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THE DIFFUSION OF DRUGS AND CHEMICALS THROUGH ISOLATED RABBIT MESENTERY

by

Latif Shawki Shenouda

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To My Parents

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INTRODUCTION

Poisonings, both accidental and intentional, claim many lives each year. Some form of poison accounts for more than 500,000 illnesses a year and approximately 5,000 deaths (1). It has been estimated that there are over 200 incidents of poisoning for each fatality. About one-half of the deaths was accidental with one-third occurring in children. The latest vital statistics reported, in 1963, 2,061 accidental and 3,399 suicidal deaths from solid and liquid substances (2), deaths from vapors and gases being listed separately.

Though many drugs and chemicals are involved in poisoning, the barbiturates and salicylates are probably the most common. Between the years of 1960 and 1963, death from barbituric acid derivatives and salicylates constituted 77-83% of the number of suicides committed through the use of analgesic and soporific substances (2-5), barbiturates contributing the largest proportion. Furthermore, statistics have shown that 28-32 percent of accidental deaths from solid and liquid substances are caused by these two kinds of poisons.

Treatment of poisoning involves the removal of the unabsorbed drug or chemical from the stomach, the administration of medicinals with antagonistic pharmacologic action, the injection of antidotes such as chelating agents in case of heavy metal poisoning, the injection of stimulants

in the case of respiratory depression, and some means of facilitating the removal of toxic materials from the blood such as promoting diuresis.

When the kidney is undamaged the promotion of diuresis may be quite helpful, yet this ordinarily does not remove the poisons as rapidly as desirable, and other methods may be attempted. Two important methods are employed for the removal of poisons, namely, hemodialysis and peritoneal dialysis. Hemodialysis employs an apparatus called the artificial kidney through which the blood is pumped, while peritoneal dialysis utilizes dialyzing fluids in the peritoneal cavity. Though slower than hemodialysis, peritoneal dialysis has many advantages. This technique can be used in every hospital (6,7). Its value was emphasized particularly in small hospitals (8). Palmer, et al. (9) suggested that the major advantages are reduced demands for special equipment, trained personnel, and the large blood supplies required for the extracorporeal dialyzer. Ellis and his associates (8) stressed the importance of this method in isolated hospitals where an artificial kidney is not available and the transfer of patients would prejudice recovery. Moreover, once established, the procedure can be managed by the nursing staff. In case of emergency, the technique can be used in a doctor's office for the treatment of acute poisoning.

Although some investigators (10, 11) have tried to increase the efficiency of peritoneal dialysis by the addition of drugs and other substances to the dialyzing

fluid, this remains as an intriguing and relatively unexplored concept.

In spite of the fact that peritoneal dialysis is quite valuable in the recovery of patients suffering from intoxication, it still remains a slow process. If the technique can be mechanically or chemically modified to achieve much faster removal of toxic substances, its value will be much enhanced. In order to proceed logically towards this goal, the mechanism of transference of drugs and chemicals across the peritoneum should be known.

LITERATURE REVIEW

Structure of the Peritoneum

In mammals, the so-called moist membranes consist of a joint product of an epithelium and its underlying connective tissue (12). They are kept moist by a watery exudate or a slimy secretion and, on this basis, are classified as serous and mucous membranes, respectively.

The serous membranes are abundant in body cavities. Each of the closed body cavities is bounded by a membranous sheet or tunica serosa. These membranes are specifically named the pericardium, pleura, and peritoneum.

The peritoneal cavity has been known since the earliest records of the dissections of animals and is described in the Eber papyrus, which was written about 3,000 B.C., as a definitely outlined cavity in which the viscera are somehow suspended (13). Detailed discussions regarding the structure of the peritoneum are presented by many authors (12-15).

Gross anatomy shows that the peritoneum consists of two parts: the parietal peritoneum which lines the external wall of tha abdominal cavity, and the visceral peritoneum which is reflected over the exposed surface of the protruding organs. The extent of the surface the peritoneum has to cover, together with the undulations and reduplications it undergoes, gives it a large surface area. It is correct to say that the area of the peritoneum is coextensive

with that of the surface of the body. Hertzler (14) and Grollman (16) gave the surface area of the peritoneum as approximately 22,000 cm^2.

The mesentery arises when the serosa leaves the abdominal wall to reflect around the gut. The parietal peritoneum, from each side, unites into a double sheet. Histologically, the mesentery has a layer of mesothelial cells on each free surface enclosing a layer of connective tissue, that is, the lamina propria. The mesothelium consists of simple, squamous, cellular plates. Their serrated edges interlock and their surface bears short micro-villi. Baron (15) has shown that the lamina propria consists of five parallel layers. These are: the basement membrane which lies under the mesothelium, followed by the superficial collagenous layer, the superficial unoriented elastic network, the deep longitudinal elastic network, and the deep latticed collagenous layer. The connective tissue varies regionally in density and thickness and is quite elastic. It carries blood vessels, lympatics, and nerves to the intestine. It also contains lymph nodes and usually considerable fat.

The dorsal and ventral mesentery of the stomach are called the omenta. The omentum resembles the mesentery in structure with the exception of holes which are present on its surface caused by the out-wandering cells. In those areas of the omentum which are not provided with holes, undifferentiated cells occur along the vessels, and macro-

phages are numerous. There are also many small lymphocytes and plasma cells, and occasionally eosinophile leukocytes and mast cells. The number of lymphocytes and plasma cells varies considerably in different animals. In certain areas the macrophages are accumulated in especially dense masses, such macroscopically visible areas often being arranged along the blood vessels as round or oval patches, called milky spots. These spots are prominent in the rabbit omentum. Baron (15) denied the presence of stomata or stigmata in the peritoneum; however, he stated that no conclusion should be drawn as to the existence of stomata in the mesothelium of this membrane. Furthermore, the same author, in 1937, detected the presence of stomata in the mesothelium of the diaphragmatic peritoneum and in some cases in that of the large intestine. Microscopic examination and electromicrographs of the peritoneum showed no pores; however, Gosselin and Berndt (17) indicated that pores might exist at the junction where three mesothelial cells meet. In spite of the enormous amount of research that has been done on these membranes, the presence of stomata and pores is still debatable.

Removal of Poisons Via the Peritoneum

The history of peritoneal lavage goes back to the latter part of the nineteenth century when Wegner (18) studied the effect of alterations of the peritoneal temperature on the body temperature of rabbits. In 1923, Putnam (19)

emphasized the importance of the living peritoneum as a dialyzing membrane. Since that time methods have been developed to utilize the peritoneum as a filtering medium for the removal of toxic materials from the blood. The first technique to be used in hospitals was continuous peritoneal dialysis. This is performed by fixing two catheters in the abdominal cavity (20) and allowing the irrigation fluid to flow continually through the peritoneal cavity. This method was used until the introduction of intermittent peritoneal dialysis by Reid et al. (21,22) in 1946, and was evaluated in this country by Grollman and his co-workers, in 1951 (23). The intermittent method consists of inserting a tube in the abdominal cavity through which the irrigation fluid, equilibrated to body temperature, is allowed to flow in by gravity. At a given time the perfusate is removed through the same tube, again by gravity. Thereafter, fresh solution is admitted. This technique offers some advantages over the continuous method (20): there is less leakage, no direct channel from inflow to outflow which diminishes the effectiveness of dialysis, less chance to perforate the intestines during the introduction of the tubes and, finally, less danger of overhydration and infection.

The early application of peritoneal dialysis was in renal disease. The technique has been widely used in the treatment of acute and chronic renal failure. The effectiveness of peritoneal dialysis in acute renal failure is

indicated by the drop in the rate of death from 60 to 45 percent (24). This technique is also valuable in the treatment of hopeless cases of chronic renal insufficiency. Ellis and his associates (8) achieved temporary clinical improvement in two out of three chronic cases. Prolongation of life of six patients suffering from chronic glomerulonephritis was reported by Cohen (25). The clinical and biochemical manifestations of uremia were ameliorated using the technique of intermittent peritoneal dialysis (26,27). In some patients suffering from advanced uremia and hyperkalemia, and in others with refractory pulmonary edema, the procedure was considered life-saving.

In addition to substituting for the kidney, dialysis is used to supplement the kidney in acute poisoning, whether the poison be exogenous or endogenous. Maher (24) listed the following criteria to judge the efficacy of dialysis in acute poisoning: 1. The poison molecule must diffuse through the peritoneum from plasma water at a reasonable rate 2. The poison should be well distributed in accessible body fluid compartments with minimal protein binding or localization in isolated fluid compartments. 3. Toxicity should bear a relationship to the blood concentration and duration of the body's exposure to the circulating poison. 4. The quantity of poison dialyzed must constitute a significant addition to the normal body mechanism for dealing the particular poison under the physiologic circumstances encountered.

The major types of exogenous poisons which can be treated by dialysis include barbiturates, glutethimide, salicylates, and methyl alcohol. Endogenous intoxications other than uremic syndrome may also be amenable to dialysis. These include hypercalcemia, uric acid poisoning, hepatic coma due to ammonia, and hyperbilirubinemia.

Clinical use of peritoneal dialysis in cases of barbiturate intoxication have been reported by many investigators (24, 28-30). Maxwell et al. (28) treated a case of secobarbital-amobarbital intoxication caused by the ingestion of 6 Gm. of these hypnotics. Application of peritoneal dialysis using 56 liters of standard dialyzing fluid for a period of 30 hours removed 1.620 Gm. with subsequent recovery of the patient. Similar results were achieved in five other patients with acute barbiturate poisoning (29). The barbiturate blood levels in these patients varied from 1.5 mg.% to 5 mg.%. The amount of barbiturate removed by dialysis was 5 to 20 times the amount excreted by the kidney. Berman and Vogelsang (30) were successful in recovering four patients suffering from secobarbital, pentobarbital, and phenobarbital intoxication by using two types of peritoneal dialysis. The popularity of peritoneal dialysis in the treatment of barbiturate intoxication is reflected in an article by Maher (24), who reviewed his experience with this group of poisons. He indicated that 15% of patients with acute barbiturate poisoning in his geographic area are referred to dialysis, and two-thirds of these undergo peritoneal dialysis.

Glutethimide is another poison which was effectively removed from the blood stream by peritoneal dialysis. It was reported (24) that 13 of 37 patients with glutethimide poisoning were treated with hemodialysis, and the rest were treated by peritoneal dialysis. The latter method was efficient in recovering 75% of the treated cases. The usefulness of peritoneal dialysis in the removal of poisons was extended to acute cases of salicylism (10,31), and methyl alcohol poisoning (32). The technique was equally effective in the treatment of boric acid poisoning (33), and hypernatremia due to accidental ingestion of sodium chloride in children (34). Since no antidote is known for boric acid, the treatment of acute cases of this poison can be accomplished by blood transfusion. However, a four-hour period of peritoneal dialysis removed approximately the same amount of boric acid as could be removed by blood transfusion.

Hepatic coma may be caused by high blood level of ammonia and bilirubin. Jones et al. (35) were successful in removing the large amounts of ammonia in the blood by peritoneal dialysis. The recovery of the patient was prompt due to the rapid diffusion of the ammonium ion across the peritoneum. The use of peritoneal dialysis in cases of hyperbilirubinemia (11,28) led either to temporary improvement or complete recovery. Hypercalcemia (28) is another example of endogenous poisoning which was treated with success using peritoneal dialysis.

From these reports, it is apparent that the living peritoneum may serve as a major channel for removal of a great variety of poisons from the body, a fact that makes it one of the most interesting membranes in the human body.

Diffusion Across the Peritoneum

Theoretical Aspects of Diffusion

Diffusion is a process by which matter is transported from one part of a system to another as a result of random molecular motion. The mathematical theory of diffusion in isotropic substances is based on the hypothesis that the rate of transfer of a diffusing substance through a unit area of a section is proportional to the concentration gradient measured normal to that section. This is expressed by Fick's first law of diffusion assuming a constant diffusion coefficient (36).

$$G = -D \frac{\partial c}{\partial x} \qquad (1)$$

where G is the rate of transfer per unit area of a section, c the concentration of the diffusing substance in a given layer of the section, x the space coordinate measured normal to the section, and D the diffusion coefficient. In order to determine the diffusion coefficient using equation (1), an arrangement should be made in which the flux, G, and $\partial c/\partial x$ are accessible to measurement.

