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The dialysis of caffeine through selected semi-permeable membranes

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THE DIALYSIS OF CAFFEINE THROUGH SELECTED SEMI-PERMEABLE MEMBRANES

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INTRODUCTION

The phenomenon of dialysis, first described by Thomas Grahmn in 1861, is being applied to numerous laboratory, industrial, and medical processes today (1). During the past 110 years, techniques have been continually refined to enhance the value of this process to the scientific community. Originally, Grahmn used dialysis to separate sucrose from gum arabic; two molecules whose molecular weights differed by a factor of nearly 1000. Today, successful separations are seen between solute particles whose molecular weights differ by less than a factor of two.

By definition, dialysis is a passive membrane transport process in which solute molecules are transferred through a semi-permeable membrane from a more concentrated solution to one which is more dilute. The only energy available for the passage of these particles is the intrinsic free energy of the molecules involved. There are a number of other membrane transport processes that have evolved from dialysis as a result of attempts to improve solute transport rates. One of these, ultrafiltration (2), uses relatively high, externally applied, pressures to amplify the diffusion rate of the molecules. According to Ferry (3), the only difference between ultrafiltration and dialysis is that dialysis ends and ultrafiltration begins at that point where the hydrostatic pressure

becomes greater than the osmotic pressure. Two other methods of membrane transport are electrodialysis and thermal osmosis (2). The former uses externally applied electrical energy, while the latter uses external heat sources to enhance diffusion rates.

The most notable disadvantage of dialysis is its passive transfer characteristic, since whenever the chemical potential between two dialyzing solutions is small, the dialysis rate becomes extremely slow. However, this has been found to be advantageous in situations where external energy might be harmful to sensitive substances such as food, biologicals, and blood components. Medically, consideration of this factor is especially important when concerned with the handling of blood (2).

Leonard (4) describes the perfect dialysis membrane as having several desirable characteristics. First, it should allow for the instantaneous passage of each essential molecule that strikes it while, at the same time, rejecting all the dispensable molecules that strike it. Further, it should be completely compatible with whatever mechanical, chemical, or biological system which might be expected to come in contact with that particular membrane. Needless to say, this membrane has yet to be formulated. To date, all those which have been formulated are found to represent compromises of these characteristics.

Until the past few years, cellulose derivatives, (e.g., cellophane, collodion, and parchment) and animal membranes (e.g., gold beater's skin) have been the only dialysis membranes employed commercially (5). Cellophane has been used

as the dialysis membrane in the artificial kidney since the machine's inception in 1914. It continues to serve in this capacity, even though, in the last few years, attempts have been made to develop better films (6).

An appreciation of both the "solution theory" and the "pore theory" is in order for this discussion. By incorporating the dynamics of these theories in the techniques of membrane formulation, improved membrane performance can be exhibited. In the following discussion which considers membrane formulation, improved membrane performance can be displayed by higher particle transfer rates and greater particle selectivity.

Solution Theory

The choice of the polymer used as the basis of the membrane is highly important in determining the diffusion properties of each specific membrane. The "solution theory" is centered on the premise that, in order to achieve selective rapid transport, the membrane's polymer units should be chosen according to their ability to act as solvents for that particular solute being removed. The dialyzing solute is dissolved by the membrane. Because the film may be considered to have the properties of a viscous liquid without any pores through which the solute might diffuse, transport takes place by normal diffusion through the liquid soluble semi-permeable membrane. Thus, the components of the solution not soluble in the "viscous liquid" are totally rejected (1). If the polymer can swell in the dialysis solvent, then it is possible to achieve a high rate of dialysis with a low degree of

selectivity among small molecules, since the swelling causes an increase in the area of mobility for the diffusing particles. At the same time, however, the polymer should be insoluble in the dialysis solvent and should be able to sustain itself upon handling. Thus, a compromise between strength and diffusional properties must be reached when employing the "solution theory" in membrane fabrication.

Pore Theory

The "pore theory" is based on the premise that only solute particles below a certain diameter can pass through the membrane pores while all molecules larger than this diameter are rejected by the membrane. Unlike the "solution theory," the dialyzing molecules are not "dissolved" by the membrane. Instead, the pores are filled with the dialyzing solution. This solution usually has a definite affinity for the membrane and "imbibes" a certain proportion of it, such that the membrane becomes swollen and less rigid, thus facilitating the particle transfer rate. Porosity of this type can be a function of the solvent as well as the nature of the membrane itself, since the degree of swelling differs with different solvents (1).

Membrane Fabrication

A diffusion membrane is produced by casting a polymer solution into a thin film by a method which varies, depending on whether the procedure is performed under laboratory or industrial conditions. In the laboratory, the solution is usually spread over a glass plate. The matrix of the film is

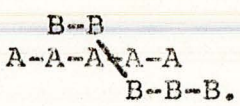
then fixed either by solvent evaporation or by coagulation in a non-solvent liquid system. On an industrial scale, the solution is forced through an orifice onto a temperature-controlled drum, or into a coagulation bath, or both. Although membranes may be cast from the same polymer solution, they may vary from lot to lot. These differences may be due to small changes in the physical conditions that are experienced in formulation. Temperature variations in the temperature-controlled drum and the environment may produce differences in the dialyzing abilities of different batches of the same membrane. In continuous commercial production, tension variations cause reorientations of the "shape" of the polymer. Thus, tension variations may also alter dialysis rates. In some formulation systems, the solvent employed appears to be especially important because very small particles are added to the casting solutions to act as the pore-formers for the membrane. After the film sets, the particles are leached out of it. Therefore, a change in the solvent might well alter the solubility of the particular pore-former used. Colloidal starch granules are a good example of pore-formers employed in this technique (2).

In an effort to achieve some degree of selective permeability, a number of techniques are employed to change pore structure. The film may be formed in the presence of the molecular species that are supposed to penetrate the pores. A second method of pore alteration involves the bombardment of the membranes with nuclear particles. However, when Leonard bombarded Visking cellulose with alpha particles, a membrane

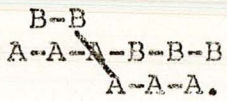
was produced having less permeability than the original membrane (4). It was hoped that the bombardment would reduce the atomic densities along the bombarded areas so as to allow for improved flow characteristics. Still another method of controlling pore size is by the use of a mixed solvent system. Initially, the polymer material used to form the membrane is dissolved in a solvent in which the polymer is highly soluble. A non-polymer solvent is then added to the original solution in quantities just bordering on the range of precipitation. The membrane is then formed by evaporating the solvents. The volatility of the two solvents may determine the polymer chain lengths and thus the pore size (2).

To overcome the problems of brittleness and shrinking upon drying, encountered with some membranes, plasticizers may be added to the solvent system. These remain in the film until they are leached out when it is put into use. An example of this is cellophane which is plasticized with glycerine (2).

To achieve a balance between physical strength and diffusional characteristics, the membrane may be cast onto an open weave fabric or thin fibers can be added prior to casting. An alternative to this technique is the casting of homogenous polymer mixtures which may be accomplished by one of three different methods. Firstly, polymer chains comprised of two monomer units, with each unit attached to itself many times, can be formed, i.e.:



Secondly, this attachment may be randomized to form a random co-polymer, i.e.:



Finally, a block co-polymer can be prepared. This film consists of two different molecular components in each chain. The first constituent is chosen for its favorable solubility towards the dialyzable species while the second component is selected for its ability to partially insolubilize the membrane so as to impart mechanical strength to the membrane. An example of this method of fabrication is exemplified by the use of polyethylene glycol as the solubilizing moiety, while polyethylene terephthalate is employed as the insolubilizing moiety (2).

As a means to overcome the swelling of the membrane to the point of self-dissolution, a number of methods have been devised. The four following methods may be employed so as to render the films partially insoluble:

1) by cross-linking the pre-formed film with alpha radiation,

2) by the addition of a catalyst to the film,

3) by esterifying the film, such as in the case of the esterification of cellulose acetate by sodium methylate,

4) by hydrolyzing the film such as the acid hydrolysis performed on vinyl esters (2).

Types of Membranes

It is common practice to divide membranes into three different categories according to the mechanism of transport:

the microporous membranes, the molecular-diffusion membranes, and the ultra-filtration membranes.

1. Microporous Membranes

The spongy structure of microporous membranes is characterized by capillary flow of the solvent through the pores; thus, operating according to the "pore theory," the liquid flows through the pores according to the dynamics of Donnan membrane equilibrium. The pore diameter, which ranges from one to ten microns, is several times larger than the mean diameter of the molecules that pass through them. The appearance of the membranes is opaque even when made of transparent material since the microporous structure scatters visible light. These membranes are used in the filtration of suspensions, ultrafiltration of colloidal matter, as diffusion barriers or as separators in electrochemical cells, and in the preparation of synthetic leathers or cloth. Even in the last application, they are considered to be membranes, as they are permeable to water vapor. Cellulose acetate is considered the best example of this particular group of membranes. The stability under varying humidity conditions, high tensile strength, and the ability to be autoclaved without alteration of pore structure make cellulose acetate the membrane of choice in this group (7).

2. Molecular Diffusion Membranes

Molecular diffusion membranes, which are also termed solution-diffusion membranes, represent the direct antithesis of microporous membranes in that they derive the dynamics of their mode of transport from the "solution theory." Since

the dialyzing solution passively diffuses through the "viscous liquid" barrier, pore size is not considered when these membranes are discussed. Because they are made from polymers with limited swelling abilities in the diffusing medium, there can be significant differences in the diffusion coefficients of even closely related molecules, such as cellulose acetate as opposed to cellulose xanthate. Solubility differences, or more precisely, compound-polymer interaction differences and diffusion coefficient differences lead to separations in the mixture by differential transport of the components. The net effect of these differentials is the selective transport of the most mobile dialyzable component. Occasionally, this group is referred to as reverse-osmosis or desalination membranes. Cellulose acetate, although previously referred to as an example of a microporous membrane, may be restructured into a molecular diffusion membrane. It consists of a thin membrane of 0.3 microns supported by a spongy microporous layer of about 50 to 100 microns of the same material. The thin layer differentiates between water transport and transport of highly mobile molecules with good flux owing to its thinness. Thus, the dialyzable components dissolve in the membrane such that the most mobile members are able to diffuse through it. Applications of this group of membranes include the desalination of brackish water and sea water, and treatment of acid mine water containing sulfuric acid, industrial waste water, domestic waste water, and individual liquid wastes in closed ecological systems (7).

