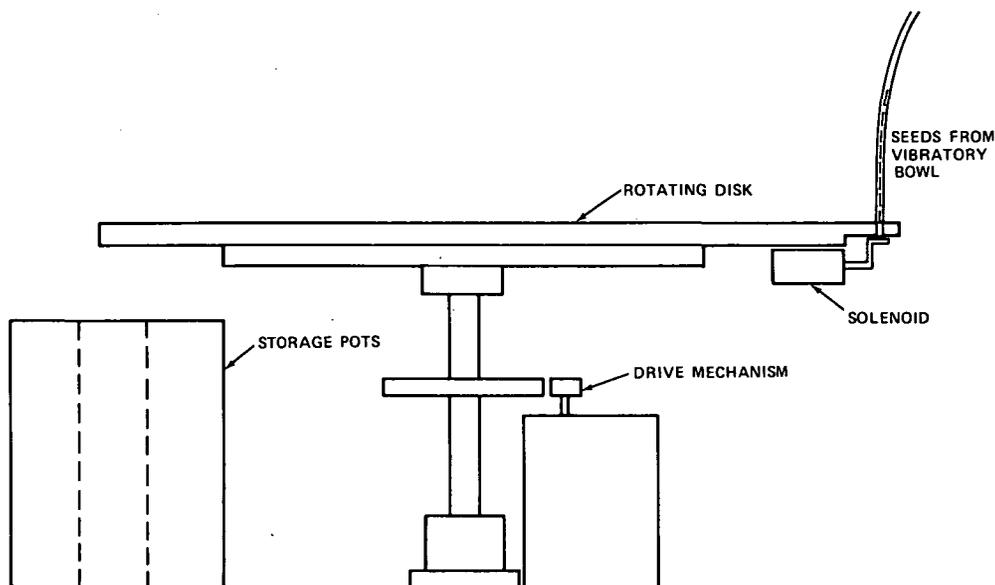


Figure 4-28. Iridium 192 Sorter

The idea has come to mind to activate the seeds after they have been placed in a magazine. This would eliminate a lot of the handling of the seeds while they are hot. It might also be possible to arrange them uniformly in this magazine so that all are exposed to the same flux in the reactor. If this is possible and if it is possible to make each seed contain the exact amount of iridium then calibration of each seed would not be necessary. Probably all that would be necessary would be a quick count of the whole magazine to make sure it is in the right range.

One problem with this would be that the magazine itself would be activated, therefore the material used to make this magazine would have to be carefully picked and must be very pure. Some alloys of aluminum would probably work, such as aluminum 1100. The impurities in this alloy seem to all have short lives with the exception of Mg which is not in high concentration.

One hang up with this idea is the fact that the seeds really can not be made as accurately as needed. If the seeds have different amounts of iridium and their different activities, the whole idea is worthless.

Since the idea of a continuous belt type of magazine cannot be produced to work in a precision sorting type of system, it may be better to consider a solid form of a magazine which will hold only a set amount of seeds.