The fundamental differential equation of diffusion in an isotropic medium is derived from equation (1) as follows (37). Consider an element of volume in the form

of a rectangular parallelpiped whose sides are parallel to the coordinate axes and are of lengths, 2dx, 2dy, 2dz. Let the center of the element be at P(x, y, z). where the concentration of the diffusing substance is c. Let A B C D and A'B'C'D' be the faces perpendicular to the x-axis as in Figure 1. The rate at which the diffusing substance enters the element through the face A B C D in the plane x-dx is given by

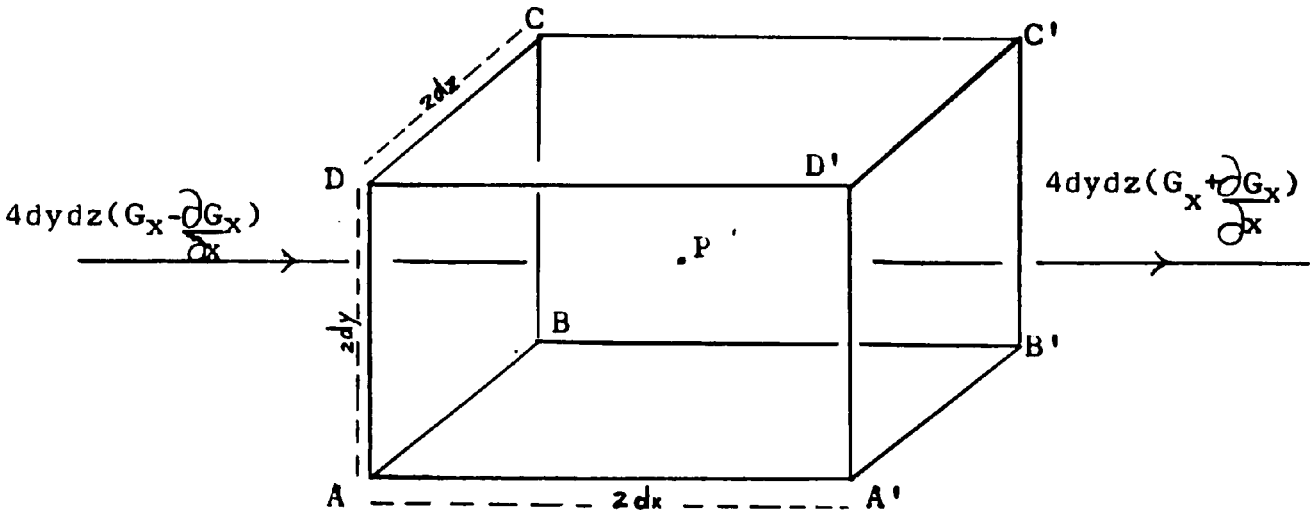


Figure 1. - Element of volume

$$4\ dy\ dz \quad \left(G_x - \frac{\partial G_x}{\partial x}\ dx\right) \tag{2}$$

where G_x is the rate of transfer through a unit area of the corresponding plane through P. Similarly, the rate of loss of diffusing substance through the face A'B'C'D' is given by

$$4\ dy\ dz \quad \left(G_x + \frac{\partial G_x}{\partial x}\ dx\right) \tag{3}$$

The contribution to the rate of increase of diffusing substance in the element from these two faces is thus equal to

$$-8\ dx\ dy\ dz\ \frac{\partial G_x}{\partial x} \tag{4}$$

Similarly from the other faces, the following quantities are obtained

$$-8\ dx\ dy\ dz\ \frac{\partial G_y}{\partial y}\ , \qquad \text{and} \qquad -8\ dx\ dy\ dz\ \frac{\partial G_z}{\partial z} \tag{5}$$

But the rate, at which the amount of diffusing substance in the element increases, is also given by

$$8\ dx\ dy\ dz\ \frac{\partial c}{\partial t} \tag{6}$$

Hence,

$$\frac{\partial c}{\partial t} + \frac{\partial G_x}{\partial x} + \frac{\partial G_y}{\partial y} + \frac{\partial G_z}{\partial z} = 0 \tag{7}$$

Assuming a constant diffusion coefficient, and substituting for $\underline{G_x}$, $\underline{G_y}$, and $\underline{G_z}$ using equation (1), equation (7) becomes,

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}\right) \qquad (8)$$

If diffusion is one-dimensional, that is, if there is a gradient of concentration only along the x axis, equation (8) reduces to

$$\frac{dc}{dt} = D\frac{d^2 c}{dx^2} \qquad (9)$$

Equation (9) is known as Fick's second law of diffusion in one dimension. It is identical with equation (1) except that it represents an expression in terms of concentrations which are experimentally measurable instead of molecular flux which must be determined indirectly.

The general solution of equation (9) is approached by many methods, one of which is the separation of variables. Let

$$c = X(x) \cdot T(t) \qquad (10)$$

where $\underline{X}$ and $\underline{T}$ are functions of $\underline{x}$ and $\underline{t}$ respectively. Substitution in equation (9) using (10) yields,

$$X\frac{dT}{dt} = DT\frac{d^2 X}{d x^2} \qquad (11)$$

which may be rewritten as

$$\frac{1}{T} \cdot \frac{dT}{dt} = \frac{D}{X}\frac{d^2 X}{d x^2} \qquad (12)$$

The expression on the left-hand side of equation (12) is dependent on t only, while that on the right-hand side is dependent on x only. They can be equal only if each one is equal to the same constant, independent of t and x. Let this constant be $-\lambda^2 D$, and hence

$$\frac{1}{T} \cdot \frac{dT}{dt} = -\lambda^2 D \qquad (13)$$

and

$$\frac{1}{X} \cdot \frac{d^2 X}{d x} = -\lambda^2 \qquad (14)$$

The general solutions of the two differential equations (13) and (14) are,

$$T = \exp (-\lambda^2 Dt) \qquad (15)$$

and

$$X = A \sin x + B \cos x \qquad (16)$$

Therefore, equation (10) after substitution becomes,

$$c = (A \sin x + B \cos x) \exp (-\lambda^2 Dt) \qquad (17)$$

The general form of equation (17) can be easily obtained by setting the initial and boundary conditions for each specific problem.

The transfer of chemicals through a membrane or barrier separating two well-stirred compartments represents the diffusion through a finite region. Considering a membrane of thickness h with the following initial and boundary conditions,

$$c = C_1 \quad \text{at } x = h \quad \text{for } t \geq 0$$

$$c = 0 \quad \text{at } x = 0 \quad \text{for } t > 0$$

and

$$c_o = 0 \quad \text{at } 0 \leq x \leq h \quad \text{for } t = 0 \qquad (18)$$

where c_o is the initial concentration within the membrane. The general solution of equation (9) for this set of conditions is

$$c = \frac{C_1 x}{h} + \frac{2C_1}{\pi} \sum_{n=1}^{\infty} \frac{\text{Cos } n\pi}{n} \text{ Sin } \frac{n\pi x}{h} \exp\left(-\frac{Dn^2\pi^2 t}{h^2}\right) \qquad (19)$$

Differentiation of equation (19) with respect to x and evaluation at $x = 0$ yields,

$$\left(\frac{dc}{dx}\right)_{x=0} = \frac{C_1}{h} + \frac{2C_1}{h} \sum_{n=1}^{\infty} \text{Cos } n\pi \exp\left(-\frac{D\, n^2\pi^2 t}{h^2}\right) \qquad (20)$$

Integration of equation (20) with respect to t yields an expression for Q_t, which is the amount diffused per unit area,

$$Q_t = \frac{DC_1 t}{h} + \frac{2C_1 h}{\pi^2} \sum_{n=1}^{\infty} \frac{\text{Cos } n\pi}{n^2} \left[1 - \exp\left(-\frac{Dn^2\pi^2 t}{h^2}\right)\right] \qquad (21)$$

Since

$$\sum_{n=1}^{\infty} \frac{\text{Cos } n\pi}{n^2} \longrightarrow \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} = -\frac{\pi^2}{12} \qquad (22)$$

Equation (21) can be rearranged and reduced to

$$\frac{Q_t}{C_1 h} = \frac{Dt}{h^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(-Dn^2\pi^2 t/h\right) \qquad (23)$$

As t becomes infinitely large, the exponential portion of both equations (23) and (19) drops out, and the reduced forms are, respectively:

$$Q_t = \frac{DC_1}{h} \left(t - h^2/6D \right) \tag{24}$$

and

$$c \approx \frac{C_1 x}{h} \tag{25}$$

The term $h^2/6D$ represents the lag time which is necessary to establish a steady state. On plotting Q_t *versus* time, a curve is obtained which becomes linear after achieving a steady state. Extrapolation of the straight line portion to the time axis gives a value of $h^2/6D$ for the lag period. This can be used to determine the diffusion coefficient of compounds through a membrane with a known thickness. On the other hand, equation (25) indicates the concentration in the membrane becomes linear with x, that is, the change in concentration is constant. Therefore, the diffusion equation in one dimension becomes

$$D \frac{d^2c}{dx^2} = 0 \tag{26}$$

Integration of equation (26) with respect to x, gives

$$\frac{dc}{dx} = \text{Constant} \tag{27}$$

and consequently

$$c = c_o + c_1 x \tag{28}$$

This means a constant concentration gradient and a linear distribution of concentration exist within the membrane, provided that the diffusion coefficient is constant (36). In other words,

$$\frac{dc}{dt} = 0 \qquad (29)$$

It is difficult to achieve the steady state experimentally, hence the concept of a quasi-steady state is adopted. This concept allows the treatment of those cases which nearly meet the steady state requirements. In a quasi-steady state neither the concentration nor the concentration gradient are exactly constant but if this change is small enough, that is,

$$\frac{dc}{dt} \approx 0 \qquad (30)$$

the assumption of constant concentration gradient does not give rise to serious errors.

This concept was applied experimentally by Lueck et al. (38) in their study of diffusion of solutes through mechanical barriers. It was also applied by Gosselin and Berndt (17) in their experiments of diffusion of Rb^+ across the isolated rabbit mesentery, and by Chang and Wilke (39) in studying the diffusion of electrolytes using the diaphragm cell method.

Mechanism of Diffusion Across the Peritoneal Membrane

It has been shown that there are two means of transference of substances across the peritoneum, osmosis and diffusion. Osmosis controls the movement of water or solutes depending on the tonicity of peritoneal fluids compared to those of body fluids. On the other hand, the transference of ions and molecules across the peritoneal membrane under isoosmotic conditions takes place by diffusion in accord with their individual concentration gradients. Putnam (19) suggested three modes of transmission either from or to the peritoneal cavity: passage of small molecules through a permeable or semi-permeable membrane, passage of small or large molecules and perhaps particulate matter through microscopic or ultramicroscopic spaces, and some form of "vital activity," of which the most obvious example is the phagocytosis of microscopic particles and fluid droplets by the serosa cells.

The mechanism of diffusion across the peritoneal membrane is not fully understood. There are two schools of thought regarding the mode of transference of chemicals through the membrane. The first claims the passage of ions and molecules through pores, whereas the second demands lipid solubility in the membrane as a means of diffusion. One series of studies on isolated mesenteric membrane of the rabbit was reported by Gosselin and Berndt (17, 40-42). These authors attempted to determine the mechanism of diffusion across the peritoneum. Measurement of the <u>in vitro</u> permeability of the

mesentery and peritoneum to ^{86}Rb, ^{32}P, and creatinine was done using a simple technique (40). The method consisted of tying a piece of rabbit mesentery, freed from fat and blood vessels, over the lip end of a glass tube 13 mm. in diameter and 100 mm. long. This tube was suspended in a 10-ml. beaker containing 8 ml. of solvent. The fluids outside and inside the tube were maintained at the same level during the experiment to avoid any difference in hydrostatic pressure. Both fluids were circulated through the counter for radioactive ion measurement. Since, with their arrangement the source concentration and sink volume were held constant, the data were analyzed using the first-order equation,

$$(C_1 - C_2) = (C_1 - C_o)\ e^{-kt} \qquad (31)$$

where C is the concentration of radioactivity in counts per minute per volume of solution contained in the well counter (approximately 0.75 ml.), C_2 is the radioactivity in the sink at any time, t, C_o is the radioactivity in the sink at zero time (usually zero in their experiments), C_1 is the concentration of the source solution, and k is the first order rate constant in $min.^{-1}$ It was believed that the mechanism of diffusion is a passive one and is not due to the net solute migration against an electrochemical gradient, or in other words, not due to active transport.