3. Ultrafiltration Membranes

When the dynamics of the "pore theory" and the "solution theory" are combined, the resultant film is termed an ultrafilter. This group represents the middle ground between microporous films and molecular diffusion membranes, because the material is partially swollen by the solvent, yet some solvent is transported by a modified porous flow. It includes various regenerated cellulose membranes, animal membranes, cross-linked membranes, and polyelectrolyte complexes.

a. Regenerated Cellulose Membranes

Derivatives of regenerated cellulose are good ultrafilters due to their excellent film-forming properties and variable hydrogen bonding. The glomerulus of the human kidney is considered a good reference point for the pore size of ultrafilters. With a mean diameter of 0.26 microns, it allows the passage of small solute particles such as urea, creatinine, and uric acid but retains the macromolecules, such as proteins, and blood cells (7).

Visking cellulose, a regenerated cellulose xanthate membrane, is marketed as a dialysis membrane for aqueous solutions. When wet, it has an average pore size of 0.24 microns. It can be swollen by treatment with zinc chloride or sodium hydroxide to yield membranes that can purify and fractionate proteins, antibiotics, surface active agents, and organic dyes (7).

Cellulose acetate is cast from a solvent such as acetone containing additives such as organic phosphates, amides, organic acids, sulfoxides, and aqueous swelling salts. If

the additives are omitted and only the solvent is used, the resulting product is a dense film with extremely low water permeability (7).

Collodion, an ether-alcohol solution of cellulose tetranitrate, was the first cellulose derivative used as a dialysis membrane. According to Ferry (3), Fick used it in his separations in 1855; Schumacher, in 1860, described a collodion sac for dialysis; and in 1891, Saranelli introduced it into his bacteriological work, which included the ultrafiltration of plasma *in vivo*. As with cellulose acetate, its porosity is controlled by additives in the casting solution. By varying the concentrations of the polymer and of the additives, the pore size can be varied from 0.2 to 2.0 microns (8).

b. Animal Membranes

Animal membranes also figured prominently in the early history of dialysis and ultrafiltration. In 1856, Schmidt found that when a solution of gum arabic or protein was filtered through an animal membrane, the filtrate was less concentrated than the original solution (3). Gold beater's skin is a good example of a commercially available ultrafilter today. It is an ox gut membrane that has been treated with alum or camphor and coated with an egg albumin film.

c. Cross-linked Membranes

Within the past ten years, cross-linked membranes have been developed. Some of these have been found to be far superior to the cellulose derivatives in their dialyzing ability. They have been the result of research directed at the development of membranes suitable for hemodialysis. In

1964, Lyman (9) prepared a block co-polymer composed of polyethylene glycol, due to its favorable urea solubility and polyethylene terephthalate because of its strengthening capability. He showed that this film was able to exhibit selective permeability. It was capable of dialyzing urea, creatinine, and uric acid faster than Cuprophane, a regenerated cellulose membrane produced by the DuPont Corporation, while at the same time dialysis of glucose, sucrose, and raffinose, was slower than with Cuprophane.

Two years later, this same researcher (10) developed a group of copolyurethane-polyethylene glycol membranes. In these, the urethane component of the block co-polymer served as the hydrophobic strengthening component. By reacting methylene bis(4-phenylisocyanate) with polyethylene glycol and 1,5-pentanediol; 1,10-decanediol; and trans-1,4-cyclohexanediol, Lyman was able to prepare three membranes in this series. In all cases, the films exhibited slower half-time escape rates for urea, creatinine, and uric acid than those shown by Cuprophane and the polyethylene glycol-polyethylene terephthalate co-polymer. These data indicated that small changes in the hydrophobic component of the co-polymer had extremely large effects on the overall performance of the membrane. Thus Lyman, in this particular study, concluded that interactions occur not only between the hydrophilic segment of the membrane but also between the hydrophobic segment and the dialyzing medium.

In 1964, Markle (5) prepared several different synthetic cross-linked membranes and evaluated them by measuring

the rates of urea dialysis. He found that cross-linked polyvinyl alcohol and cross-linked polyvinyl pyrrolidone dialyzed urea faster than cellophane. The same hydrophobic segment as used in Lyman's second report above, i.e., methylene bis(4-phenylisocyanate), was included in these membranes. One of the polyvinyl pyrrolidone membranes exhibited a dialysis rate greater than three times that observed with cellophane. However, the most interesting point of all was that the rates of dialysis for polyvinyl pyrrolidone and polyvinyl alcohol were related to the amount of crosslinking or hydrophobic segment added. Thus as the crosslinking decreased, the rate of dialysis increased (5). It seems apparent that cross-linked membranes will hold great promise for the future since their dialyzing efficiency has frequently been proven superior to that of the cellulose derivatives.

d. Polyelectrolyte Complexes

Polyelectrolyte complexes represent the result of crosslinking two highly but oppositely charged polyelectrolytes. Individually, the polyelectrolytes are highly water soluble but, when combined, they undergo only limited swelling in water and electrolyte solutions. The complexes are highly permeable to water and small solute molecules and are said to exhibit selective permeability. Formation of the complexes is accomplished by reacting the salt of a polyacid with the salt of a polybase. Appropriate derivatives of polystyrene can be used as the reactants. For example, poly-(sodium styrenesulfonate), serving as the polyanion, can be reacted with the polycation poly-(vinyl benzyl)-trimethyl-ammonium chloride to form a thermally stable hydrogel. The

most stable polyelectrolyte complexes are made from polyelectrolytes whose monomeric acids and bases have pK values less than 2.0. Their gross structure resembles that of the cellulose acetate membranes used for desalination by reverse-osmosis. Due to their high degree of water permeability and selective permeability, they are now commercially available and widely used (11).

Although a number of different materials have been available for dialysis for many years, and the past decade has witnessed the proliferation of new membrane materials having significant dialyzing ability, there does not seem to be any extensive study of a comparative evaluation of the many membranes available. Before new materials suitable for dialysis can be evaluated, it is mandatory to have some comparative data of the presently available materials to which new films may be compared. This study was initiated to assemble this necessary data. The choice of solute for this work was made, keeping in mind the possible medical use of the findings. The most common medical application of dialysis is the artificial kidney. Of the group of human waste products that the machine dialyzes out of the blood, uric acid is the largest. Thus, uric acid initially seemed to be the dialyzable solute of choice for the study. However, early experience showed it to be unstable in aqueous solutions in the concentrations necessary for spectrophotometric analysis, the method of assay employed in this work. Caffeine closely resembles uric acid and is more stable in solution; therefore, it seemed that this compound would be suitable for this investigation. By

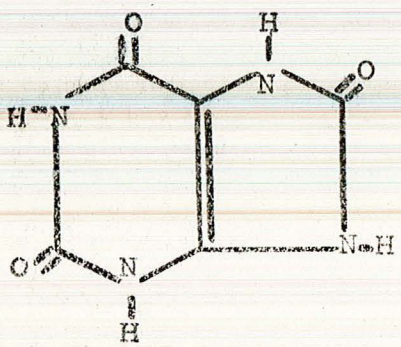
determining the rates at which caffeine would be dialyzed through the selected membranes, it was felt that a clear indication of the type of membrane most useful for dialyzing uric acid might become apparent.

Chapter II

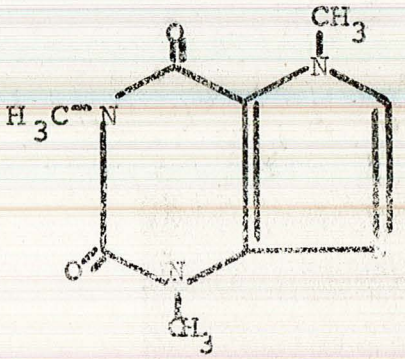
EXPERIMENTAL METHOD

The human kidney excretes primarily three waste products, urea, creatinine, and uric acid. The ability to dialyze these three molecular species determines the success or failure of any artificial dialyzing apparatus used for hemodialysis. Obviously, the most important component of this system is the membrane that separates the blood from the external dialyzing medium, since it, to the greatest extent, controls the rate of dialysis of the solutes. The molecular weights of urea, creatinine, and uric acid are respectively 60, 113, and 168. For a dialyzing unit to be truly efficient, it must be able to remove all of the blood's waste products. Any good, effective membrane then must be able to remove uric acid since it represents the largest solute particle of the group. If one could measure the rates at which uric acid in an aqueous system is dialyzed through various semi-permeable membranes, it might be possible to select one that may be used as the membrane of choice in an artificial kidney. However, the low solubility of uric acid in water restricted the usefulness of this compound in the study. Using spectrophotometry as the method of analysis, the solutions could not yield adequate reproducible spectrophotometric responses. Exploratory work revealed an apparent drop in the concentration of the uric acid solutions upon standing.

Realizing that the instability of uric acid in aqueous solutions would tend to skew the results in the evaluation of the membranes under study, a search for a suitable congener was initiated. Caffeine was chosen as the solute of choice for the investigation. A comparison of the molecular structure of the two readily illustrates the similarity of caffeine to uric acid.



URIC ACID



CAFFEINE

The respective molecular weights of uric acid and caffeine are 168.11 and 195.19. The greater solubility of caffeine in water (1:46 parts) facilitated the preparation of solutions of such concentrations that could be handled well by spectrophotometric analysis. Experience showed these solutions to be stable on standing. Standardization of the Anhydrous Caffeine, USP,[a] employed in the study, was performed by means of a melting point determination and a molecular weight determination.

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a - Ruger Chemical Co., Irvington-on-Hudson, New York, N. Y.

Using a Thomas Hoover Capillary melting point apparatus[a], the melting point range of the compound was found to be 235-236.8 °C., while a USP standard sample[b] melted in the range of 235 to 236.5 °C. Employing the Rast Camphor method and using the melting point apparatus, the molecular weight of caffeine was calculated as 195.2 as compared to the USP designated value of 195.19.

Since the experiment involved the measurement of the change in concentration of a solution over a given period of time, spectrophotometry was the method employed in the evaluation. The Bausch and Lomb Spectronic 600[c] equipped with an automatic recorder and continuous flow-through cuvette was selected as the most suitable instrument.