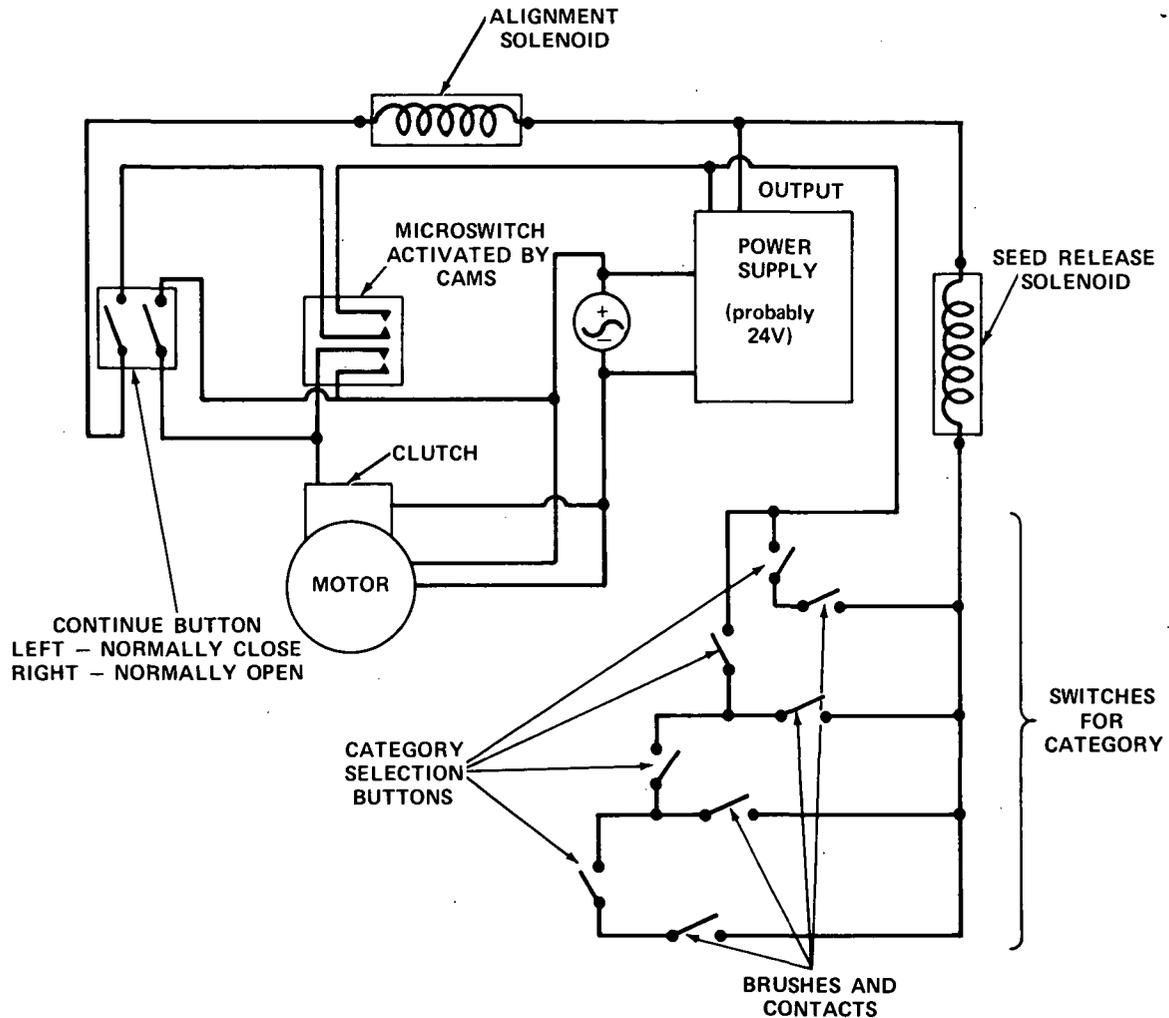
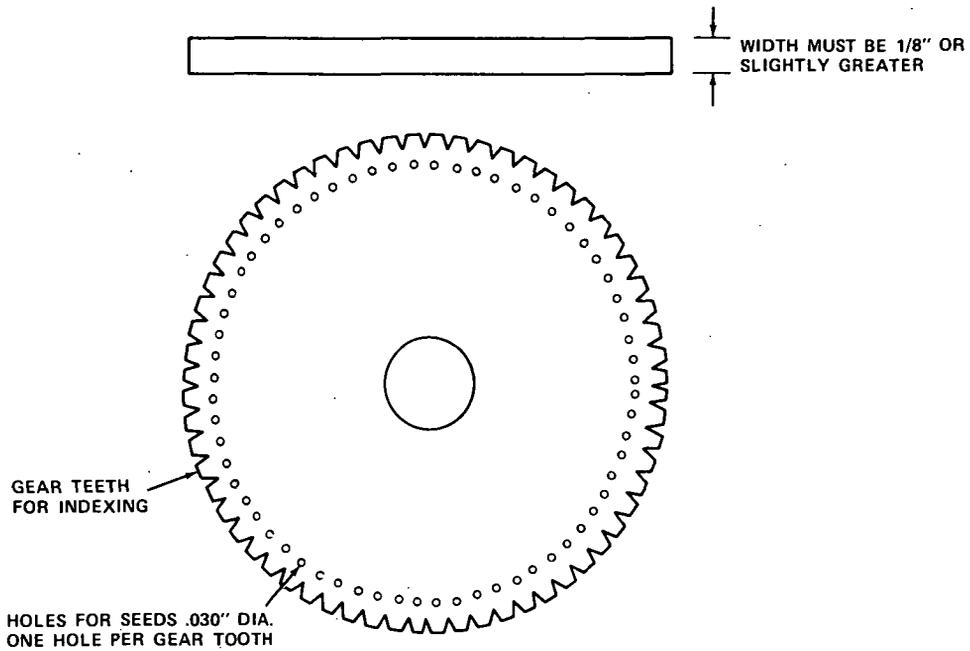


Figure 4-29. Switching Circuit for Disk Movement Control

A circular design with the seeds stored around the perimeter lends itself to loading and use in an implantation or ribbon loading device (Figure 4-30).

This could be made of either plastic or metal with gear teeth around its circumference for indexing purposes. Should this become a widely used item the plastic would provide a cheap disposable magazine while the metal would be a reusable product which also offers some shielding of the seeds' activity. The decision of which material would be more advantageous will be left until the basic idea itself is proven.

An experimental prototype may be made by drilling holes to accommodate the seeds in a commercially available gear of the appropriate dimensions.



Drawing is of 2" gear with 32 diametral pitch. It will accommodate 64 seeds. Using a finer pitch will allow a magazine holding an equal number of seeds to be of smaller diameter.

Example: 1-1/3" gear with 48 diametral pitch

Figure 4-30. Circular Seed Magazine

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PERSONAL CONTACTS

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Dr. David Mahan, Department of Radiotherapy, Howard University.

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Aaron Fisher, Materials Engineering, G.S.F.C.

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Dr. James Goft, Department of Packaging, Michigan State University.

Art Clark, Superior Electric Co., Sales Representative, College Park, Md.

COMPANIES WHICH SUPPLIED CATALOGUES AND LITERATURE ON PRODUCTS

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SECTION 5
INDIRECT BLOOD PRESSURE MEASURING DEVICE

Submitted by:

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Technical Advisors:

Mr. Leonard Kleinberg
Mr. Edward Twine

ORIGINAL PROBLEM STATEMENT

As paraphrased by:

Written by:

Dr. John Downing, M.D.

Eugene M. DeLoatch, Ph.D.

TITLE: AN IMPROVED NON-INVASIVE METHOD OF OBTAINING PULSATILE PRESSURE DATA FROM INFANTS AND CHILDREN

STATEMENT OF THE PROBLEM

The present blood pressure cuff serves some useful purpose in the measurement of systolic and diastolic pressure levels. This method, while practical in adults, exhibits drawbacks during pediatric usage. An improved method, which remains non-invasive, is desired in clinical pediatric settings.

SCOPE OF WORK

It is thought that with the increased interest in ultrasonic techniques that the correlation of this method of pressure recordings with invasive and cuff recordings is possible. The work here would include the best information available from NASA files on blood pressure monitoring with an accompanying necessary modification of devices (electrode positioning, size, etc.) to make the technique applicable to infants and children.