The diffusion of ions and molecules through the mesentery was analyzed in two different ways (17). First, the membrane was treated as a single homogeneous barrier in which the process of diffusion was expressed by the following empirical equation.

$$\frac{dQ}{dt} = KA\,(C_1 - C_2) \qquad (32)$$

where dQ/dt is the instantaneous flux, A the area of the specimen in cm^2., $(C_1 - C_2)$ the concentration difference across, and K is the permeability constant (cm./sec.). If diffusion is the sole mode of transport, therefore,

$$K = \frac{D\epsilon}{h} \qquad (34)$$

where D is the diffusion coefficient of the substance, ϵ the aggregate cross-sectional area of the diffusion channels per square centimeter of the mesentery, and h the average length of these channels. Using an estimated value of 2.5×10^{-5} $cm.^2/sec.$ for the diffusion coefficient of Rb^+, experimental values for K indicated that the pores should have an aggregate cross-sectional area of at least 10% of the macroscopic surface area of the mesentery (porosity of 0.10).

In the second analysis (17), the mesenteric membrane was treated as a three phase system through which solutes diffuse in series. If C_1 and C_2 are the solute concentrations maintained at the two free surfaces of the membrane, the concentration gradient in the first layer is $\Delta C/h_1$, and is

denoted by $\underline{g_1}$ provided that a steady state is established within each layer. Therefore, the following relations exist:

$$\frac{1}{A} \cdot \frac{dQ}{dt} = D_1 \epsilon_1 g_1 = D_2 \epsilon_2 g_2 = D_3 \epsilon_3 g_3 \qquad (35)$$

and

$$h_1 g_1 + h_2 g_2 + h_3 g_3 = (C_1 - C_2) \qquad (36)$$

By eliminating g's the flux per unit area becomes:

$$\frac{1}{A} \cdot \frac{dQ}{dt} = \frac{D_1\epsilon_1 \cdot D_2\epsilon_2 \cdot D_3\epsilon_3 (C_1 - C_2)}{h_1(D_2\epsilon_2 \cdot D_3\epsilon_3) + h_2(D_1\epsilon_1 \cdot D_3\epsilon_3) + h_3(D_1\epsilon_1 \cdot D_2\epsilon_2)} \qquad (37)$$

This relation is analogous with equation (32), hence

$$K = \frac{D_1\epsilon_1 \cdot D_2\epsilon_2 \cdot D_3\epsilon_3}{h_1(D_2\epsilon_2 \cdot D_3\epsilon_3) + h_2(D_1\epsilon_1 \cdot D_3\epsilon_3) + h_3(D_1\epsilon_1 \cdot D_2\epsilon_2)} \qquad (38)$$

For the mesentery, layers 1 and 3 are equivalent and it is believed that the diffusion is considerably impeded by them. In other words $\underline{D_1\epsilon_1}$ and $\underline{D_3\epsilon_3}$ are presumably small. The loose areolar connective tissue which constitutes layer 2 does not hinder diffusion and as a result $\underline{D_2\epsilon_2}$ is comparatively large. Due to the symmetric nature of the mesentery, the following relations are considered valid:

$$h_1 = h_2, \text{ and } D_1\epsilon_1 = D_3\epsilon_3 \qquad (39)$$

By substituting and rearranging the terms in equation (38),

$$D_1\epsilon_1 = \frac{2 K h_1 (D_2\epsilon_2)}{D_2\epsilon_2 - K h_2} \qquad (40)$$

All the terms on the right hand side of equation (40) can be estimated with reasonable confindence. The authors (17) assigned a vlaue of one micron for h_1 and consequently h_2 has a value of 36.5 microns, since the average measured thickness of the mesentery of ten pieces obtained from six different young albino rabbits is 38.5 microns (standard error 1.9). It was assumed that layer 2 behaves like a gel and that diffusion of ions through this layer is similar to their behavior in free solution, and hence, ϵ_2 is assumed to be unity. Calculations showed that the pores should occupy an average of 0.6% of the area of the mesentery, provided that the Rb^+ passes freely through the pores that lie between the mesothelial cells. In a similar treatment of the parietal peritoneum which consists only of layers 1 and 2 and whose average thickness is 38.1 microns, the estimated pore area of this membrane was 0.2% of the total area. Although this treatment of the three-phase system is interesting, its results contradict the authors' observations (17) concerning the transudation of the red blood cells through these membranes. Consequently, it appears that there must be pores which transversely cross the mesenteric membranes, thus allowing the passage of erythrocytes having mean diameter of 7.2 microns (43) and small ions such as rubidium whose diameter is 2.96 Å (44). If the membrane is analyzed as a single homogeneous barrier, the calculated porosities for the mesentery and the peritoneum are approximately 0.11 and 0.07, respectively.

Knochel and his associates (45) believed that it is the undissociated form of phenobarbital that diffuses through the peritoneum. They arrived at this conclusion from their studies of the effect of THAM, tris (hydroxymethyl) aminomethane, on the rate of removal of phenobarbital in experimentally intoxicated dogs by peritoneal dialysis.

Though passive diffusion can be the sole mode of transport across the peritoneum, it is still not decisive whether this porcess takes place through holes and/or lipid solubility. This is because the peritoneum does not discriminate between lipid-soluble and lipid-insoluble substances, ions or molecules.

The rates of diffusion of substances may be different depending on their diffusion coefficients, ionic and molecular sizes, steric factors, and the degree of hydration. In natural membranes, pores might be normally distributed. For a measurable rate of diffusion, it is required that a certain percentage of the whole population of the pores should have diameters many times greater than the largest molecules that were found to diffuse freely through these membranes. However, it is worthy to mention that a case of restricted diffusion might occur if the pore diameter is of comparable value to that of the diffusing molecule (46). Rappenheimer (46) indicated that capillary permeability decreased with an increase in the size of the diffusing molecules and ions, and a similar situation may occur with the peritoneal membrane.

Diffusion Rates of Various Substances

Diffusion rates have been described in terms of peritoneal clearance or clearance ratio. Clearance is calculated from the Van Slyke's formula (20):

$$C = \frac{UV}{P} \tag{41}$$

in which C is the clearance in ml. per minute, U is the concentration in the irrigation fluid (outflow), P is the blood or plasma concentration, and V is the dialysant in ml. per minute.

The peritoneal clearance of urea is used as a reference point to compare the peritoneal clearance of other substances. The clearance ratio R of a substance x is its clearance C_x, expressed as percentage of urea clearance.

$$R = \frac{C_x}{C_{urea}} \times 100 \tag{42}$$

Most of the clinical data for many substances in the literature are expressed in terms of their clearance ratios. Diffusion curves of various substances are established by plotting the concentration ratio, which is the ratio of concentration of substance in the peritoneal fluid to that of the blood, *versus* time. Figure (2) illustrates the simultaneous diffusion curves obtained from clinical data in one patient for urea, creatinine, and uric acid (47). Boen (47) used the peritoneal clearance to compare different

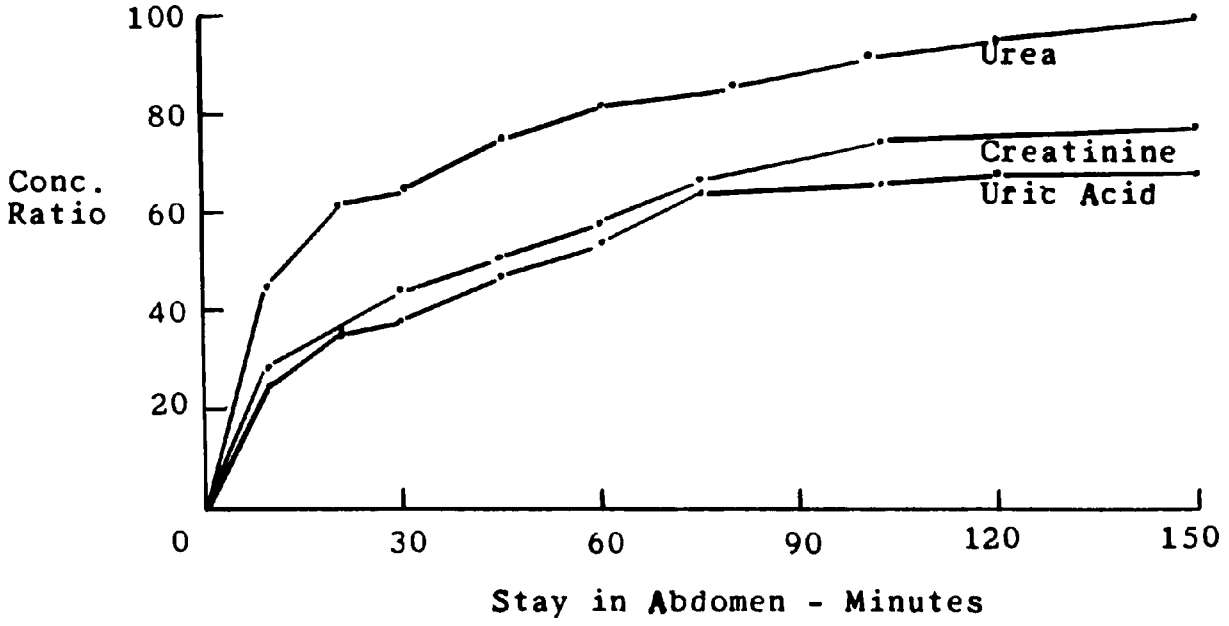


Figure 2. - Simultaneous diffusion curves for urea, creatinine, and uric acid. [From Boen (47)].

diffusing ions and molecules. He listed the peritoneal clearance of various substances in decreasing order: urea > K > inorganic phosphate > creatinine > uric acid Ca^{++} > Mg^{++} > indican. This is consistent with the diffusion curves, shown in Figure 2, which compares the diffusion of creatinine and uric acid to that of urea.

However convenient clearance values may be to the clinician, its interpretation is confined by the fact that it is not a constant which can be applied directly to a

variety of conditions. From equation (41) it is seen that the clearance will change with the rate of flow of the dialysis fluid and, since the measurement is not limited to equilibrium conditions, it will vary with the blood level. Also, the technique used for peritoneal dialysis involves generally not a constant flow of dialysis fluid but an intermittent flow with variable dwell time. Thus, only when the procedure is standardized can clearance values be directly compared.

Factors Affecting Rate of Peritoneal Dialysis

There are many factors that seem to control the rate of diffusion across the peritoneal membrane. Generally speaking, the higher the concentration in the blood, the higher the rate of removal. The volume of the dialysis fluid is another factor. It has been shown (47) that clearance increases with increase in the volume of dialyzing fluid until it reaches a maximum value that is characteristic for a specific compound and then it starts to decrease. For example, the clearance of urea is 25 ml./min. and 28.5 ml./min. for volumes of dialyzing fluid of 2.5 and 3.5 L., respectively. A drop in the urea clearance was observed when the volume of the dialyzing fluid was increased beyond 3.5 L. Furthermore, the peritoneal clearance is valuable in calculating the amount of a drug removed during continuous dialysis. Boen (47) showed that if the blood urea level is kept constant at 3 Gm./L. during the

whole dialysis, and if the peritoneal clearance is 25 ml./min., 4.50 Gm. of urea will be removed per hour according to equation (41).