An ultraviolet scan of caffeine was performed revealing the lambda maximum of caffeine at 272.5 millimicrons (Fig. 1). It was found that a concentration of 0.004% w/v was the maximum concentration that could be measured by the spectrophotometer. Dilutions of this solution were made in order to prepare a standard Beer's plot (Graph 1).

Membranes

1. Visking Cellulose

The selection of a representative group of commercially available ultrafiltration membranes suitable for dialysis was

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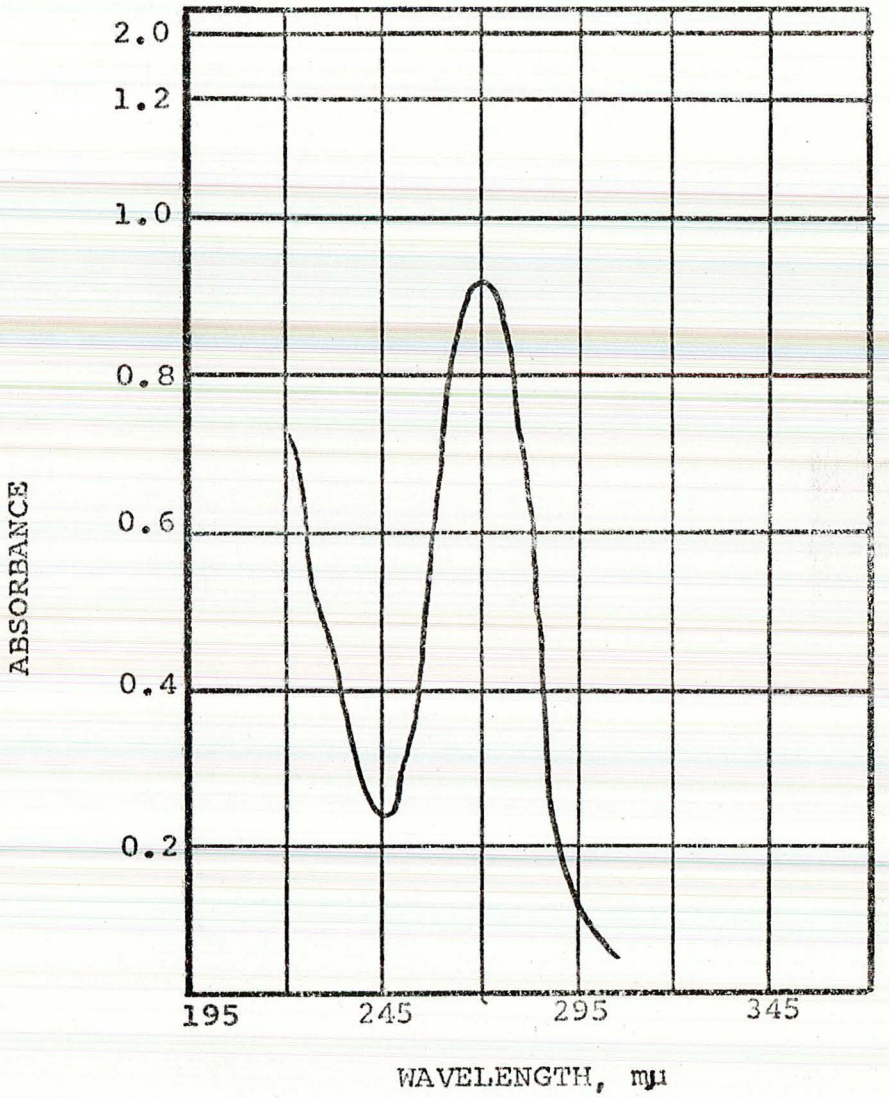
a - Arthur H. Thomas Co., Philadelphia, Pa.

b - Arthur H. Thomas Co., Philadelphia, Pa.

c - Bausch and Lomb Optical Co., New York, N. Y.



Anhydrous Caffeine, 20 mcg./ml.

Fig. 1. U. V. Spectrum of Anhydrous Caffeine
(λ max. 272.5)

of prime consideration in the investigation. First, a standard had to be chosen in order to place the results of the work in a meaningful context. Visking cellulose (cellulose xanthate) was chosen as the reference membrane due to its wide use in clinical hemodialysis and its being a well standardized membrane. According to Leonard (4), it is thought to be the membrane used in the Travenol Twin-Coil kidney and in some MacNeill-Collins kidneys (4). Subsequently, an Ultra-Flo 145 Dialyzer disposable coil unit[a] (5M-02-32) was obtained. Thus, the coil provided the criterion membrane to which the others could be compared.

2. Gold Beater's Skin

Animal membranes were first used experimentally as dialysis membranes over 115 years ago. In order to gain some concept as to how far ultrafiltration technology has advanced over the years, gold beater's skin was selected as a representative of this class of materials. Gold beater's skin[b] is the outside membrane of the large intestine of the ox, used primarily by gold beater's to separate sheets of gold while beating them into gold leaf.

3. Millipore Membranes

A group of synthetic membranes, produced by the Millipore Corporation[c], provided examples of other types of membranes. This group included:

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a - Artificial Organs Div., Travenol Co., Morton Grove, Ill.

b - Central Scientific Co., Chicago, Ill.

c - Millipore Corp., Bedford, Mass.

1) MF-Millipore,

2) Celotate,

3) Polyvic,

4) Pelicon ultrafilter membranes.

The MF-Millipore filter, the "standard" Millipore filter, is composed of "pure, biologically inert esters of cellulose." The membrane is also described as consisting of "mixed esters of cellulose" thus implying that the film is composed of cellulose acetate butyrate and cellulose acetate propionate mixture. Although available in pore sizes ranging from 8 microns to 0.025 microns, the type having a mean pore size of 0.22 microns was singled out for the study. This pore size is slightly smaller than the smallest bacteria and is recommended for use in sterilization by filtration. This becomes an important factor when considering these films as potential hemodialysis membranes (12).

Celotate, a membrane composed of cellulose acetate, was obtained in the smallest available pore size of 0.2 microns. Normally it is recommended for the microfiltration of lower molecular weight alcohols, such as methanol and ethanol (12).

Polyvic, a film fabricated from polyvinyl chloride, although similar in many aspects to the cellulose membranes, possesses the additional properties of increased flexibility, strength, and solvent resistance which make it extremely useful in many research and industrial applications. It was obtained in the smallest pore size available, 0.6 microns (12).

While all of the previously mentioned membranes may be considered under the heading of simple ultrafilters, the Pelicon membrane appears to fit the classification of a solution-diffusion membrane or a molecular diffusion membrane. It is a two-component system, consisting of an extremely thin, polymeric matrix, supported by a porous substrate. The matrix, or membrane proper, is considerably less than one micron thick, thus permitting high fluxes. The substrate, a porous sheet of mixed cellulose esters, i.e., cellulose acetate butyrate and cellulose acetate propionate, approximately 130 microns thick, provides a high degree of mechanical durability and stability. They are not described as having any particular pore size, instead they are spoken of as having a nominal molecular weight cutoff. The membranes obtained for this study had a nominal molecular weight cutoff of 1000, the lowest cutoff available. Thus, the passage of any solute particles having a molecular weight greater than 1000 is prevented by these films (12).

4. Diaflo Membranes

Diaflo[a] ultrafiltration membranes represent polyelectrolyte ultrafilters that have been restructured to act as molecular diffusion membranes. These consist of a very thin (0.1 to 1.5 micron) layer of extremely fine pore size (0.02 to 1.0 micron) superimposed on a much thicker (50 to 250 microns) layer of an open-celled, microporous sponge. The thick skin and microporous substructure produce a unique combination of selectivity, high throughput, and non-clogging

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a - Scientific Systems Div., Amicon Corp., Lexington, Mass.

characteristics. The Diaflo UM-05 membrane was utilized in this project. It has a net anionic charge on its polymeric backbone. This makes possible the separation of neutral solute particles from charged species of comparable molecular size and structure. Therefore, the membrane has the capability to dialyze a caffeine molecule, which is cationic, and reject the passage of glucose which is neutral. Thus, the membrane exhibits the property of selective permeability which is highly desirable. The molecular weight cutoff for this membrane is 500 (13).

5. Fiberfilm Material

Teflon-coated membranes have recently come into prominence. Fiberfilm T10G6N[a], which is thin tissue-glass paper impregnated with Teflon, was tested. However, it failed to allow for any dialysis to take place as it is hydrophobic in nature. Even though hydrophobic, aqueous suspensions may be readily filtered if the interfacial tension between the filter and the liquid is overcome. In the particular system used in this work, it was not overcome, so the membrane was deemed unsuitable for evaluation (14).

Membrane Evaluation System

When means of evaluating the membranes were being considered, it was felt that it would be far easier to construct a dialysis chamber that was suited to the methods and materials involved in the study than to imitate another previously designed membrane evaluating system. Figure 2 shows the

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a - Pallflex Products Corp. Div., Pall Corp., Putnam, Conn.