INTRODUCTION

Our task was the design of a non-invasive system for the continuous monitoring of pulsatile blood pressure in infants and small children. Blood pressure measurements are classified under two major headings—invasive (direct) and non-invasive (indirect). A non-invasive method is desired because although invasive methods are the most accurate means of measuring blood pressure, they require the insertion of a long tube called a catheter into the artery. After about twenty-four hours in the artery, the catheter becomes coated with blood and must be replaced. Other drawbacks of the direct techniques are the pain experienced by the patient, the fact that an operation must be performed by a physician under extreme sterile conditions, and the possibility of permanent damage being done to the catheterized artery. These factors prompted us to completely disregard all direct techniques.

In 1733 Stephen Hales, an English scientist, measured the mean blood pressure in an unanesthetized horse. He inserted one end of a glass tube into its artery and observed how high the level of the blood rose in it with respect to the

left ventricle of the horse's heart. Amazingly enough, this produced results accurate to within ± 10 mm Hg of those obtained by present-day techniques. This experiment marked the beginning of measuring a physiological parameter of paramount importance. Not only can malfunctions of the heart be detected through blood pressure measurements, but diseases of the blood-carrying ducts—arteriosclerosis and congenital cardiovascular anomalies to name a few—must be substantiated by an accurate blood pressure record. Moreover, pulmonary and endocrine disorders, as well as those of the nervous system, can manifest themselves in blood pressure variations, as can many other organic ailments. The most significant factors involved in determining blood pressure are (1) cardiac output; (2) peripheral vascular resistance; (3) volume of contained blood, (4) blood viscosity, and (5) elasticity of the arterial walls. Unfortunately, like most life science parameters, there is no way of quantitatively developing a mathematical model from these factors which would facilitate the calculation of blood pressure. Also, factors such as blood viscosity are more difficult to measure than blood pressure using present-day technology.

To begin the attack on our problem, we surveyed the state-of-the-art techniques to see if we could possibly modify them to meet our needs. We discovered that the most commonly used techniques can be placed into two categories: palpatory and auscultatory. Determination of blood pressure by the palpatory technique is as follows:

1. A pressure cuff is placed around the extremity and inflated to occlude the artery. Occlusion is determined by the doctor by placing his fingers on the skin over the artery and noting when he can no longer feel the pulse.
2. The pressure in the cuff is then decreased at the rate of about 2 mm Hg per heart beat.
3. The pressure at which the doctor feels the pulse return is taken as the systolic pressure.

When using this technique, there is no way of determining diastolic pressure.

Determination of blood pressure by the auscultatory technique is as follows:

1. A pressure cuff is placed around the extremity and inflated to occlude the artery.
2. A stethoscope is used to detect the appearance and disappearance of the Korotkoff sounds.

3. The pressure in the cuff is then decreased at the rate of about 2 mm Hg per heart beat.
4. The appearance of the Korotkoff sounds marks the systolic pressure. As the pressure is continually decreased, the sounds disappear. This pressure marks the diastolic level.

Both of these methods place a good deal of reliance upon human judgement. In addition, the auscultatory technique relies upon the detection of the Korotkoff sounds which are usually faint or indistinct in infants and small children. Also, ambient noise such as a baby crying would make it even more difficult to hear these sounds. Moreover, both methods require the use of a pressure cuff. The major disadvantage of this is the space utilized. To assure accuracy, the width of the cuff should be 20% greater than the diameter of the extremity where the pressure is to be measured(1). Another factor which is extremely applicable to small and weak infants and children is the extreme discomfort the cuff causes if pumped more than 30 mm Hg above systolic level.

After examining the shortcomings in the state-of-the-art, we determined some of the major engineering considerations in the development of an improved system to be:

1. Safety — system should cause no pain or deleterious effect upon patient.
2. Size — part of system which comes in physical contact with patient should be as small as possible.
3. Administration — should be easy to apply and use by any hospital technician.
4. Accuracy — results should correlate with direct methods to within ± 2 mm Hg.
5. Precision — the results should be repeatable.
6. Output — results should be easy to interpret, possibly in the form of a digital readout.
7. Storage of data — should be some provisions made for the permanent storage of the data, possibly using magnetic tape.
8. Automation — system should require little as possible interfacing with hospital personnel.

The crux of our problem then became one of determining some external transducible property which could be correlated with intra-arterial pressure. We decided upon skin fluctuations caused by pulsatile pressure in a vessel as the transducible property. We then experimented with two devices which were able to detect these movements and convert them to an electrical signal proportional to those displacements. The first such device consisted of a material called polyvinylfluoride (PVF) which can be processed to become piezoelectric. The device was rectangular, approximately 1/2" x 1/4", and of negligible thickness. A coaxial cable came from one side where the output was taken. The major advantages of the device were that it was self-generating and required no balancing network. Its major drawback was its extremely high output impedance (10^{12} ohms). In an attempt to overcome this problem, we used a matching network which consisted of a FET in a source follower configuration. This arrangement has no gain but does possess high input impedance and low output impedance. Unfortunately, the gain of this system was still too low and when we patched in an amplifier with 40 db gain, our signal-to-noise ratio became intolerable. We therefore abandoned this device for a strain gauge transducer.

With the strain gauge transducer, skin movements were detected by means of a cantilever device placed on the skin above an artery. The cantilever was a 10 mil strip of copper-beryllium (1" x 1/2") which served as the force-sensing element. At one end of the cantilever 3/16" from the edge, a 0.2" dia. aluminum shaft was centered. This shaft was 3/8" long and extended 1/16" past the housing for the cantilever. The knob was to make the beam move with the oscillations of the arterial wall. The beam was clamped at the other end to complete the cantilever design (see figure 5-1).