To illustrate the effect of the volume of dialysis fluid, consider a hypothetical case of uremia in a patient whose level of urea is kept constant at 3 Gm./L. during the process of intermittent dialysis, assuming that the patient has a body weight of 60 Kg., and urea distribution of 60% of his weight. The surface area of the peritoneum, $\underline{A}$, is 22,000 cm^2 (16). Assume that the human peritoneum is similar to that of the rabbit, therefore, the reported values, by Gosselin and Berndt (17), for membrane porosity, $\underline{\epsilon}$, of 0.10 and membrane thickness of 38.5 microns are used. The diffusion coefficient of urea, $\underline{D}$, is 1.018×10^{-3} $cm^2/min.$ (this work). Therefore, the rate of diffusion across the peritoneum can be expressed by equation (31) in which C_o, initial concentration of the peritoneal fluid, is equal to zero. In rearranging equation (31), the following equation results;

$$1 - \frac{C_2}{C_1} = e^{-kt} \qquad (43)$$

where $\underline{C_2}$ is the concentration of urea in the peritoneal fluid, $\underline{C_1}$, is the constant concentration of urea in blood and body fluids, $\underline{k}$, is the first order rate constant defined as

$$k = \frac{DA\epsilon}{hv} \qquad (44)$$

D, A, ϵ, h, are defined before, and v is the volume of the dialysis fluid. Equation (43) is used to calculate the amount of urea diffused, during intermittent dialysis, for two values of v, that is, 2.5 and 3.5 L. The calculated results are shown in Table 1. The table indicates that equilibrium is reached within a short time, 30 minutes when the volume of dialysis fluid is 2.5 L., 45 minutes when the volume of dialysis fluid is increased by one liter. At equilibrium 7.50 and 10.50 Gm. of urea should be removed for the two volumes respectively.

These theoretical calculations suggest that short dwell times are required for better efficiency of intermittent dialysis. This is impractical because at least 15 minutes is needed for the introduction and withdrawal of the dialysis fluid to and from the peritoneal cavity.

In case of continuous dialysis, the rate of diffusion across the peritoneum should be independent of the volume of the dialysis fluid, that is, v is infinitely large. At any time, t, during the dialysis C_2 is much smaller than C_1 ; hence equation (43) reduces to

$$C_2 = C_1 \, kt \tag{45}$$

where k is defined as,

$$k = \frac{DA\epsilon}{h} \tag{46}$$

Calculations show that 104.700 Gm. of urea should be removed in a period of one hour, which is equivalent to about 97% of the total amount of urea in the body.

TABLE 1

THEORETICAL AMOUNTS OF UREA DIFFUSED AT A CONSTANT BLOOD LEVEL OF 3 Gm./L. DURING INTERMITTENT PERITONEAL DIALYSIS FOR TWO DIFFERENT VOLUMES OF DIALYSIS FLUID AT 37°C.

Time Min.	2.5 L. Dialysis Fluid		3.5 L. Dialysis Fluid	
	Conc. of Urea in the Dialysis Fluid Mg./Ml.	Total Amount of Urea Diffused Gm.	Conc. of Urea in the Dialysis Fluid Mg./Ml.	Total Amount of Urea Diffused Gm.
5	2.065	5.162	1.692	5.992
10	2.708	6.770	2.430	8.505
15	2.909	7.272	2.751	9.628
20	2.971	7.427	2.892	10.122
25	2.991	7.477	2.953	10.335
30	2.997	7.492	2.979	10.426
35	.....	.....	2.991	10.468
40	.....	.....	2.996	10.486
45	.....	.....	2.998	10.493

The amount of urea removed in a period of one-half hour is about five and seven times larger than the corresponding amount diffused during the same period of intermittent dialysis for volumes of dialysis fluid equivalent to 3.5 and 2.5 L. respectively. This indicates that continuous dialysis, though not popular nowadays, can be more effective than the intermittent process.

According to these calculations, the total amount of urea diffused per hour by the continuous process is approximately the same as the quantity removed per day as calculated by Boen using equation (41). This suggests that the experimentally determined peritoneal clearance using the Van Slyke's formula may not represent the true rates of diffusion of urea across the peritoneum. Furthermore, the fact that these clearances decrease beyond a certain point in a continuous dialysis is an indication that diffusion is not occurring at its maximum rate but is limited by other factors.

Mixing within the peritoneal cavity may also affect the dialysis rate. During intermittent dialysis, the dialysis fluid within the peritoneum is subject to slight mixing. The mixing is due to movement of the diaphragm during breathing and possibly some peristaltic action of the intestines. Poor mixing of the dialyzing fluid inside the peritoneal cavity may decrease the rate of diffusion. This may be due to the stagnation of the fluid in the neighborhood of the membrane, and hence there

will be an additional diffusion layer barrier next to the membrane which may slow the diffusion process. If the dialysis procedure is designed in such a way as to provide better mixing in the form of turbulence inside the peritoneal cavity, the rate of removal of substances may be improved. Therefore, better mixing may give values for the amount removed by peritoneal dialysis which are comparable to those obtained by hemodialysis. This is because of the fact that the peritoneum provides a larger surface area (22,000 cm^2) for dialysis than the Kloff twin-coil artificial kidney with surface area of 18,000 cm^2 (28).

The effect of pH on the diffusion of ^{86}Rb (41) and phenobarbital (45) across the peritoneum has been studied. In case of Rb^+ diffusion, an increase or decrease of pH by one unit from 7.4 showed a marked increase in permeability that varied between 20% and 100%. It should be noted that only the pH of the source solution was changed. The pH's of both the source and the sink solution were not checked at the end of each experiment. According to the author's observation (41) the pH did modify the permeability of the membrane. However, it would have been more informative if the solutions on both sides of the membrane had the same hydrogen ion concentration.

Further experiments to demonstrate the effect of pH of the dialyzing fluid on the removal rates of phenobarbital in experimentally intoxicated dogs were conducted by Knochel *et al.*(45). Using THAM as a buffer in a con-

centration of 0.074 molar in the dialyzing fluid resulted in a solution of pH 10. It was found that the amount of phenobarbital removed with this buffer was almost double the amount removed using a standard dialyzing fluid at pH 5. At the pH of the plasma, the undissociated form of phenobarbital, whose pKa is 7.2, amounts to 61.3%. It was believed that the increased rate of removal caused by THAM was due to the diffusion of the undissociated form through the peritoneum which in turn dissociated in the new environment of different pH. This dissociation in the alkaline peritoneal fluid would maintain a concentration gradient favoring further diffusion of undissociated drug from the blood to the peritoneal cavity. The peritoneal pH at the end of 30 minutes of dialysis was 8.4. Alkalinization of the urine, increase of the urine flow, and increase in the osmolar clearance were observed when THAM was incorporated in the dialysis fluid, however, none of these effects was noticed when a dialysis fluid at pH 5 was employed. These same renal effects were produced when THAM was introduced into the blood stream (48). The authors (45) believed, to be consistent with their explanation for phenobarbital removal, that the absorption of THAM from the peritoneal cavity should be completely retarded due to the highly polar nature of its molecule and its poor lipid solubility. However, their observation indicated that a significant amount of THAM was obsorbed from the peritoneal cavity which might have produced

those effects. THAM has been detected in the red blood cells (48) whose cell membrane is characterized by minute pores of an equivalent diameter of 8.4 A^{o} (49). Diffusion of THAM into the erythrocytes might have taken place through these pores. This may suggest a similar mechanism for the transference of THAM across the peritoneal membrane. Furthermore, Engel and Kerekes (50) stated that the diffusion of a compound from the blood stream into the peritoneal cavity depends primarily upon the chemical structure of the compound rather than its physical state. They concluded that lipid solubility is not the determining factor because lipid soluble acid dyes were transferred across the peritoneum. Generally, basic dyes such as methylene blue and acid dyes like indigo carmine, eosin, phenol red, and Evans blue were detected in the peritoneal fluid following intravenous injection. Some dyes, both basic and acidic, however, did not diffuse into the peritoneal fluid, for example, gentian blue, malachite green, and congo red. This was probably because of some other factor involved such as protein binding.

Another factor affecting diffusion of drugs across the peritoneum is protein binding. The binding of drugs and chemicals to plasma proteins has been recognized since the earliest days of chemotherapy. The extent of binding not only varies from one chemical to another, but it may also be different between individual compounds

in a group of similarly related chemical structure. For instance, barbital, phenobarbital, and thiopental bind with plasma proteins to an extent of 0, 40, and 75%, respectively (51).

Binding of drugs to plasma proteins may decrease the hazardous effect produced by a drug in case of poisoning However, at lower blood level of the drug, the binding may diminish its pharmacological effect. Sulfonamides and penicillins when bound to plasma proteins lose their antibacterial effect (52). Apart from the effect that binding may have on the duration of action of a drug, it has been thought that the physicochemical factors involved may be of relevance to the behavior of the compound at the site of action.

Potential poisons which bind with plasma proteins lead to the reduction of the effective concentration in the blood and body fluids. It is believed that the diffusion of chemicals across the peritoneum is a function of the concentration of the unbound form which represents the only form that diffuses across the peritoneal membrane. One contradicting observation is that of Northover (53), who stated that it was the albumin bound form of Evans blue which diffused through the peritoneum of mice. In case of poisoning where the drug is partially bound to plasma proteins, the amount of the drug removed per unit time during peritoneal dialysis is relatively small. However, the addition of albumin to the dialysis fluid

results in the increase of the rate of removal of drugs. This is due to the binding of the diffused drug to the albumin in the dialyzing fluid, thus favoring the formation of more unbound form in the blood stream. This property of binding of drugs to plasma proteins has been utilized by many investigators to enhance the removal of poisons by peritoneal dialysis. The effect of albumin on the rate of dialysis of drugs was studied experimentally by Ettel-dorf et al. (31), Bourne and his associates (54), clinical-ly by Grollman and Odell (11), Berman and Vogelsang (30), and others (10).

Substances which produce a direct effect on the peritoneal membrane have been studied. Chambers and Zweifach (55) demonstrated the important role of calcium ion in the regulation of capillary permeability. As a result, the effect of this ion on the permeability of similar membranes of the same embryonic origin, that is, the mesentery, was attempted (41). Three levels of calcium were used: normal, subnormal, and above normal. The experiments consisted of four consecutive periods of 45 minutes using two different membranes of a rabbit. It was observed that reduction in calcium ion concentration caused an increase of 19% in the permeability of the mesentery, as measured by the rubidium flux. However, the data indicated the irreversibility of such effect. The elevation in the permeability persisted even when the calcium concentration was returned to normal or raised

to twice normal. There was no explanation given for this effect. Furthermore, it was observed that certain drugs, like histamine, 5-hydroxytryptamine, epinephrine, and sometimes acetylcholine, increased the permeability of the mesentery to ^{86}Rb and ^{32}P (40-42). Similar effects were produced by vasopressin (56). It has been suggested that the effect of vasopressin in promoting passive water reabsorption in the mamalian kidney may be due to the opening of pores. Since the migration of ^{86}Rb and ^{32}P across the rabbit mesentery appears to be a passive process, one might predict that the number or size of channels available for diffusion would be increased by vasopressin. However, its ability to discriminate between these two tracers cannot be explained solely by alteration of pore geometry. This is because vasopressin caused a persistent increase in the rate of diffusion of ^{32}P through the mesentery, but a decrease in diffusion of ^{86}Rb. Nicotinamide is another substance which is said to have a direct action on the peritoneal membrane. It was reported (57) that nicotinamide accelerates the absorption of the colloidal or semi-collodial coloring matters (Congo Red) through the peritoneal membrane. Albanus and Fries (58), on the basis of their experimental observations, suggested that hyaluronidase injected intraperitoneally cannot accelerate absorption of fluids from the peritoneal cavity.