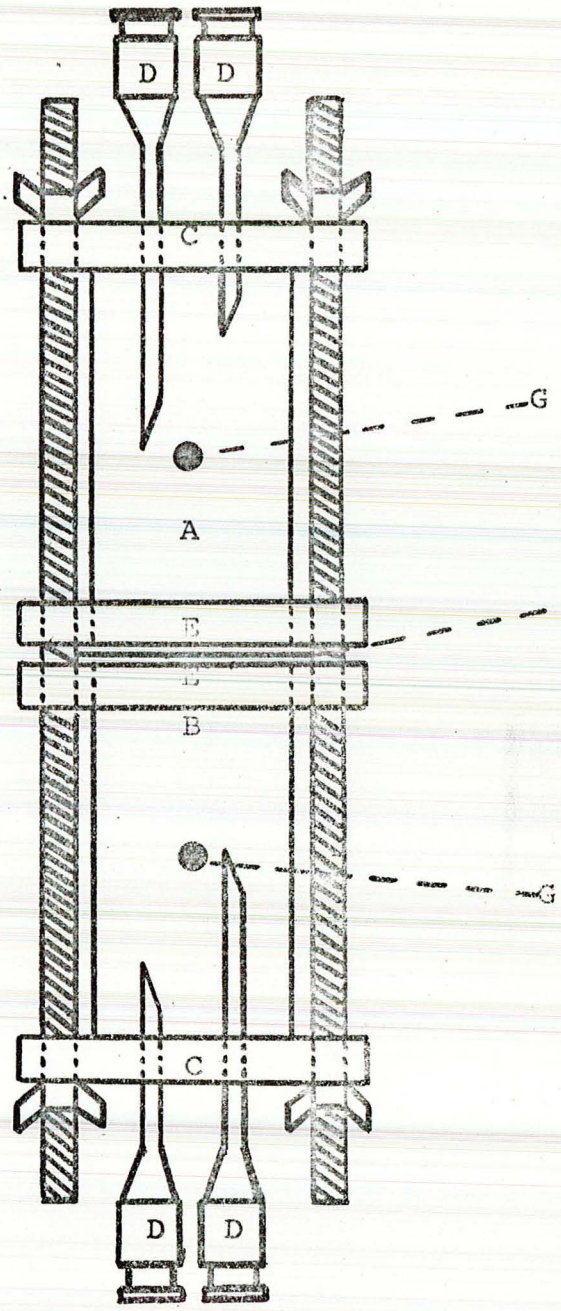

Figure 2. Design of the Dialysis Chamber. A. chamber A; B. chamber B; C. solid Plexiglass backing; D. Leur-Lock needles; E. hollowed Plexiglass backing; F. membrane; G. filling holes.

design of the dialysis chamber constructed for this experiment on a 1:1 scale. It actually consists of two separate chambers (A and B) between which a membrane was placed. The cylinders of the two chambers, made of Plexiglass, are 2" long and have an internal diameter of 1 1/8 inches. Each chamber was glued to a 1 7/8" Plexiglass square (C) that has been impaled by two 12 gauge Leur-Lock hypodermic needles[a] (D) of 1" and 1 1/2" in length. The chambers and their backings were glued to a second pair of 1-7/8" Plexiglass squares (E) from which a circular area of 1 5/16" diameter was cut out from their center. The membrane (F) under consideration after being placed between two gaskets composed of plastic bed-sheeting is positioned between chambers A and B and then the entire dialysis chamber is bolted together by means of 5 1/2" bolts and 1/4" wing-nuts. So that the chambers can be filled with their appropriate solution, 1/4" holes (G) were drilled in the top of each chamber. These openings were then stoppered by two needle adapters[b] obtained from disposable intravenous Venopak sets. The needle adapters were filled with plastic cement so that they would act as plugs. The needle adapter plugs were then used to stopper the chambers after they were filled with their appropriate solutions at the beginning of each evaluation.

Figure 3 shows a schematic diagram of the components of the system and illustrates how they are integrated to form the entire complex. Since the method of analysis in the

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a - Becton-Dickinson Co., Rutherford, N. J.

b - Clay-Adams Co., New York, N. Y.

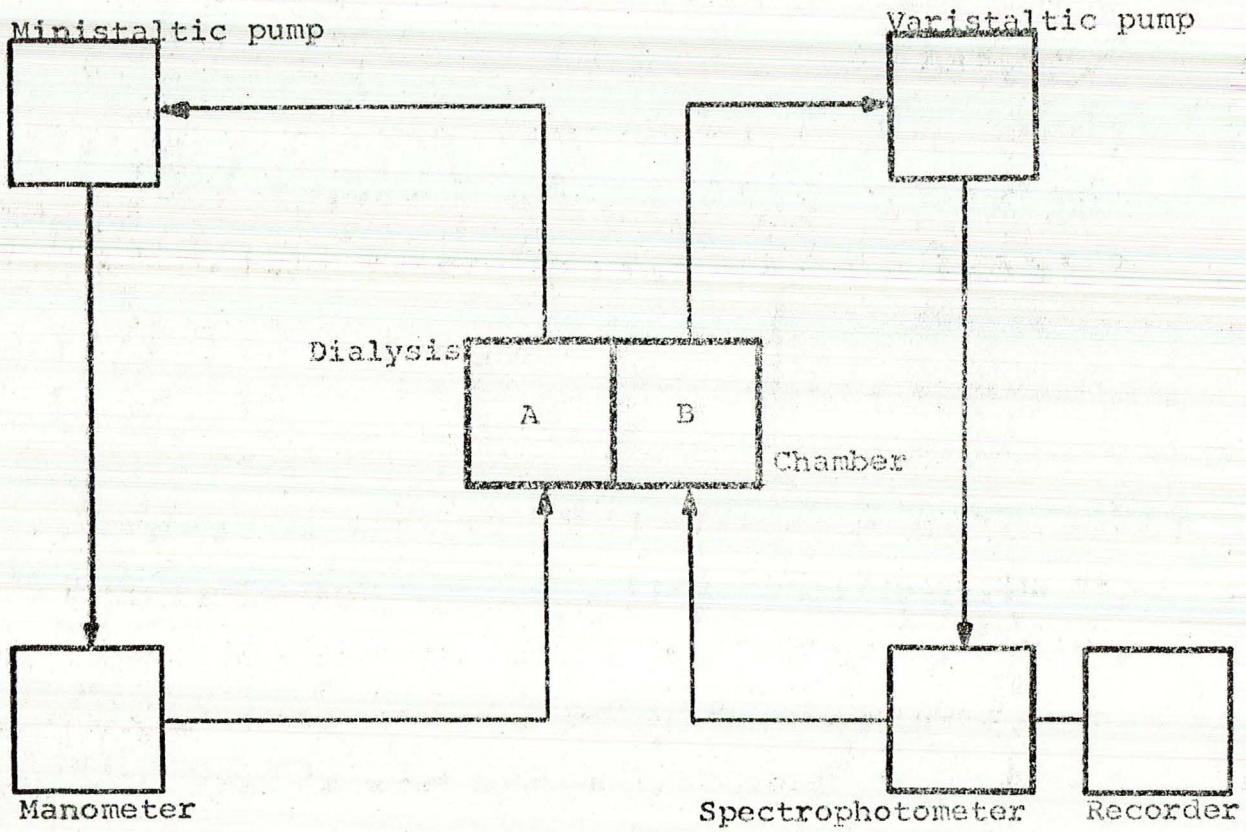

Figure 3. Block Diagram of the System.

study involved the use of spectrophotometry the dialysis chamber was designed keeping in mind the fact that the contents of one of the chambers had to be sampled at varying intervals without disturbing the system to any appreciable extent. The Bausch and Lomb Spectronic 600 equipped with a Linear/Log Varicord 43 recorder[a] was utilized because it contained a flow-through cuvette that facilitated the sampling procedure. So that the liquid could be circulated through chamber B during the sampling intervals, a Varistaltic[b] pump was employed. The solution contained in chamber A was continuously cycled during the entire evaluation period by means of a Ministaltic[c] pump. This pump was specifically designated for the chamber A half of the system since it was able to impart higher pressures for longer periods of time than the Varistaltic pump. An open system mercury manometer was constructed for the chamber A side in order to determine the amount of pressure being exerted by the Ministaltic pump against the membranes being tested. The tubing used to connect all of the components of the complex was extra-capacity plastic tubing (0.120" I.D.) obtained from a number of disposalbe intravenous Venopak[d] sets. The tubing was connected to the chamber needles by way of plastic tubing

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a - Photovolt Corp., New York, N. Y.

b - Manostat Corp., New York, N. Y.

c - Manostat Corp., New York, N. Y.

d - Abbott Laboratories, North Chicago, Ill.

adapters[a] that were modified to accomodate the large tubing.

With the test membrane in place and the components of the system fully assembled, compartment A was filled with a 0.004% w/v solution of caffeine. Compartment B was then filled with distilled water, produced by a Corning AG-1 distillation unit[b]. Dialysis was then allowed to proceed for the prescribed 4 hour interval, with intermittent samples being analyzed during this period. Prior to any recording of a particular caffeine concentration in chamber B, the Varistaltic pump was allowed to operate for several minutes so as to insure uniform mixing of the solution. The Ministaltic pump was adjusted to maintain a constant membrane pressure differential of 25 mm. Hg between the two chambers (the same pressure differential as found in the human kidney). At the end of each observation, the concentration of caffeine in chamber B was recorded. This data is presented in both tabular and graphic form in Chapter III.

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a - Clay-Adams Co., New York, N. Y.

b - Corning Scientific Instruments, Cambridge, Mass.