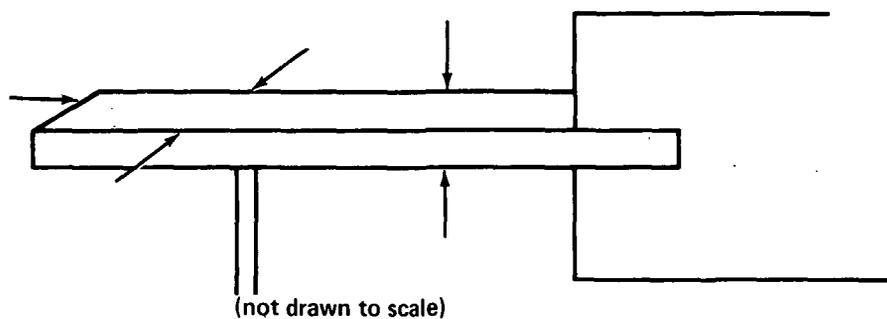


Figure 5-1

Since the maximum strain occurred at the point where the beam was clamped to the housing the strain gauges were mounted as close as possible to this point. The highest output voltage that can be obtained from a bridge containing only one strain gauge is $V_o = \frac{V_i \Delta R}{R}$ where V_o and V_i are the output and input voltages respectively and R is the resistance of the gauge. Therefore, four gauges were

employed in a full bridge configuration to give a maximum possible output of $V_i \Delta R_R$. The gauges used were Micro-Measurement constantan foil strain gauges, type EA-13-062 A-120. The gauges were operated through a Budd Strain Indicator. This produced a 1.1 VRMS, 1 KHZ square wave to energize the Wheatstone bridge. Increased sensitivity could have been obtained if the input voltage were increased. However, satisfactory resolution was obtained with the present voltage. Thermal effects were negligible for the gauges used for voltages up to 4 VRMS.

The design mentioned is not the only method nor is it the best design for pressure measurements. Other types of strain gauges could be used such as capacitive, inductive or semiconductor gauges which have greater sensitivity. The use of a diaphragm could be employed in place of a bending beam cantilever.

After having the device fabricated, our next task was to run some tests on it. Two tests were run, and the first was entitled, "The Dog Experiment" and is described as follows:

DOG EXPERIMENT

PURPOSE

The purpose of the experiment was to determine if we could get wave forms from the strain gauge transducer, which would be placed upon the skin above the artery, similar to those obtained using direct techniques.

EQUIPMENT

Surgical tools, anesthetizing agent, dual channel Sanborn strip chart recorder, Sanborn Pressure Transducer P2, Model 267AC (control), Sanborn carrier pre-amplifier, strain gauge transducer (experimental device), Budd Strain Indicator, #7 catheter.

PROCEDURE

A dog was shaved and anesthetized where the experimental transducer was to be placed. After this, the dog was placed on the surgical table with all legs strapped. Dr. Downing performed a femoral cutdown using the left femoral artery. This incision was for the purpose of inserting a catheter into the artery. The other end of the catheter was then connected to the Sanborn pressure transducer. To obtain accurate readings, the Sanborn transducer was positioned on the same level as the dog's chest. The output of this transducer was fed to one channel of the strip chart recorder via the carrier preamplifier. The strain gauge transducer, under test, was held manually on the skin above the right

femoral artery. The transducer's output was taken from a Budd Strain Indicator and fed a second channel on the Sanborn recorder, which permitted simultaneous recording of the left femoral artery pressure and the force developed above the right femoral artery, as indicated by the control and experimental devices.

CONCLUSION

Since at the time of the experiment we had no means of calibrating the strain gauge transducer, we could draw no quantitative conclusions from the results. Nevertheless, we were pleased to see that the two curves were similar in shape and that the phase difference between the two is small.

A sample of the data may be found in Figure 5-2.

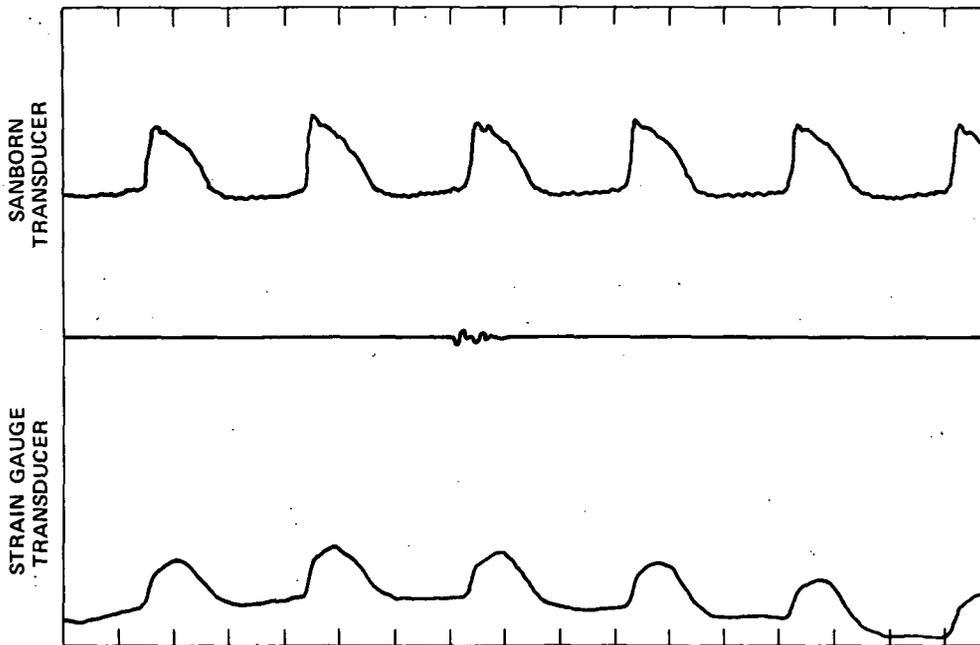


Figure 5-2. Sample Data From "Dog Experiment"

To be able to accurately reproduce a blood pressure curve, the transducer should have a frequency response of at least 0-50HZ. To check the response of our device we used a mechanical oscillator whose output was held constant at 0.1 VP-P and varied the frequency from 2.38 to 52.5 HZ. A graph of the response can be found in Figure 5-3.