Rates of metabolism and urinary excretion of a poison have been generally ignored by most experimenters and clinicians when treating their data on peritoneal dialysis. Recently, Bloomer (59) questioned the significance of peritoneal dialysis in the removal of poisons compared to the normal body mechanisms for their disposal. His study of a case of pentobarbital intoxication indicated that, in relation to hepatic degradation, relatively small quantities of pentobarbital are removed by the renal and peritoneal routes. Both the metabolite and parent compound have been detected in the peritoneal fluid, whereas only the former was predominant in the urine.

It should be noted that most of the work on peritoneal dialysis in the literature has used methods of analysis which do not discriminate between unchanged drug and its metabolites. For example, spectrophotometric methods have been used to measure the amounts of barbiturates in both serum and dialysis fluid in studies of dialysis of this class of drugs. Failure to distinguish between the drug and its metabolite may lead to erroneous conclusions regarding the rate of removal and the time required for attainment of equilibrium. Also, if the metabolite should diffuse faster than the unchanged drug, the data may falsely indicate a high degree of effectiveness of dialysis. This problem was emphasized by Bloomer (59) and by Bourne et al. (54).

Other factors that may possibly affect the rate of diffusion across the peritoneal membrane are the attachment of the diffusing ion or molecule to the membrane, and the adsorption of ions on the surface of the membrane that may cause repulsion or attraction depending on the charge of the diffusing ions.

Analysis of the Literature Data

It is of interest to see whether basic diffusion equations may be applied to clinical and animal data on peritoneal dialysis. One equation which may be used for this purpose is that derived by Lueck et al. (38). His equation was used for the diffusion of compounds through ointment barriers separating two very well-stirred compartments. Consider the diagram in Figure (3). This diagram consists of two compartments I and II separated by a membrane of surface area A cm^2, porosity ϵ, and thickness h cm. The volumes of solutions in compartments I and II are V_1 and V_2 ml., and the respective concentration of the penetrant are C_1 and C_2 in any suitable units. In Lueck's case the diffusion of the solute took place by lipid solubility in the membrane which is governed by the partition coefficient, K, of the diffusing substance. In this application simple diffusion through pores and/or lipid solubility are assumed. If the membrane is very thin the establishment of the quasi-steady state will be very rapid. According to Fick's law, the flux across the membrane, G, is represented by,

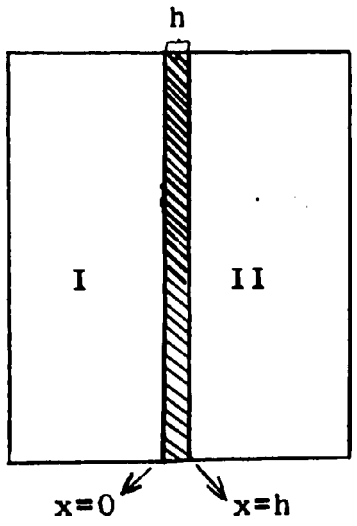


Figure 3. - Schematic diagram of a diffusion model.

$$G = \frac{KDA\epsilon}{h} (C_1 - C_2) \tag{47}$$

At zero time (t = 0) the following conditions are valid:

$$C_1 = C_o, \quad C_2 = 0, \text{ and } \quad C_h = 0 \tag{48}$$

Equation (47) may be rewritten in the following form,

$$-V_1 \frac{dC_1}{dt} = V_2 \frac{dC_2}{dt} = \frac{KDA\epsilon}{h} (C_1 - C_2) \tag{49}$$

The first two equalities of equation (49) are multiplied by dt and integrated between the limits,

$$\int_0^{C_2} dC_2 = - \frac{V_1}{V_2} \int_{C_o}^{C_1} dC_1 \tag{50}$$

Therefore,

$$C_2 = \frac{V_1}{V_2} (C_o - C_1) \qquad (51)$$

On substituting for C_2 in equation (49), it becomes

$$-V_1 \frac{dC_1}{dt} = \frac{KDA\epsilon}{h} \left[\left(1 + \frac{V_1}{V_2}\right) C_1 - \frac{V_1}{V_2} C_o\right] \qquad (52)$$

The final form of equation (52), after integrating between the limits and rearranging, is

$$\log_{10} \left[\left(1+\frac{V_1}{V_2}\right) C_1 - \frac{V_1}{V_2} C_o\right] = -\left(\frac{V_1+V_2}{V_1V_2}\right) \frac{KDA\epsilon}{2.303h} t + \log_{10} C_o \qquad (53)$$

On plotting the left hand side of equation (53) <u>versus</u> time a straight line should result the slope of which is

$$-\left(\frac{(V_1+V_2)}{V_1V_2}\right) \frac{KDA\epsilon}{2.303h} \qquad (54)$$

In special cases where $V_1 = V_2$ equation (53) reduces to

$$\log_{10} (2C_1 - C_o) = -\frac{2KDA\epsilon}{2.303Vh} t + \log_{10} C_o \qquad (55)$$

A few publications have been selected for testing the application of equations (53) and (55) to <u>in vivo</u> data on dialysis. The rate of removal of salicylates in experimentally intoxicated dogs was studied employing an ordinary dialyzing fluid with and without 5% purified human albumin (31). Assuming the plasma volume to be 5% of the body weight in all dogs, these data are analyzed and the corresponding

rates using equation (53), without correcting for binding, are calculated. These rates are listed in Table 2. In equation (53), V_1 represents the volume of the plasma, V_2 is the volume of the dialyzing fluid. C_o is variable in each lavage, and is always equal to the amount of salicylate in the plasma at the end of each lavage, that is, at the end of one hour dialysis. These calculations are based on the assumption that rapid equilibrium is established between the bound and unbound salicylic acid so that the fraction of unbound salicylic acid remains constant. However, this does not seem to be the case. Examination of the calculated rates in Table 1 show a persistent decrease in the rate with the exception of Dog E. This may

TABLE 2

CALCULATED RATES OF REMOVAL OF SALICYLATES USING MODIFIED GROLLMAN'S SOLUTION

Rate of Removal of Salicylates hr.$^{-1}$*				
Animal	Lavage I	Lavage II	Lavage III	Lavage IV
E	0.1348	0.0668	0.2092	...
F	0.2466	0.1675	0.0706	...
G	0.2953	0.2334	0.0738	0.0832

*Calculated from the data of Etteldorf et al. (31)

be explained on the basis of binding. Generally a drug like salicylic acid binds with the unoccupied sites of plasma proteins according to the following equation,

$$C + P \underset{k_2}{\overset{k_1}{\rightleftharpoons}} CP \qquad (56)$$

where C is the concentration of the unbound drug, P is the concentration of plasma proteins, CP is the concentration of the bound drug, k_1 and k_2 are respectively the rate constants for the forward and reverse processes. At equilibrium, the fraction of the total drug which is bound to plasma proteins (β) is expressed by,

$$\beta = \frac{1}{1 + \frac{1}{Knl} + \frac{C}{nl}} \qquad (57)$$

where K is the equilibrium constant, k_1/k_2, and n is the number of binding sites per protein molecule. It should be noted that β decreases with increase in C. Therefore, as the concentration of the free drug decreases due to its removal by peritoneal dialysis, the fraction of bound drug increases, and hence the concentration of the unbound drug will be different at the start of each lavage. Since the rate of removal is dependent on the concentration of the unbound form, decrease in the rate will be expected as the concentration of the free drug declines. This might qualitatively explain the results listed in Table 2.

In another series of experiments, these investigators (31) employed 5% albumin in the dialyzing fluid. A volume of dialysis fluid, which is equal to the calculated volume of blood plasma in each animal, was introduced in the peritoneal cavity. Samples from the blood and dialysis fluid were simultaneously withdrawn at one hour intervals for a period of four hours. A plot of these results using equation (55) is shown in Figure 4. This figure indicates that the rate is the same in the first two dogs up to two hours of dialysis. However, equilibrium was reached in the second dog after three hours. In a third dog, equilibrium was established in one hour.

Similar results as to the effect of albumin on the rate of removal of pentobarbital were observed (54). The data indicated that the rate of removal of pentobarbital increases with increase in the percentage of albumin in the dialyzing fluid. This observation was expected, according to equation (57), because the fraction of bound drug in the dialysis fluid will increase. Consequently this will affect the concentration gradient across the peritoneum resulting in an increase in the rate of removal. The results obtained from one dog are plotted using equation (53) and shown in Figure 5.

Bilirubin is an example of a substance that is strongly bound to plasma proteins (11). Intermittent dialysis, in a case of jaundice employing an ordinary dialysis fluid, removed about 10 mg. of bilirubin in a period of two hours

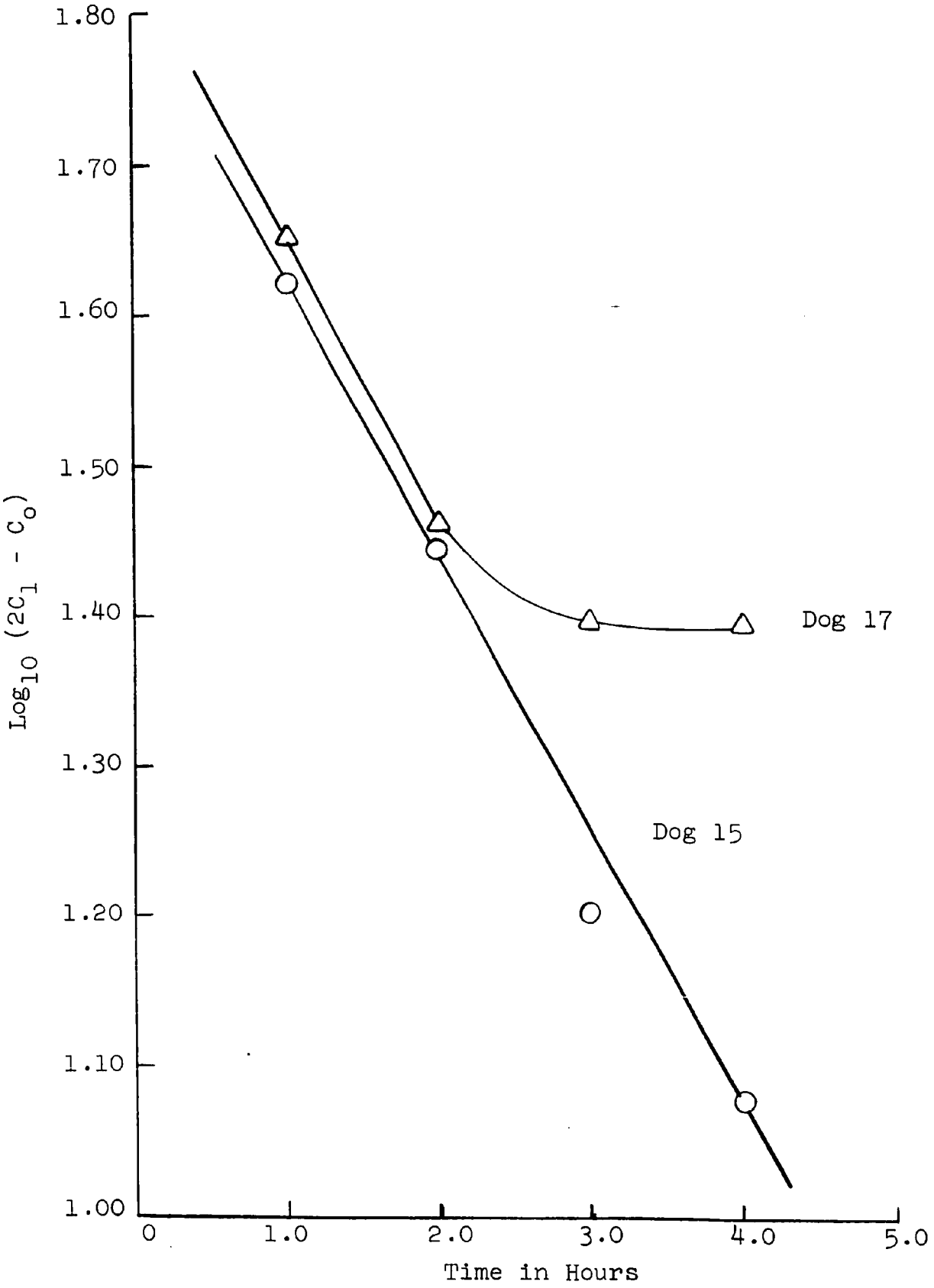


Figure 4.- Plot representing the removal of salicylic acid across the peritoneum of dogs. The dialysis fluid contains 5% human albumin. [From the data of Etteldorf *et al*. (31)].