Chapter III

RESULTS

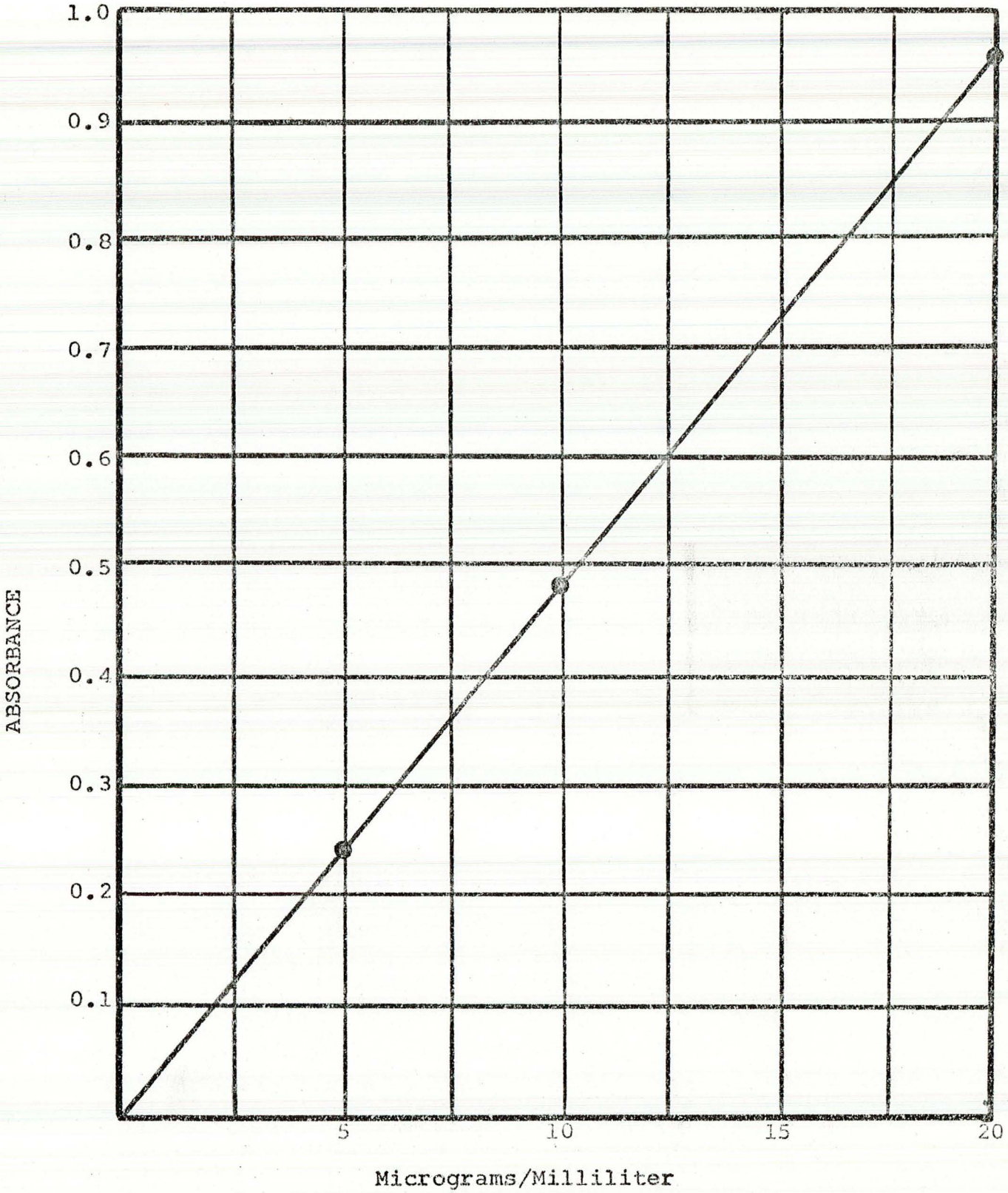
The data collected in this study represents a determination of the ability of seven selected membranes to dialyze a 0.004% w/v (40 mcg./ml.) aqueous solution of Anhydrous Caffeine USP. They include three replicate determinations for each membrane during a period of four hours with three spectrophotometric readings taken at random intervals prior to the final reading taken at the end of the period.

Standard Beer's Plot

A one percent w/v solution of anhydrous caffeine served as a stock solution. Using the stock solution, various concentrations of caffeine were prepared and values of the ultraviolet absorbance of each dilution determined. These absorbance values plotted against concentration expressed in mcg./ml. yielded the Standard Beer's plot shown on page 30 (Graph 1).

Dialyzing Efficiency Determinations

It was assumed that the system, if dialyzing correctly, would naturally dialyze until the equilibrium value of 20 mcg./ml. was reached. As can be readily deduced, dialysis would cease at a point where both halves of the chamber reached a concentration of 20 mcg./ml. Since it was impractical to dialyze to equilibrium in several cases, because of



Graph 1

Standard Curve for Anhydrous Caffeine

the lengthy time period involved, a time limit of four hours was established as the duration of each evaluation. Thus, 20 mcg./ml. was designated as the value of 100% dialysis. Assuming this, the mean percent dialysis values were determined for each membrane. These values are shown in Table I along with the individual and mean dialysis concentrations reached in the experiment for each membrane.

Dialysis Rate Curves

This set of data was compiled to determine whether the system was functioning correctly. Graphs for each determination were plotted. If the plot produced a straight line, the system was considered to be functioning correctly since dialysis follows a logarithmic pattern (1). It must be kept in mind that the logarithmic absorbance values were converted to a linear pattern by the conversion of the absorbance readings to concentration readings using the Beer's plot. Thus, a straight line actually indicates the existence of a logarithmic curve and that the system is indeed behaving as expected. Graphs 2 through Graph 8, with each being a composite of the three determinations for each membrane, substantiate the fact that the system actually did behave correctly.

Statistical Evaluation of Results

In order to attain some idea as to the validity of the results, a statistical evaluation was organized. Student t-values were determined using Visking cellulose as the test standard. Table II, which contains this data, attempts to convey some indication as to the general character of the work.

It is an indicator used to reinforce the validity of the results presented in Table I.

TABLE I

DIALYZING EFFICIENCY DETERMINATIONS

Membrane	Anhydrous Caffeine Concentration mcg./ml.	Anhydrous Caffeine Mean Concentration mcg./ml.	Percent Dialysis[a]
Visking Cellulose	7.4 7.6 7.1	7.37	36.85
Gold Beater's Skin	4.9 5.0 5.0	4.97	24.85
Milipore-MF	8.4 8.5 9.2	8.7	43.50
Celotate	8.7 9.0 9.4	9.03	45.15
Polyvic	10.0 10.0 10.0	10.0	50.0
Pelicon-PS	8.2 8.2 8.5	8.3	41.5
Amicon UM-05	0.4 0.6 0.6	0.53	2.65

a - The percent equilibrium reached by the dialyzing solution.