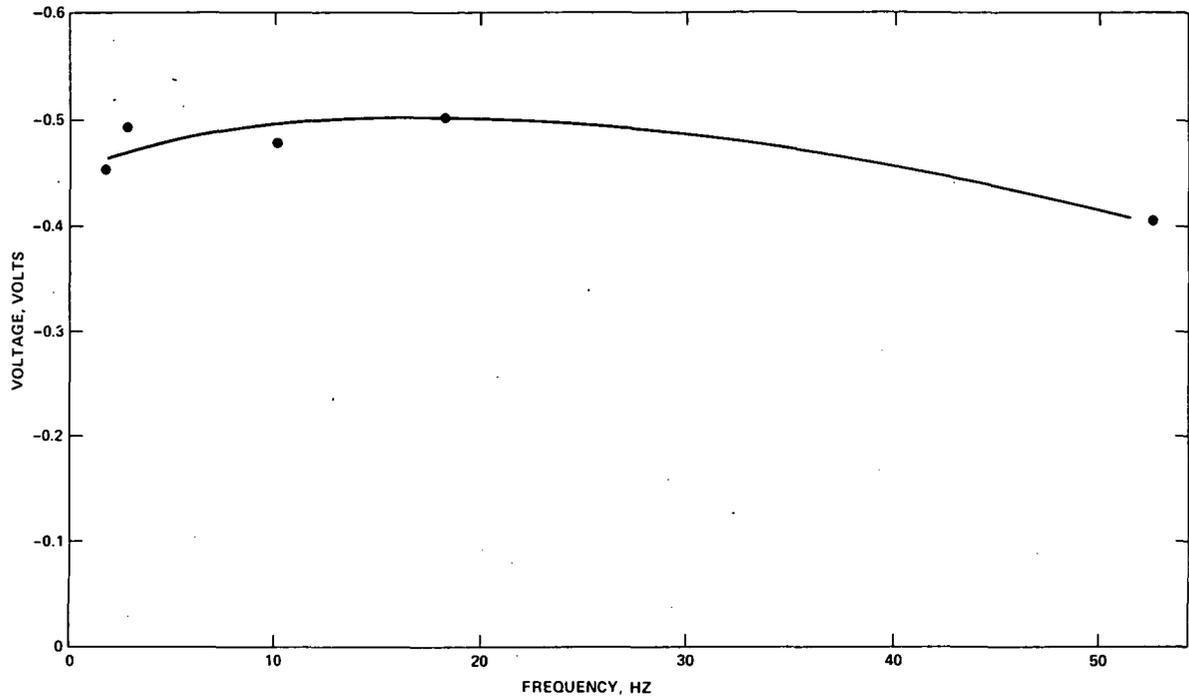


Figure 5-3. Frequency Response Curve of Strain Gauge Transducer

Although not absolutely necessary, linearity in a transducer is also desirable. This transducer yields a linear change in its resistance for variations in force applied to it from 0 to 90 grams.

A means of calibration is yet to be determined. It is hoped that calibration could be achieved by using known weights on the transducer and varying the static pressure in a 3/16" O.D. x 1/8" I.D. latex tubing that the transducer would rest upon. The tube would simulate the artery and a known liquid could flow through it to create the pressure. The amount of strain on the beam should then be dependent on the applied force holding the transducer and the pressure exerted by the tubing. Since the difference between the two forces acting on the device would cause the beam to deflect, the pressure should be able to be interpreted by the amount of strain.

In conclusion, there are still some problems that must be resolved before this system can be implemented. The major problems are calibration of the transducer and how to filter out extraneous signals such as movement artifacts caused by muscle movement. We feel that once these problems are cleared up, we have a device which will give an adequate measure of blood pressure using noninvasive techniques.

ADDENDUM TO THE REPORT (Submitted by: Clifton Cole, January, 1973)

On December 11, 1972 in the animal research facility at Howard University, a third dog experiment, with the same purpose in mind as the previous two, was attempted. This time we were fortunate to have a calibrated standard pressure transducer, but again we were not able to overcome some of the old problems from the past experiments. Specifically, we had no means of eliminating those motion artifacts caused by breathing and nervous tension. Also the mechanical design of the experimental transducers seems to be ill-suited for our purposes (see Figure 5-4). It is too bulky and the sensing knob does not project far enough below the housing of the device to permit it to be placed directly upon the artery.

As a means of developing a static calibration curve for the experimental device, known weights were placed on the device's sensing knob and the resultant strains were recorded. This resultant curve proved to be a check on the linearity of the device. The graph of this data can be found in Figure 5-5. For the same value of weights the peak output voltage was also recorded. The output voltage was then plotted vs. the force as depicted in Figure 5-6.

Due to the non-linear nature of this voltage-force curve, we must note the extreme importance of being knowledgeable of this particular curve. Since the pressure is translated to a force and then to a voltage, it would be preferable that linearity exist all the way through the system. Another possibility relative to the curve in Figure 5-6 is that more data along with some curve fitting scheme might yield a linear vs. non-linear fit.

Consideration was given for work on a model to yield some knowledge as to how the arterial pressure wave is altered as it is transmitted from the interior of the artery through the various layers of muscle and skin tissue to the outer surface of the skin where it is detected by the transducer. A Block diagram of this phenomenon is depicted below in Figure 5-7.