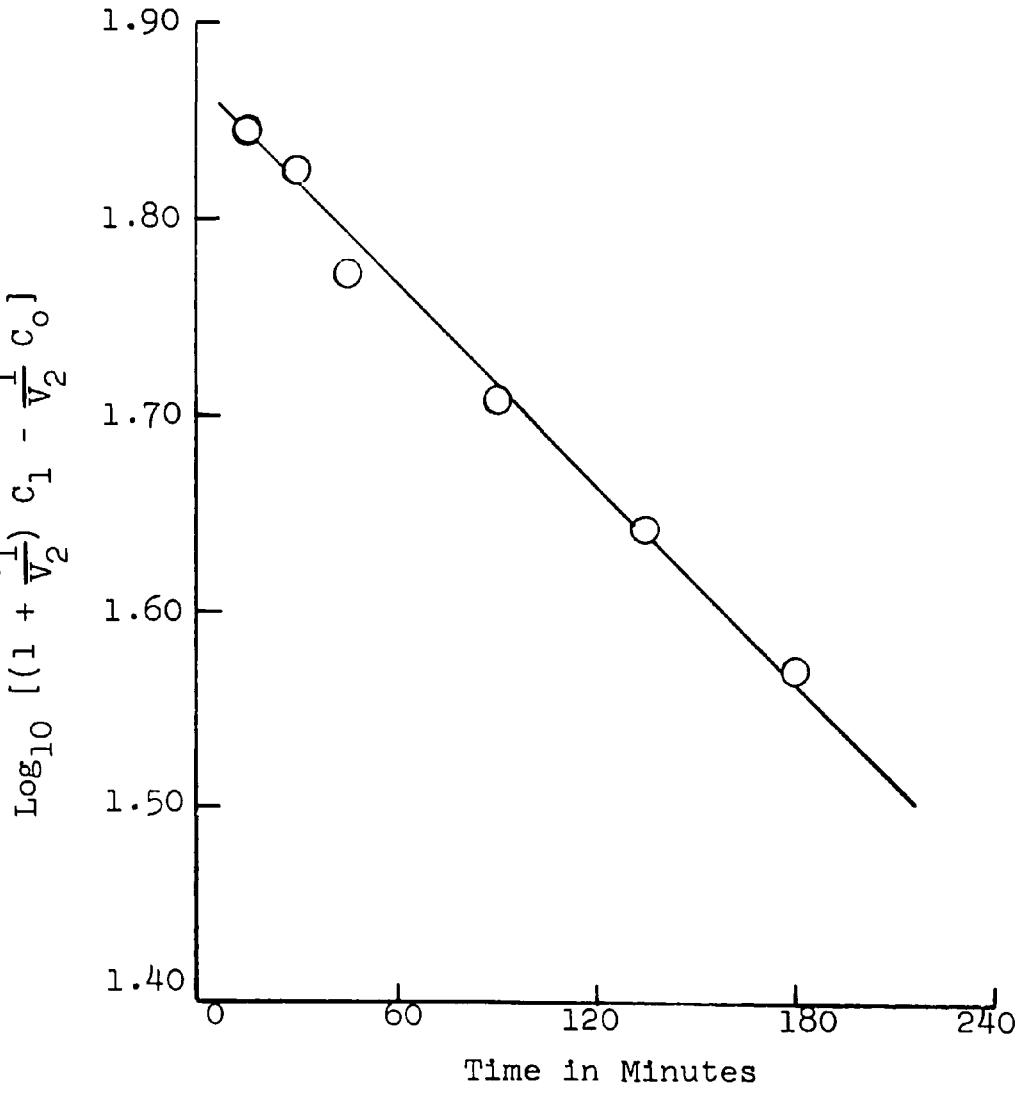


Figure 5.- Plot representing the diffusion of Pentobarbital across the peritoneum of the dog. The dialysis fluid contains 5% albumin. [From the data of Bourne and his associates (54)].

in spite of its high plasma level. This may be due to the limited peritoneal permeability to the compound or to the presence of small concentrations of the unbound substance in the plasma. Observations confirmed the latter, since the rate of removal of bilirubin is greatly enhanced by the addition of albumin to the dialyzing fluid. Five peritoneal lavages were performed. One of these lavages served as a control employing an ordinary dialyzing fluid. The other four were conducted for various periods of 1, 2, 3, and 4 hours utilizing peritoneal fluid containing albumin. The plasma concentration of bilirubin, during each of these dialyses, remained constant at an average level of 50.6 mg./100 ml. $\pm$ 2%. Equation (31), which was used by Berndt and Gosselin (40) was used in its logarithmic form to calculate the rate of dialysis of bilirubin. These rates are listed in Table 3. It is apparent from Table 3 that the rate of removal of bilirubin has increased in presence of albumin. The drop in the rate of removal in the four hour lavage is due to the establishment of equilibrium as shown in Figure 6. These calculated rates cannot be accepted as absolute values since no correction was made for protein binding.

Since there was no data available for the rates of metabolism and urinary excretion of these poisons, it was assumed, in the previous analysis, that negligible amounts were removed *via* these routes.

TABLE 3

CALCULATED RATES OF REMOVAL OF BILIRUBIN BY PERITONEAL DIALYSIS IN PRESENCE AND IN ABSENCE OF ALBUMIN*

Experiment	Period of Dialysis hr.	Rate of Dialysis $hr.^{-1}$
Control	2	0.005
1	1	0.074
2	2	0.077
3	3	0.081
4	4	0.063

* Calculated from the data of Grollman and Odell (11)

From these examples it appears that the mathematical equations, derived previously for application to the *in vitro* data, can be used to explain the data obtained from experimental animals. These equations are applicable only when the degree of protein binding, rates of metabolism and urinary excretion are known.

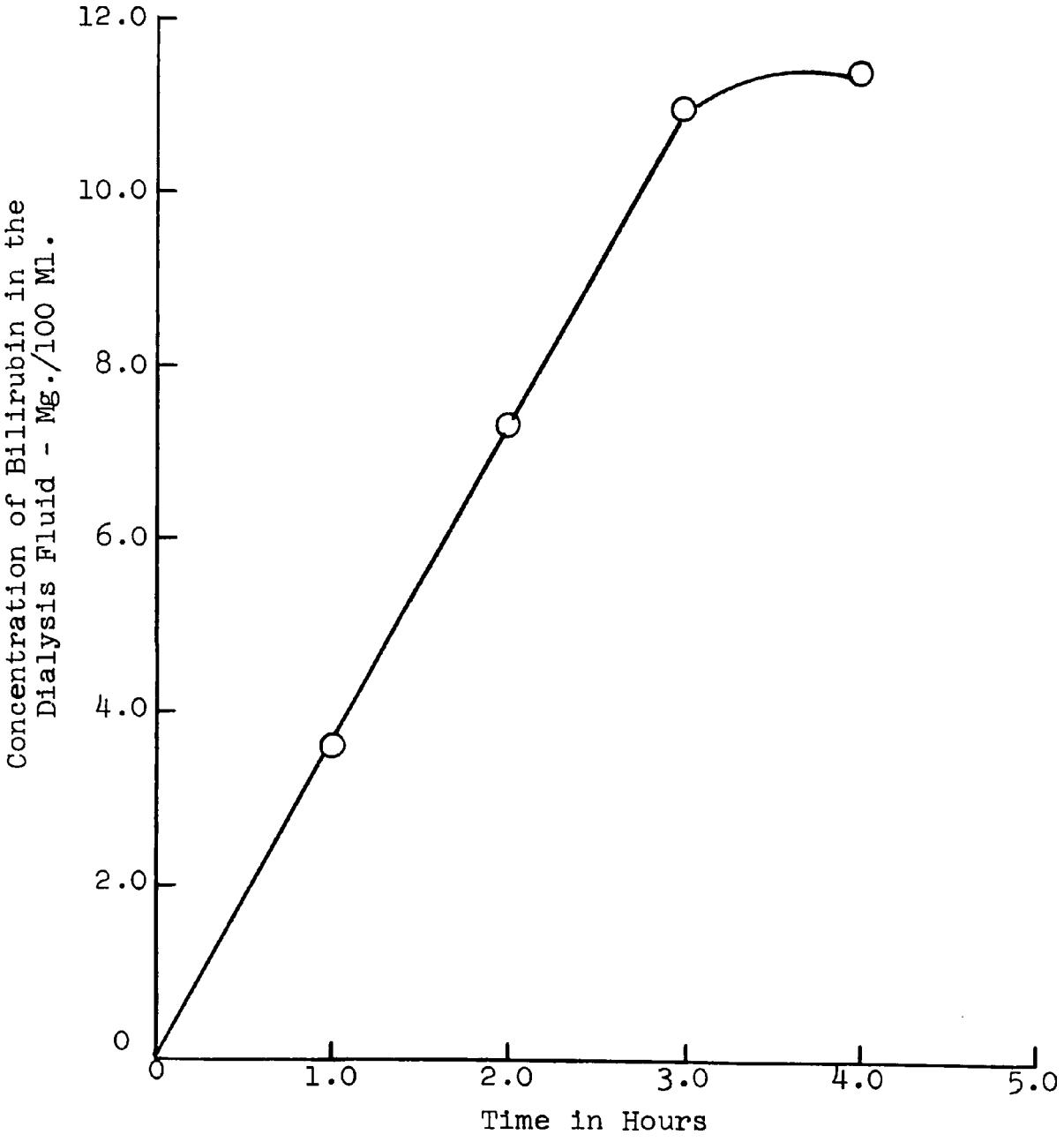


Figure 6.- Plot showing the establishment of equilibrium, in the presence of albumin, for the dialyzed bilirubin in the peritoneal cavity. [From Grollman and Odell (11)].

STATEMENT OF THE PROBLEM

Although a great deal of work has been done on the removal of poisons by peritoneal dialysis, a means of increasing the rate of dialysis is sorely needed. Attempts at imporving the process by chemical means have been limited by lack of knowledge of the dialysis process. Specifically it is not known whether drugs depend chiefly upon lipid solubility for diffusion across the peritoneal membrane and whether ionic forms of drugs will diffuse.

It was the purpose of this research to learn more of the nature of drug diffusion across the peritoneum *via* direct measurements on isolated membranes. The mesentery was selected for study, since it is the portion of the peritoneum thought to be most effective in dialysis. Three chemicals were selected for diffusion measurement which are frequently encountered in poisoning; namely, pentobarbital, salicylic acid, and urea.

It was planned to determine the rate of diffusion of these chemicals in solution containing different proportions of ionized and non-ionized molecules to determine relative rates of diffusion. Thus these studies might indicate the maximum rates of dialysis which may be achieved under a given set of conditions and serve as a basis for improving *in vivo* dialysis

EXPERIMENTAL

Determination of the Diffusion Coefficients

Materials and Methods

Selection of Experimental Conditions and Substances for Study

Three chemicals of different ionization constants were selected. These were pentobarbital sodium, salicylic acid, and urea. For radioactive measurement pentobarbital -2- ^{14}C*, salicylic acid -1- ^{14}C**, and urea - ^{14}C*** were used. The specific activities of these compounds were 1.635, 14.2, and 3.67 mc./mM., respectively. Analytical grade of potassium chloride was employed for calibration purposes. All other chemicals involved were of analytical quality.

White grid Millipore membranes were selected for measurement of diffusion coefficients. It was felt that they would provide faster rates of diffusion than the slow diaphragm cell method used by many investigators (39, 60, 61). The Millipore membranes used in this study had the following specifications: 37 mm. diameter, 0.45 $\pm$ 0.02 micron pore size, 150 $\pm$ 10 microns dry thickness, and porosity of 0.79 (62).