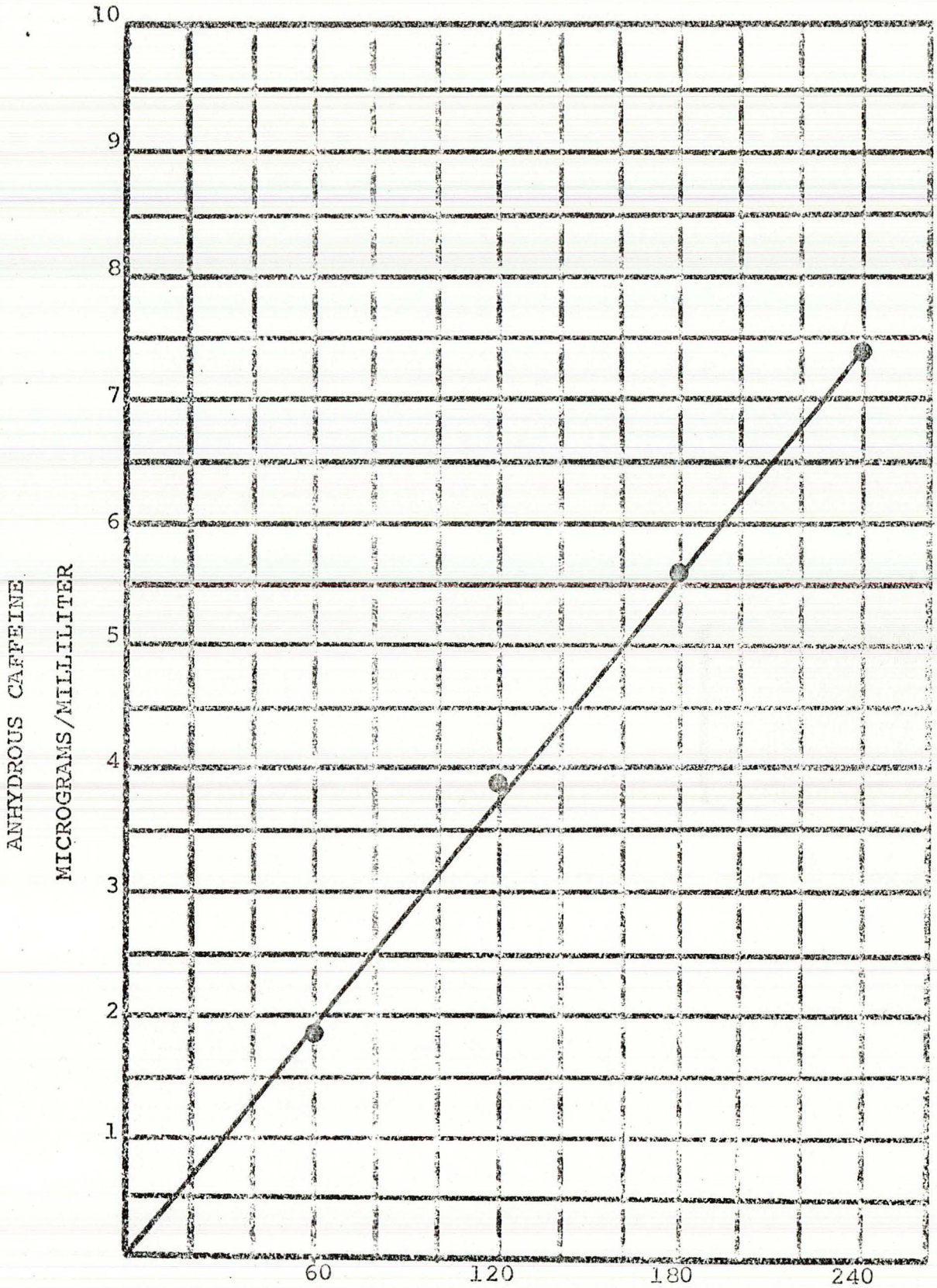
TABLE II

STATISTICAL EVALUATION

STUDENT "t" TEST*

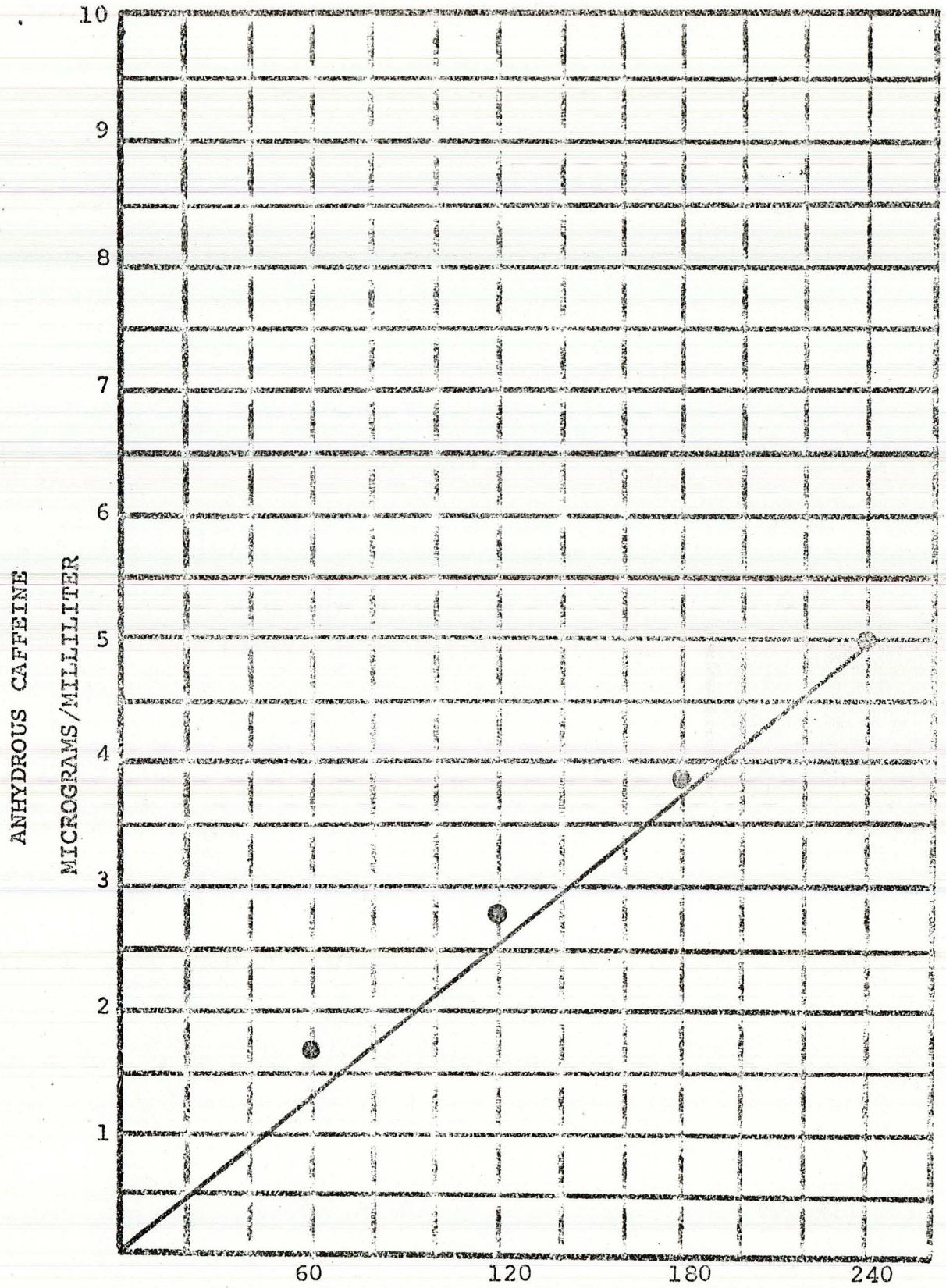
Membrane	"t" Value	Probability Value
Gold Beater's Skin	16.06	< 0.001
Milipore-MF	4.57	< 0.005 > 0.001
Celotate	6.64	< 0.005 > 0.001
Polyvic	18.05	< 0.001
Pelicon-PS	5.26	< 0.005 > 0.001
Amicon UM-05	42.67	< 0.001

*Statistical evaluation of differences between dialysis rates observed using each of the membranes in comparison with Visking cellulose.