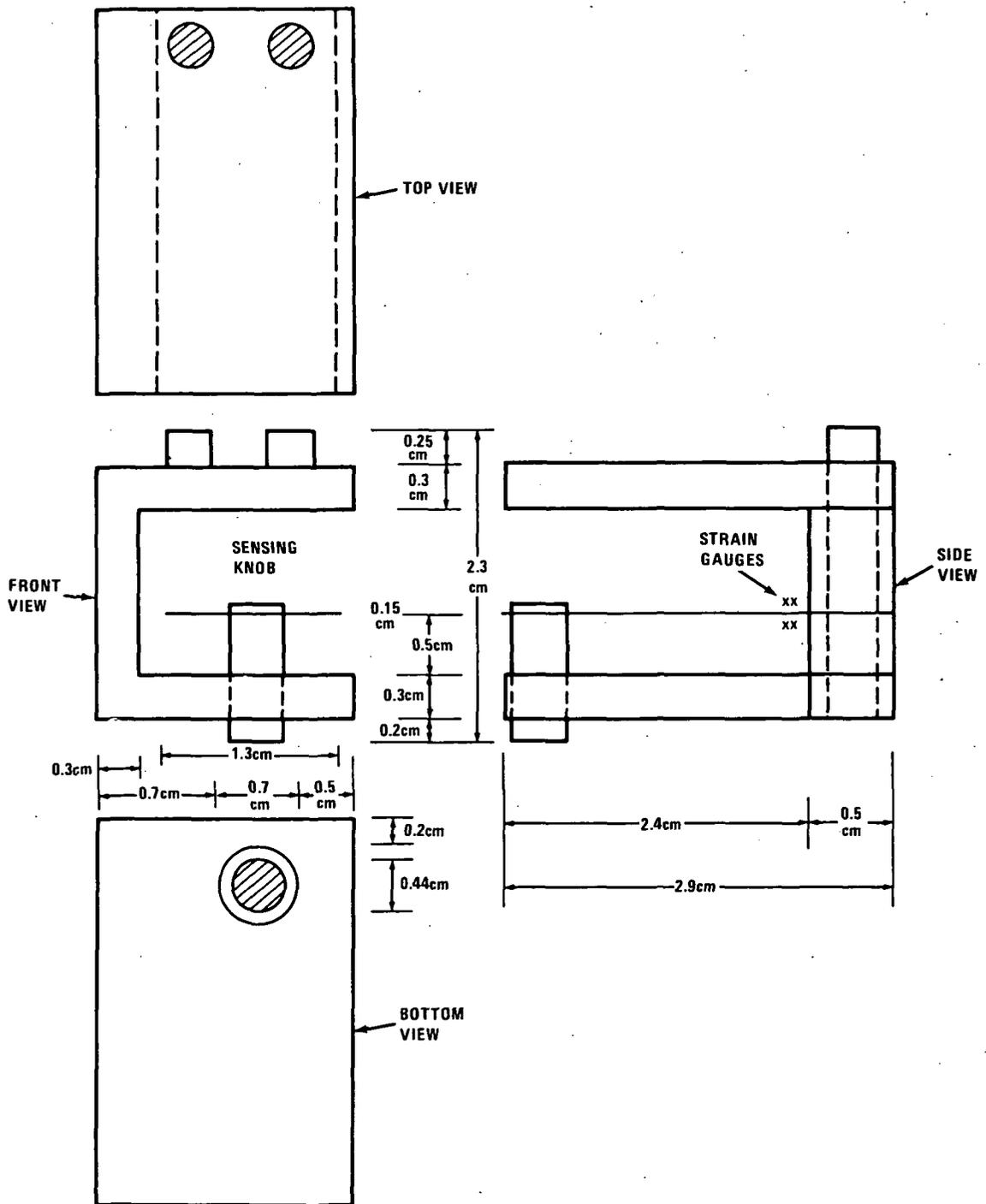


Figure 5-4. Mechanical Layout of Experimental Transducer

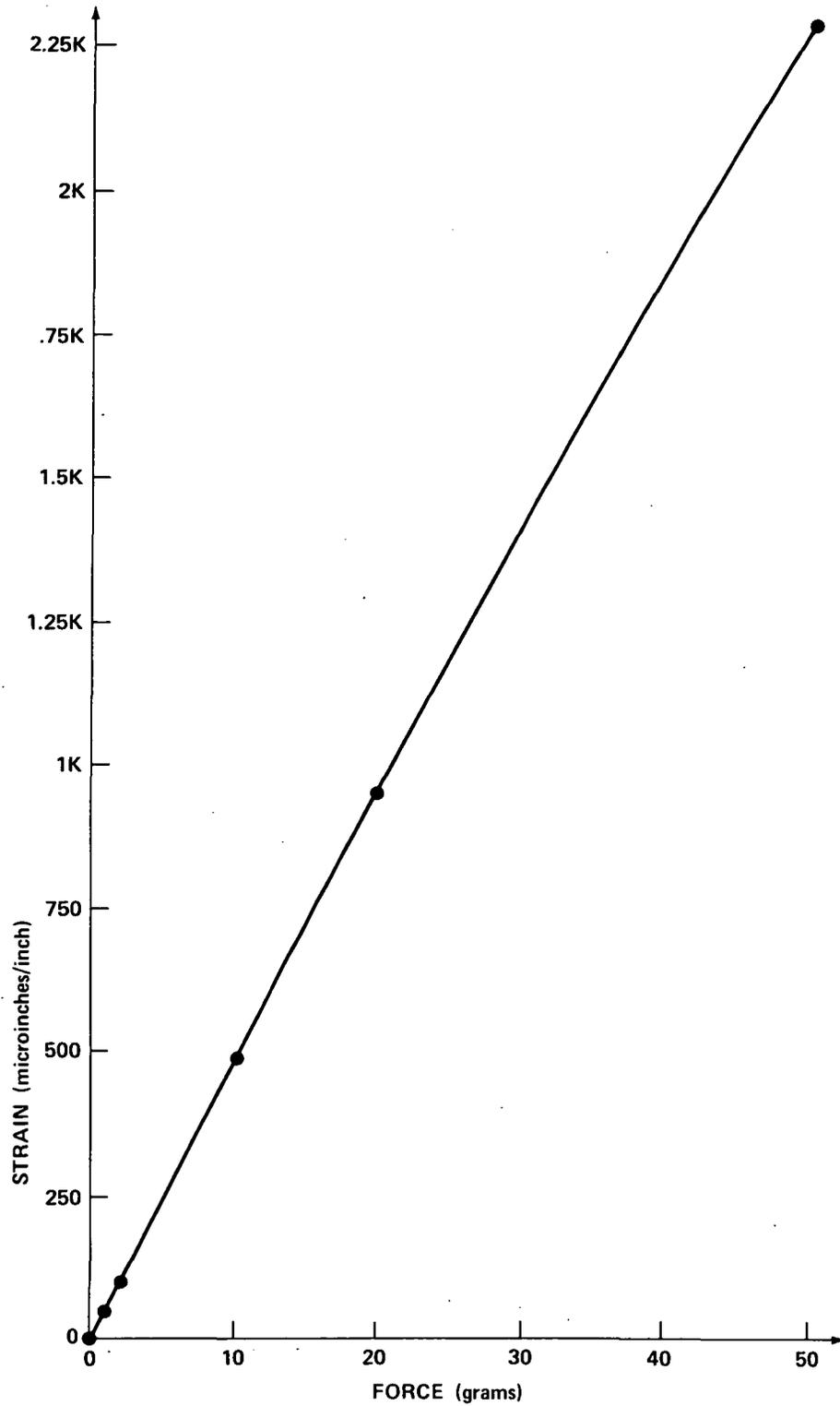


Figure 5-5. Strain vs Force for Experimental Transducer

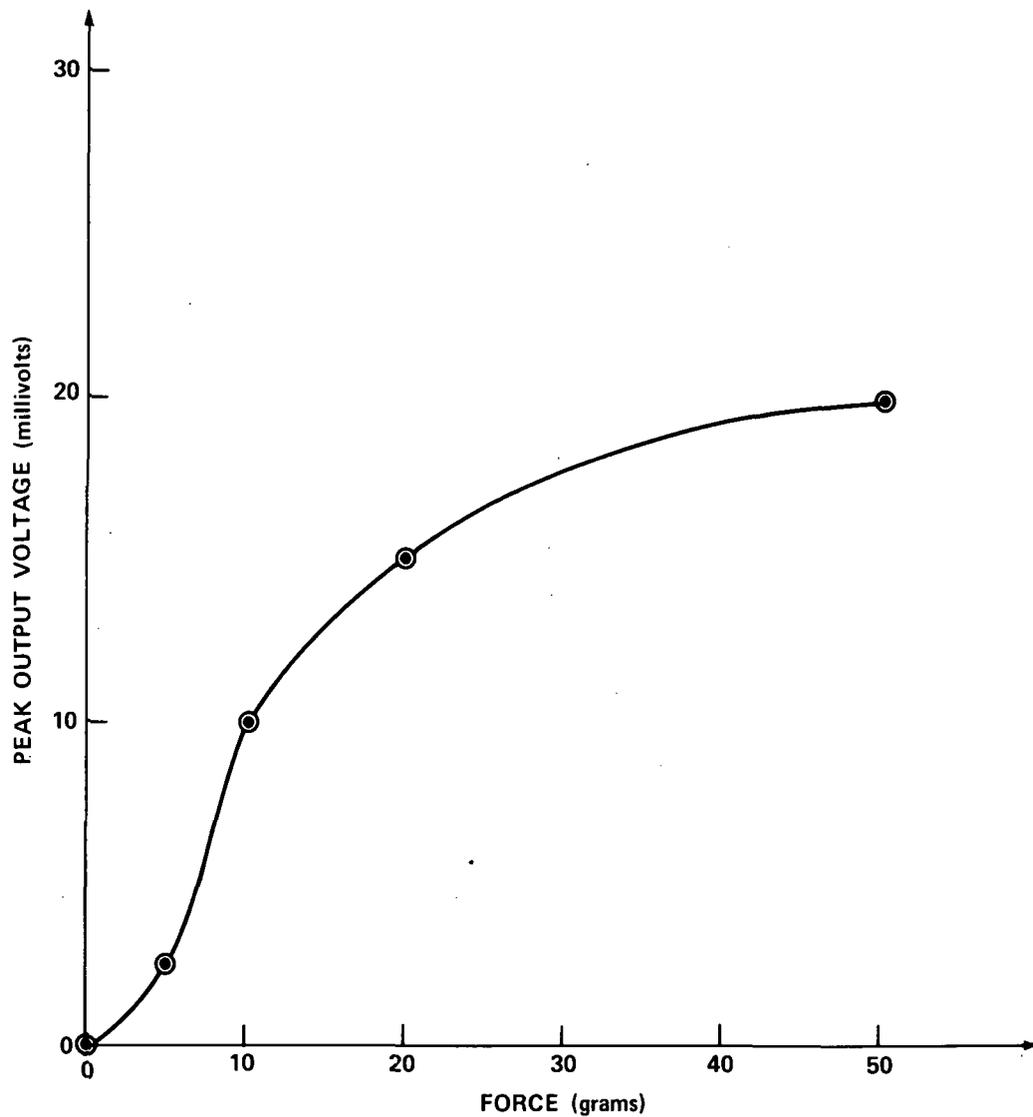


Figure 5-6. Output Voltage vs Force for Experimental Transducer

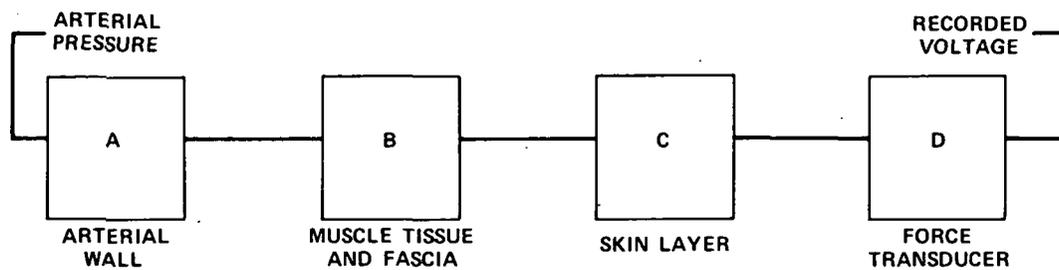


Figure 5-7

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THE FOLLOW-UP EFFORT

Work on each of the projects is still under way at some level of activity. In each case, there is a dual coordination of the progress being effected at both Howard University and the Goddard Space Flight Center. The rapidity of the follow-up effort in no way compares with the progress made during the actual ten-weeks of the Institute. This is due to a lack of daily continuity, which was lost when the student investigators as well as faculty coordinators could no longer devote their full-time efforts to Institute problems. The state of the problems is as follows:

The Electrophoresis Study requires the design and construction of a power supply specific to the needs of the four cell unit. Some work on this design is projected during the Spring of 1973.

A design and its specifications for the comparison viscometer has been discussed with the fabrication personnel at Goddard. It is proposed that a prototype be built and then tested by the Howard University team hematologists.

The anesthetic valve has been loaned to Freedmen's Hospital where the protocol for a series of animal tests is to be developed. Following these results, it is anticipated that some charcoal cannister models might be built.

The Iridium-192 Seed handling scheme has been turned over to the Department of Radiotherapy at Freedmen's. Quite a bit of design work still remains on that problem, but a Mechanical Engineer has been hired who will serve as the catalyst in bringing the design to fruition.

The problem of Indirect Blood Pressure Measurements was a very difficult undertaking. Although a device has been fabricated, its future as an answer to this problem is much in question. A continued in-depth research effort, funded internally by Howard University's Biomedical Interdisciplinary Project, is to proceed on the central concept of the problem beginning in February of 1973.

COMMENTS AND RECOMMENDATIONS