Isotonic solutions were used in all studies involving pentobarbital, salicylic acid, and urea. These solutions

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* Supplied through the courtesy of Abbott Laboratories, North Chicago, Illinois.
** Nuclear Chicago, Des Planes, Illinois
*** Tracerlab, Waltham, Mass.

contained 0.005 M/L. of $Na_2HPO4.7H_20$, calculated quantities of IN HCl or NaOH to bring the solution to the required pH, and sufficient NaCl to adjust isotonicity.

Diffusion Apparatus

The diffusion apparatus was constructed from two 125 ml. Erlenmeyer flasks. A 4-cm. tube with an expanded ground joint was sealed to the side of each flask. These joints could be clamped together enclosing the membrane, by either a threaded or screw type clamp. The inside diameters of the joints were accurately measured. Stirring in both flasks was effected by glass stirrers driven by variable speed motors controlled by rheostats. The diffusion apparatus was mounted in a water bath at 37°C.± 0.1°C. A diagram of the diffusion apparatus is shown in Figure 7.

Experimental Procedure

A Millipore membrane, which had been previously soaked in water for at least twelve hours, was mounted between the two compartments and the joints were clamped. Then the apparatus was immersed in the water bath, levelled and locked in position with a clamp. One hundred and fifty milliliters of potassium chloride solution in double distilled water, or of the drug in the isotonic solution of particular pH was introduced into one compartment. A volume of double distilled water, in the case of KCl diffusion, or isotonic solution of the same pH, in the case of other substances, was introduced into the second

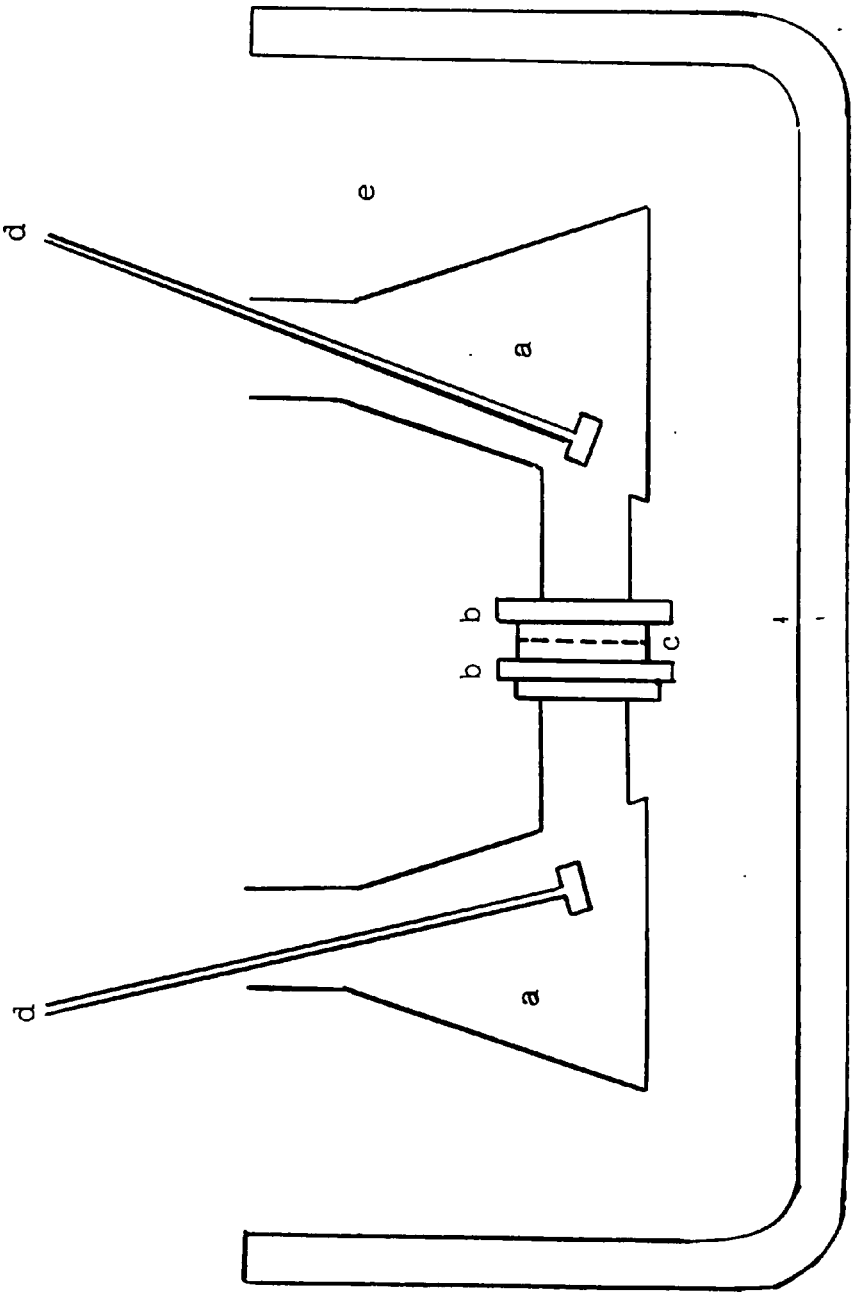


Figure 7.- Diffusion apparatus. a - Erlenmeyer flasks. b - Threaded aluminum clamp. c - Membrane. d - Glass stirrers. e - Water bath.

compartment. Three different volumes were used in the latter compartment, depending on its capacity, in order to bring the liquid inside to the same level as that of the source solution. These volumes were 150, 155, and 158 ml. All solutions were equilibrated to 37°C. before use. The liquids in both compartments were stirred vigorously without creation of a vortex. Periods of 30 minutes, in the case of diffusion of potassium chloride, urea, and salicylic acid, and 90 minutes in the case of pentobarbital, were allowed before the first sample was withdrawn from the sink compartment for analysis. Additional samples were removed every ten minutes for a period of ninety minutes in the case of potassium chloride diffusion, every fifteen minutes for a period of 2½ hours in the case of urea, every thirty minutes for a period of four hours in the case of salicylic acid, and every thirty minutes for a period of six hours in the case of pentobarbital.

It was observed that if the levels in both compartments were not the same, hydrodynamic flow would occur in either direction depending on the heights of liquid in the compartments. Magnetic stirrers caused large variations due to the formation of vortices, which caused varying fluid levels in the cells, and resulted in hydrodynamic flow. The use of slow speed stirring gave highly variable results, variations in diffusion rate being as much as six or seven times that expected from differences in membranes. This

led to investigation of the effects of stirring with the thought that inadequate stirring might allow stagnant layers to exist on either side of the membrane. It was found that by using much more rapid stirring these variations were diminished to the limit expected from normal membrane differences. Hence, maximum stirring speed was used in subsequent experiments.

Methods of Assay

Potassium Chloride Assay. --The Fajan's titration method was adopted to determine the amount of potassium chloride diffused. The method is based on the principle of titrating the chloride ions with standard silver nitrate using alcoholic solution of fluorescein as an indicator (63). The end point is indicated by the appearance of a pink color on the precipitated silver chloride particles due to adsorption of negative fluorescein ions.

Standardization of 0.1N silver nitrate was done by titration against 0.1N potassium chloride which had been prepared by accurately weighing and diluting reagent potassium chloride. At least ten samples of 0.1N potassium chloride were titrated. Each sample of 0.128 ml. of 0.1N KCl was delivered by means of a calibrated pipet into one of the shallow cavities of a porcelain spot plate. To each sample a drop of 0.2% alcoholic fluorescein solution was added. The solution on the spot plate was stirred with a small magnetic stirrer of 5 mm. length made by sealing a small iron wire in a capillary tube.

The stirred solution was titrated with silver nitrate solution delivered from a calibrated microburette operated manually. This microburette was standardized to deliver volumes as small as 10^{-4}ml. The tip of the microburette was kept in contact with the solution during the titration until the end point was reached. This method provided a precision of more than 99%.

The unknown samples, withdrawn during the diffusion studies of one molar potassium chloride, were assayed in the same manner.

Carbon^{-14} Measurement. --Radioactivity measurement was utilized in determining the concentrations of samples during the diffusion studies of pentobarbital, salicylic acid, and urea. Two standard solutions of the same concentration were prepared by diluting 1 to 10 the solution of the radioactive compound which was used in the diffusion studies. Dioxane phosphor was prepared by dissolving 7.0 Gm. PPO, 2,5-diphenyloxazole*, Scintillation grade, 0.05 Gm. POPOP, (1, 4-bis-2, 5-phenyloxazolyl)-benzene*, Scintillation grade, 50 Gm. naphthalene, and 50 ml. water in p-dioxane, spectroquality, to make one liter of solution (64). The water was included in order to maintain a clear solution upon addition of the samples. Ten ml. of the dioxane phosphor was pipetted into screw capped low potassium vials to which 0.128 ml. of the unknown samples and 0.13 ml. of the standard samples were added. Four

* Packard Instrument Co., Inc., La Grange, Illinois.

standards, two from each of the standard solutions, were prepared. The vials were counted in a Tri-Carb liquid scintillation counter in triplicate for 50 minutes or 10^4 counts, whichever came first. A vial containing the same volume of the dioxane phosphor and 0.13 ml. of isotonic solution was counted to serve as a blank. The average counts were then used to calculate the amount of substance diffused during a time interval, assuming that both the radiolabelled and unlabelled substance diffused at the same rate.

Results and Treatment of Data

Membrane Constants

The results of experiments on the diffusion of 1M potassium chloride solution through Millipore membranes are listed in Table 4. For each experiment a curve of $\log_{10}\left[(1 + V_1/V_2)C_1 - (V_1/V_2)C_o\right]$ versus time was plotted according to equation (53) and slopes were calculated by least squares. From these slopes were calculated the rates per unit area, membrane constants, and membrane thickness. Results of these calculations are presented in Table 5, and a typical graph of a diffusion experiment is shown in Figure 8.

Upon examination of Table 4, it is apparent that the concentrations of the first samples taken are somewhat variable and usually higher than expected. This is partly due to lack of control of flow in the system during the

TABLE 4

RESULTS OF DIFFUSION MEASUREMENTS OF IM POTASSIUM CHLORIDE THROUGH DIFFERENT MILLIPORE MEMBRANES AT 37°C.

Time Min.	Concentration Potassium Chloride in Sink-M./L.*				
	Membrane 1	Membrane 2	Membrane 3	Membrane 4	Membrane 5
30	0.0357	0.0359	0.0308	0.0376	0.0386
40	0.0472	0.0464	0.0431	0.0495	0.0509
50	0.0587	0.0579	0.0534	0.0607	0.0610
60	0.0688	0.0682	0.0651	0.0708	0.0724
70	0.0786	0.0785	0.0754	0.0809	0.0824
80	0.0901	0.0883	0.0868	0.0905	0.0928
90	0.0982	0.0975	0.0970	0.1006	0.1023
	Membrane 6	Membrane 7	Membrane 8	Membrane 9	
30	0.0360	0.0360	0.0359	0.0355	
40	0.0472	0.0477	0.0476	0.0467	
50	0.0579	0.0593	0.0588	0.0573	
60	0.0689	0.0704	0.0695	0.0677	
70	0.0789	0.0802	0.0806	0.0780	
80	0.0892	0.0905	0.0908	0.0880	
90	0.0995	0.1001	0.1002	0.0971	

* V_2 = 155 ml.

TABLE 5

SLOPES, RATES PER UNIT AREA, MEMBRANE CONSTANTS, AND MEMBRANE THICKNESS FROM DIFFUSION OF IM POTASSIUM CHLORIDE THROUGH MILLIPORE MEMBRANES AT 37°C.

Membrane	Slope* $Min.^{-1} \times 10^4$	Rate/Unit Area $Cm.^{-2}\ Min.^{-1} \times 10^4$	Membrane Constant $Cm.^{-2}$	Membrane Thickness $Cm. \times 10^2$
1	10.3357	1.9540	0.7199	3.30
2	10.1892	1.9263	0.7097	3.35
3	10.8785	2.0566	0.7578	3.14
4	10.4071	1.9675	0.7249	3.28
5	10.5428	1.9932	0.7344	3.24
6	10.4250	1.9709	0.7262	3.28
7	10.5500	1.9945	0.7349	3.24
8	10.4534	1.9763	0.7381	3.27
9	10.0739	1.9045	0.7017	3.39

* Calculated by the method of least squares.