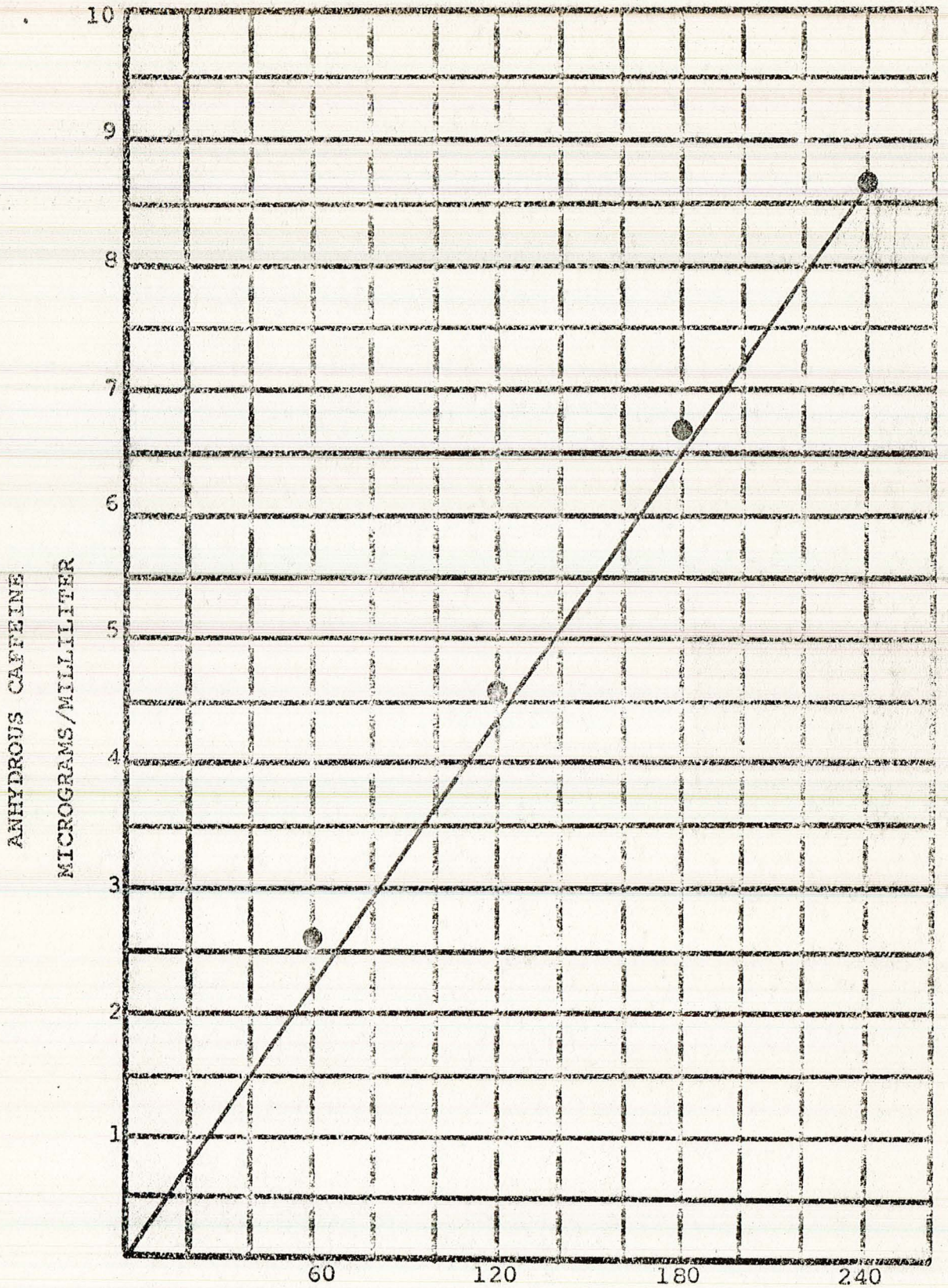
Graph 2

Dialysis Rate Curve for Visking Cellulose



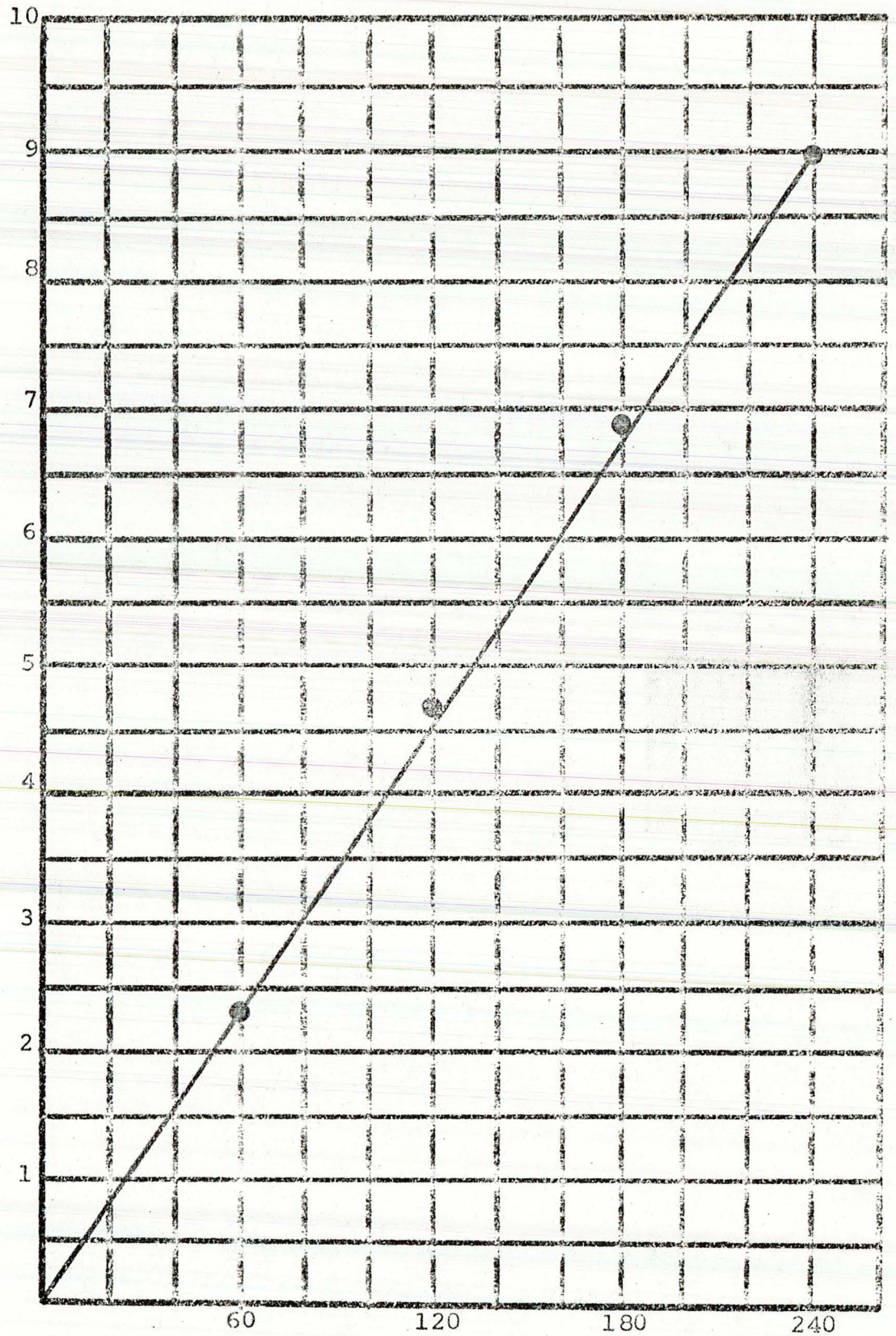
Graph 3

Dialysis Rate Curve for Gold Beater's Skin



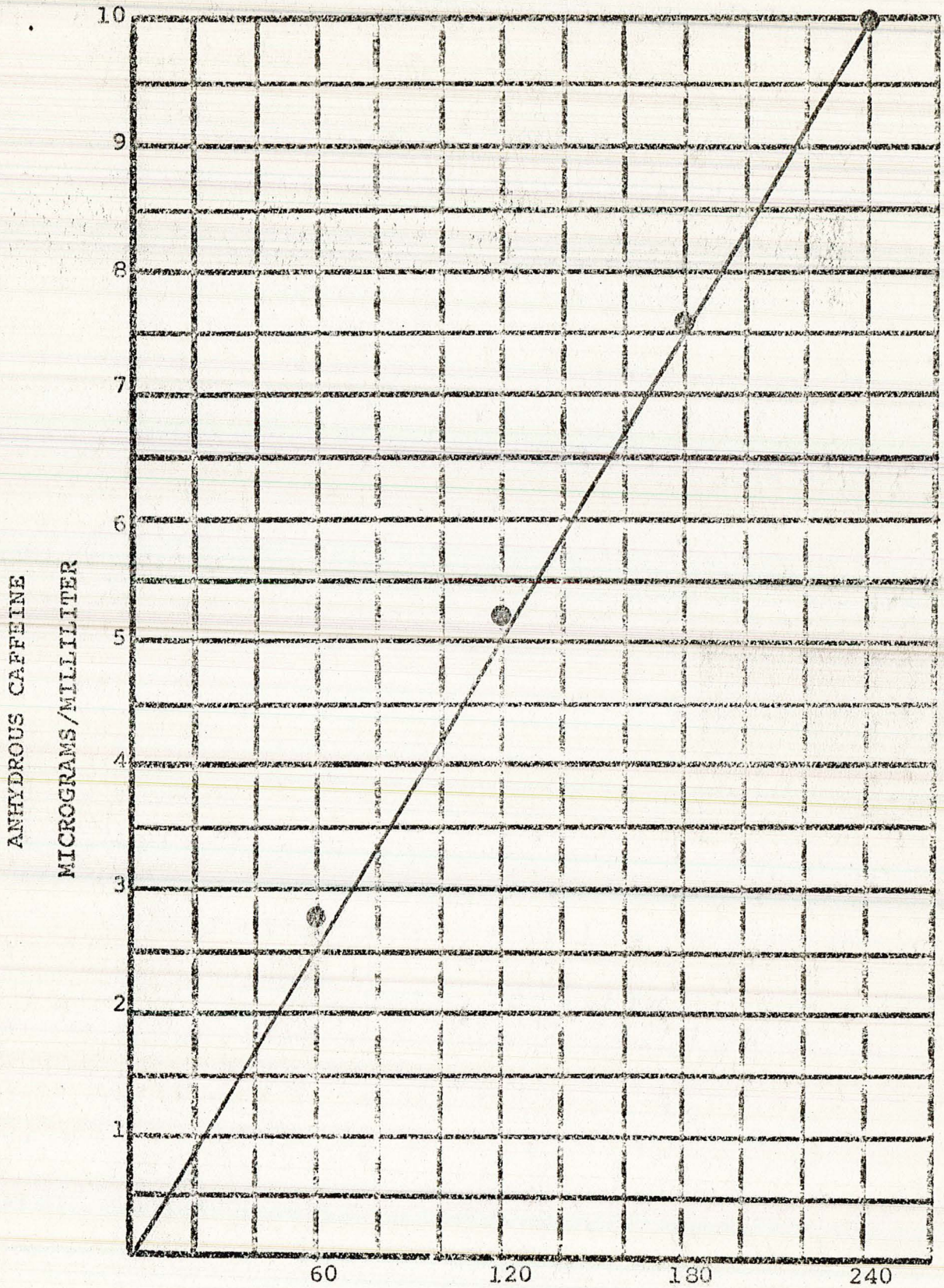
Graph 4

Dialysis Rate Curve for Millipore-MF



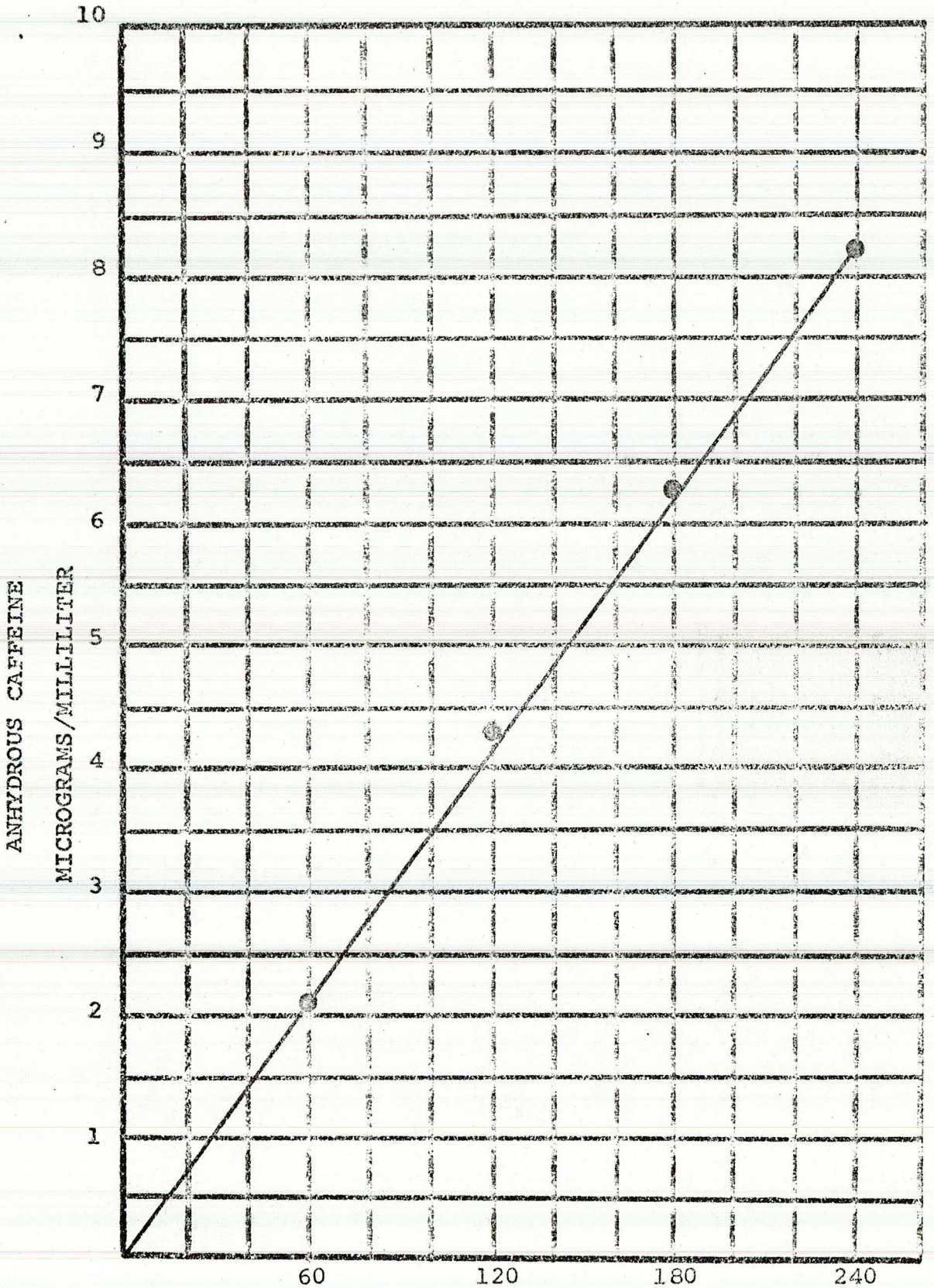
Graph 5

Dialysis Rate Curve for Celotate



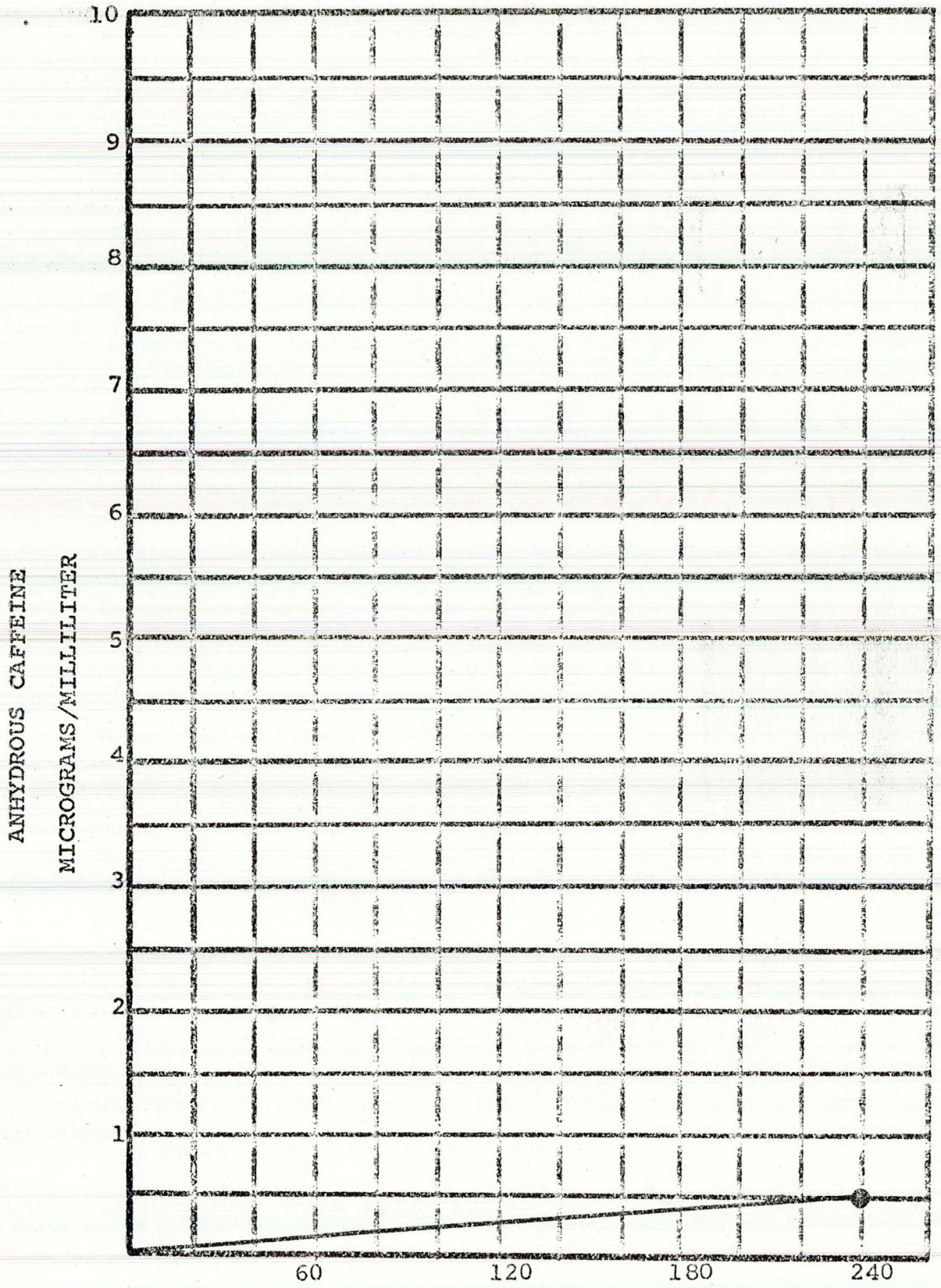
Graph 6

Dialysis Rate Curve for Polyvic



Graph 7

Dialysis Rate Curve for Pelicon-PS



Graph 8

Dialysis Rate Curve for Amicon UM-05

Chapter IV

DISCUSSION

A review of the results presented in Tables I and II is in order so as to critically evaluate the data such that a number of valid conclusions may be drawn from the experiment. Table I shows Polyvic, with a dialyzing efficiency rating of 50%, to be the best membrane overall. However, it must be remembered that it has an average pore size of 0.6 microns. Celotate, with an average pore size of 0.2 microns, and Millipore-MF, with an average pore size of 0.22 microns, have respective dialyzing efficiencies of 45.15 and 43.50%.

These figures give rise to several questions. First, one would wonder whether the Polyvic membrane would still have a 50% efficiency rating if it had an average pore size of 0.22 microns. It seems quite probable on first glance that this would not be the case. One method that could be devised to answer this question would be to test either a 0.5 micron Celotate film or a 0.65 micron Millipore-MF film and to see if they yield significantly higher dialysis rates. If so, one could then qualitatively infer with justification that a 0.22 micron Polyvic membrane probably would exhibit lower dialyzing efficiency rates than its 0.6 micron counterpart utilized in this study.

The degree of significance between the differences in

dialyzing rates of the Visking cellulose standard and the other more efficient films is a second area of concern involving the data. When gold beater's skin is compared to Polyvic and the latter is seen to be twice as efficient, the difference appears to be quite significant. However, when comparing the more efficient membranes to the Visking cellulose standard, there is seen at best, as in the case of Polyvic in comparison to Visking cellulose, only a 26% improvement figure. The degree of improvement reached by these membranes over the standard does not appear to be striking.

Comparing the two molecular-diffusion membranes, Pelicon-PS was far superior to the Amicon UM-05. However, it showed only about a 10% improvement over the cellulose standard. Some explanation should be given as to the reason for the unimpressive performance of the Amicon UM-05 in this particular study. According to Amicon literature (13), this membrane functions as an ultrafilter at pressure ranges of 10 to 100 psi. The very low pressure of 0.5 psi used in this study accounts for the apparent failure of this membrane to perform satisfactorily.

The Student "t" test values given in Table II indicate that the membranes fall into two groups. The first consists of Polyvic, gold beater's skin, and Amicon UM-05 with the probability value, P, being less than 0.001, while the second group consists of Celotate, Millipore-MF, and Pelicon-PS having P values ranging from less than 0.005 to greater than 0.001. The values imply that there actually is a difference among the dialyzing abilities of these six membranes compared to the standard, Visking cellulose. The results of the first

group appear to be very significant while the latter group, although somewhat less striking, are still significant.

The dialysis rate curves were organized as a block of evidence to confirm whether the dialyzing system was operating correctly. As has already been seen, it did indeed act as expected. Beyond this, the films exhibited a rather interesting feature. The solute particle transfer rates proceed in a slightly distorted logarithmic fashion. The rates of dialysis initially increase faster than normal and then decrease sooner than would be expected if the rates paralleled a normal logarithmic curve. This observation could be of great practical significance if applied to artificial hemodialysis technology. If this phenomena does occur in a blood system as it does in an aqueous system, then the simple method of serial dilution could be implemented as a tool to speed up the rate at which artificial hemodialysis proceeds in man. Thus, instead of only one large dialyzing bath being employed in the procedure to extract the waste products of the blood, several small fresh dialyzing baths may be utilized in successive fashion to reduce the time necessary to dialyze the blood. It is felt that this observation is of a good deal of practical importance.

There are a number of directions that are available as areas of extention for this preliminary work. After observing the success that this membrane testing method has had using aqueous solutions, a logical continuation of this inquiry would be to evaluate them in a situation in which they are exposed to blood on the dialyzing side of the film.

Thus, it would be of interest to determine how well the results of a blood system correlated with the data collected in this aqueous system. If they did parallel with each other, this method could act as a simple standardized procedure for the screening of new membrane materials suitable for hemodialysis.