As written in the original proposal, the stated purpose of the Biomedical Engineering Institute was:

1. To provide an opportunity for undergraduate engineering students to engage in the study of carefully selected biomedical engineering problems, which heretofore have not been solved.

2. To bring to bear the application of NASA technology on problems in medical related phenomena, which may have impact on the national health.
3. To provide a mechanism to expand the channels of communication between engineering and medical professionals.
4. To ascertain the strengths and weaknesses of the engineer as a potential member of a medical and health sciences team.
5. To provide a mechanism by which the existence of the NASA Technology Utilization Program can be communicated to a number of engineering colleges as well as various medically oriented environments.
6. To measure the impact of such a Summer Program on the national health industry, so that consideration can be given to adoption of similar Summer Institutes at other NASA centers.

The technical reports remain as documented evidence of how well items 1, 2 and 3 have been met.

Considering the length of time afforded each student team and its advisory staff, any questions about the success of this program are obviously non-debatable. The Institute Staff, during its planning and projection, had resigned itself to accepting less than was actually received, based on the characteristic "start-up" delays normally expected of interdisciplinary study teams. We were fortunate in experiencing little or no problems of this nature.

As a measure of how well the concept of the Institute has been and will be communicated, several inquiries about the existence of other Institutes have come in prior to completion of this report. The students inquiring about and wishing to become a part of future Institutes, as well as the medical doctors with clinical problems and problem ideas, were all refereed by participants in the 1973 program or heard about information concerning the preliminary results.

The most encouraging aspect of this experimental effort was the way in which members of the engineering profession were received by their medical counterparts. The medical doctors in this program displayed a complete willingness to cooperate and a sincere desire to provide the information needed to adequately study the technical aspects of their problems. It is believed that this was accomplished partially by the technical personnel who went to the doctors and partly by the patience displayed while the problems were being translated from their medical settings into compatible medical/technical descriptions.

As a result of the Institute it is thought that programs such as this possess unlimited potential for pin-pointing one avenue through which the medical crisis in this country might be corrected. This method relies on maximizing the attack on existing problems by bringing properly trained personnel into the environment where the problems are found to exist. In our case, we saw how young, enthusiastic engineers and scientists were able to shorten problem study and design time by merging their knowledge face-to-face, with that of the medical doctors. At the same time, a complete two-way transfer of information is thought to have been the most important single ingredient in insuring success.

Although the Institute proved that great studies can be made with the proper coordination of personnel and information, we must be made aware of another extremely important aspect of this program. That was the existence of a prolific technical store-house of knowledge developed by the NASA programs during the past fifteen years as well as the unbelievable shop capabilities which exist for the purpose of device fabrication. It is impossible to see how the success of this type of program can be guaranteed without similar facilities for constructing prototypes of the devices for present and future testing.

It is to this aspect that all parties in this nation, concerned with the problems of health care, must give their attention. Money must be provided for device fabrication and testing, one of the more costly phases of the development process. The Technology Utilization Office at the Goddard Space Flight Center has, through this program, indicated the potential returns of good medical/technical interface. It is suggested that similar efforts could be attempted by private industry. It might be advantageous to have an academic/governmental/industrial/medical study effort put together to test industry's ability and willingness to come to grips with the present problems of delivering adequate health care to all the citizens of this nation at a cost which the poor as well as the middle income groups can afford.