Another second direction that might be of interest to pursue is the formulation of new membrane materials. The method utilized by Lyman (9, 10) and also Markle (5), where hydrophobic and hydrophilic polymers are combined in varying proportions to produce a cross-linked polymer certainly deserves further attention. A method of approaching this particular piece of work would be to gather together a group of urea soluble polymers along with a group of hydrophobic polymers and then cross link them systematically by varying the polymer concentrations. This area of investigation, according to the literature, certainly does not seem to be exhausted at this point. The reason for this seems to be that usually after an investigator discovers a fairly promising membrane, he then usually turns to blood studies to determine whether it possesses coagulant characteristics. If it does cause blood coagulation, they then may attempt to heparinize the polymers to decrease the membrane's clotting character. This was the chain of events that occurred in both Lyman's and Markle's investigations.

When trying to become acquainted with the dialyzing ability of various films, one cannot help but become familiar with the fundamentals of artificial hemodialysis. Probably

the most striking fact about this medical procedure is the paradox of how such a relatively simple process can be transformed into such an expensive operation. With this in mind, a number of ways occurred to the author on means to improve the machine's efficiency. Realizing that a normal human kidneys' nephrons have a surface area of about 15,000 $cm.^2$ and the artificial kidney coil also has about the same surface area, it seems that a method for compacting this surface area could act as a space-saving procedure. One way to implement this idea would be to produce, instead of the cylindrical dialysis tubing, convoluted tubing. In cross-section, it would have a starfish-like appearance. This quite probably would drastically reduce the size of the kidney coil.

The serial dilution technique previously described on page 44 would have a dual effect on the artificial kidney. First, it would probably decrease the amount of dialyzing fluid necessary for the procedure and in turn decrease the amount of space required to house the machine. Finally, it would decrease the time necessary for the procedure. All of the suggestions mentioned above point to the same goal which is the reduction of the amount of time and space required for artificial hemodialysis.

Chapter V

SUMMARY AND CONCLUSIONS

The work described here produced a number of results worthy of note. First, a relatively simple method was devised to evaluate the dialyzing efficiency of different membranous materials. More important, however, the results indicate the method yields reproducible results, as is rather emphatically indicated by data produced gold beater's skin and the Polyvic membranes. Also the statistical evaluation indicated the quality of the procedure, as the difference in dialyzing efficiency of the different trials was certainly not due to simple chance variations.

When compared to the Visking cellulose standard, five of the six films exhibited better dialyzing capacities. Only gold beater's skin, as expected, was found to be inferior to the standard. At this juncture, some consideration ought to be given to the economics of hemodialysis. Visking cellulose is by far the least expensive of all the films available. Normally sold as sausage casing, a very small percentage of it is sold to manufacturers of artificial kidneys. Despite its relative low cost, the coil purchased for this evaluation cost approximately $35.00. Table III shows the prices paid for the membranes used in the experiment. After noting these figures, it is easy to determine why Visking cellulose is still being used as the membrane of choice in kidney coils.

TABLE III

MEMBRANE COST INDEX

Membrane	Cost ($¢/cm.^2$)
Visking Cellulose	0.24
Millipore-MF	1.04
Celotate	1.04
Polyvic	1.04
Pelicon-PS	14.45
Amicon UM-05	37.60

If any of the three least expensive membranes were used, it would cost over $150.00 for the membrane material alone. When the economics of the procedure are balanced against dialyzing efficiency abilities, one would have to tend to let the economic advantages of the Visking cellulose overrule the efficiency advantages of the Millipore-MF, Celotate, Polyvic, and Pelicon films. Although these membranes had experimentally significant results, it is questionable whether the economic considerations would allow for their use in hemodialysis. The cost of new membranes should be at least on a par with that of Visking cellulose. Also if membranes are produced that can be reused, this would greatly aid in the overall cost reduction of this procedure.

This study was initiated with the intention of producing data that would allow for the comparative evaluation of

newer membrane materials having improved dialyzing capacities. With the increased demand for hemodialysis units, it is imperative that work be continued along these lines to develop and evaluate new membrane materials, as well as to design and fabricate improved techniques for removal of waste and toxic products from the blood.

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