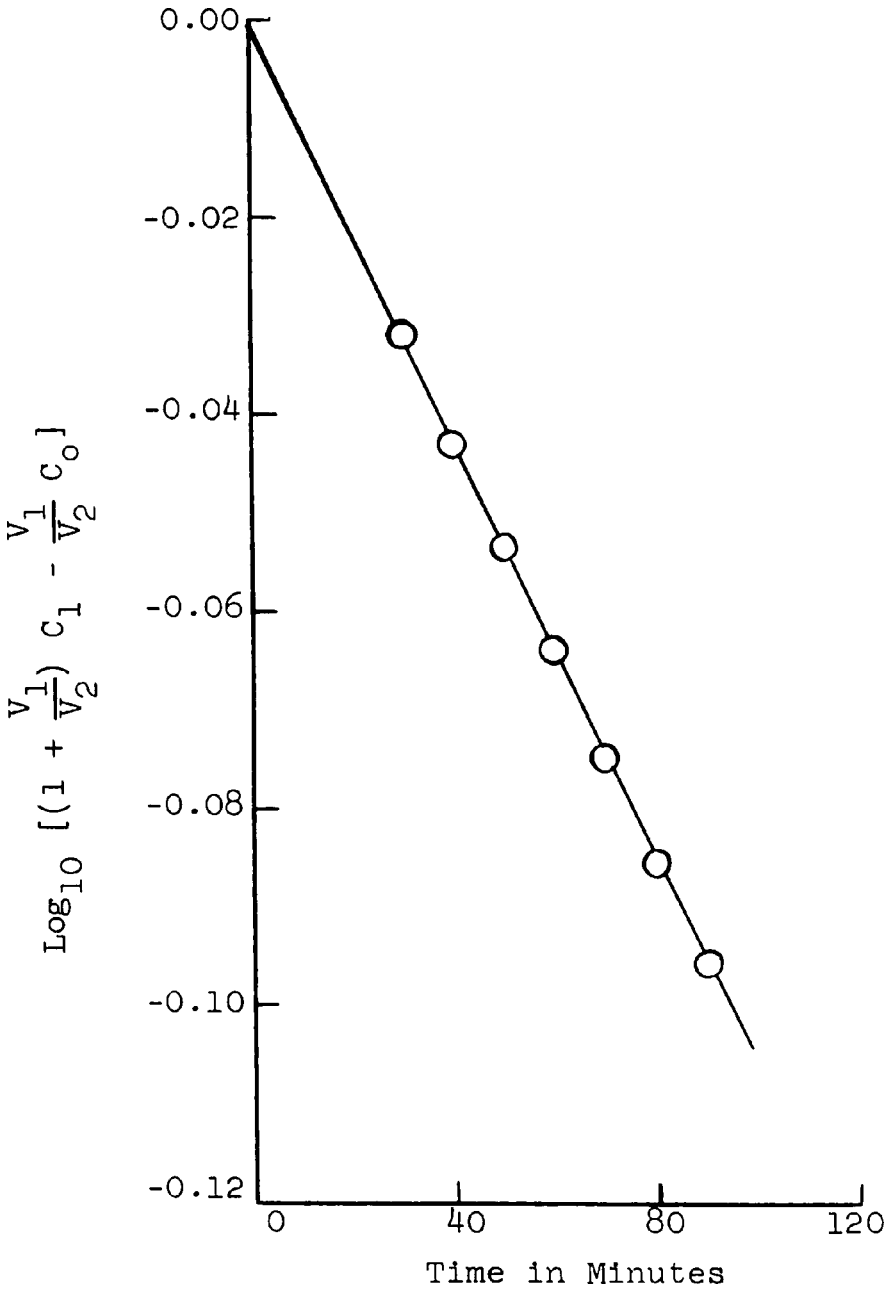


Figure 8.- Typical diffusion curve of 1M potassium chloride through Millipore membrane at 37°C.

introduction of solutions into the diffusion apparatus. Also, timing was begun at the midpoint of the filling of the apparatus and some unpredictable amount of diffusion took place prior to the beginning of timing. Because of this, the slope was calculated from the logarithmic data without forcing the intercept through the theroetical point at time zero. This method gave small finite values for the intercept, reflecting the fact that the concentration in the source flask was slightly different at zero time from that originally placed in the flask. Errors in slope from this source were small, being less than 2%.

Two values for the diffusion coefficient of 1M potassium chloride at 25°C. were reported in the literature as determined by the diaphragm cell method (60,61). These values are 1.1479×10^{-3} and 1.1458×10^{-3} cm.^{2}/min., agreeing within 0.2%. These are considered as integral diffusion coefficients since they result from measurements covering a range of concentrations, in contrast with the differential diffusion coefficient which is valid for a particular concentration. Failure to distinguish between the two kinds of coefficients by many experimenters has caused much confusion in the field of diffusion. The integral diffusion coefficient, $\underline{D_i}$, is related to the differential diffusion coefficient, $\underline{D}$, by the following equation (65).

$$D_i = \frac{1}{\Delta C} \int_{C_2}^{C_1} D \, dC \qquad (58)$$

where $\underline{\Delta C}$ represents the difference between the initial and final concentrations, that is, $(C_1 - C_2)$. Plotting the values for the differential diffusion coefficients of potassium chloride which are listed in Table 6 *versus* concentration yields the curve shown in Figure 9. Graphical integration of Figure 9 between $C_2 = 0$ and $C_1 = 1M$ yields a value for $\underline{D_i}$ of 1.1154 $cm.^2/min.$ This value agrees within 3% with the reported values determined by the diaphragm cell method (60,61) and also serves as a check for the experimentally determined values of the integral diffusion coefficient of IM potassium chloride.

The temperature dependence of the differential diffusion coefficient of IM potassium chloride was demonstrated by Longsworth (66). His data, together with those of Chang and Wilke (39) for the integral diffusion coefficient of 0.IM potassium chloride as a function of temperature are listed in Table 7. On plotting $(\log_{10} D)$ *versus* the reciprocal of the absolute temperature $\frac{1}{T}$, a straight line is obtained which is shown in Figure 10. All the points fit one line indicating that there is no apparent difference in the dependence of the two types of diffusion coefficients on temperature in either dilute or concentrated solution of potassium chloride. Therefore, it is considered permissible to calculate the integral diffusion coefficient of IM potassium chloride solution at 37°C. using the following relation.

$$\frac{(D)_{37}}{(D)_{25}} = \frac{(D_i)_{37}}{(D_i)_{25}} \qquad (59)$$

TABLE 6

DIFFERENTIAL DIFFUSION COEFFICIENTS FOR POTASSIUM CHLORIDE AT 25°C.

Concentration M./L.	D $Cm.^2/Sec.$ x 10^5
0.0000[a]	1.996
0.0126[a]	1.908
0.0265[a]	1.879
0.0500[b]	1.864
0.1000[b]	1.844
0.2000[b]	1.838
0.3000[b]	1.838
0.5000[b]	1.858
0.7000[b]	1.866
1.0000[b]	1.892

a - adopted from the data of Harned & Nuttall (68)
b - adopted from appendix 11.2 in Robinson & Stokes (69).

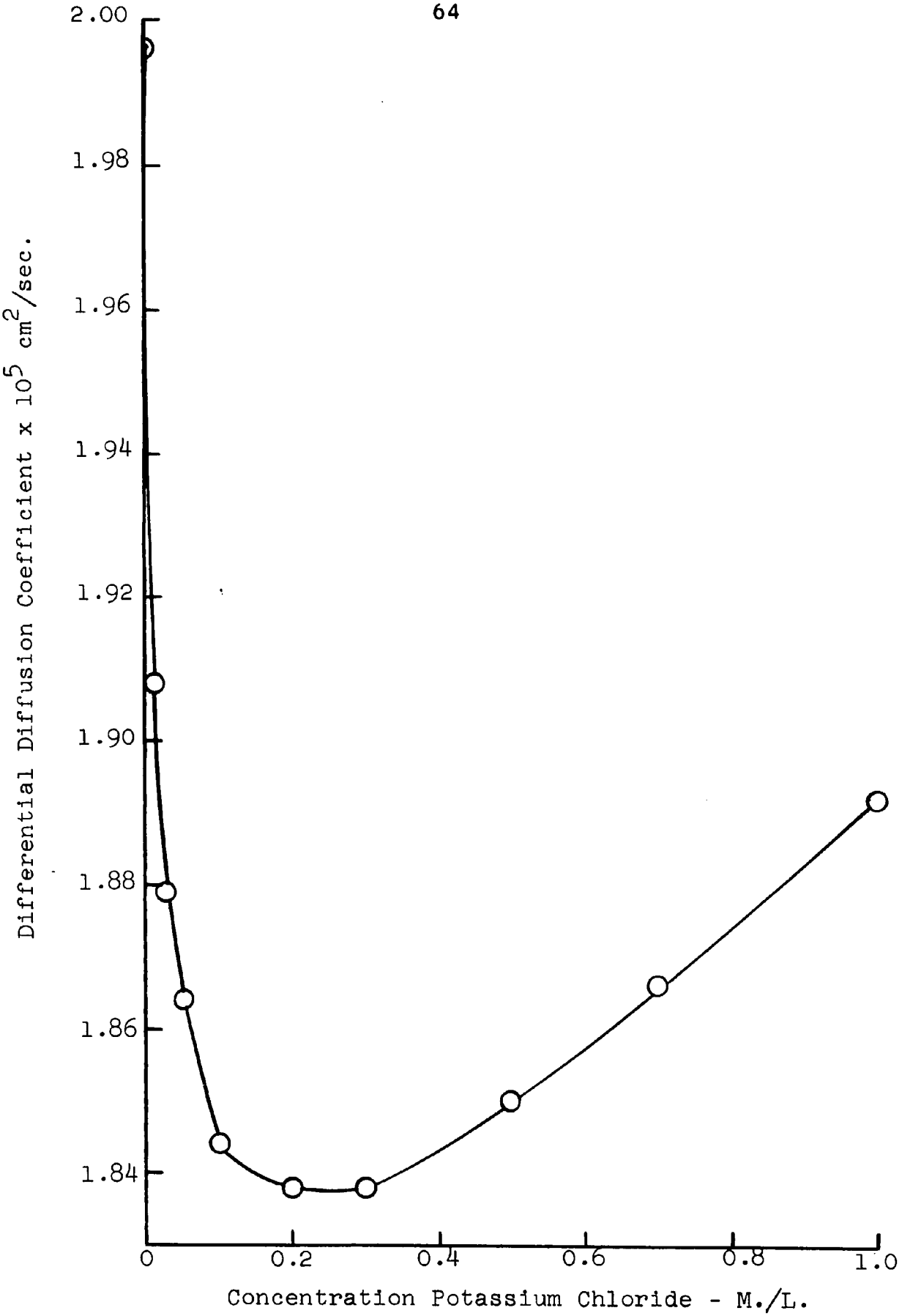


Figure 9.- Plot of the differential diffusion coefficients of KCl versus concentration at 25°C. [From references 68 and 69].

TABLE 7

DIFFERENTIAL AND INTEGRAL DIFFUSION COEFFICIENTS OF IM AND O.IM POTASSIUM CHLORIDE SOLUTIONS AS A FUNCTION OF TEMPERATURE

Temperature	Differential Diffusion Coefficient of IM KCL $Cm.^2/Sec. \times 10^5$	Integral Diffusion Coefficient of O.IM KCL $Cm.^2/Sec. \times 10^5$
288.16	1.483	...
298.16	1.890	1.870
303.16	...	2.093
308.16	2.340	...
318.16	2.825	...
324.16	...	3.200

From the data of Chang and Wilke (39), and Longsworth (66).

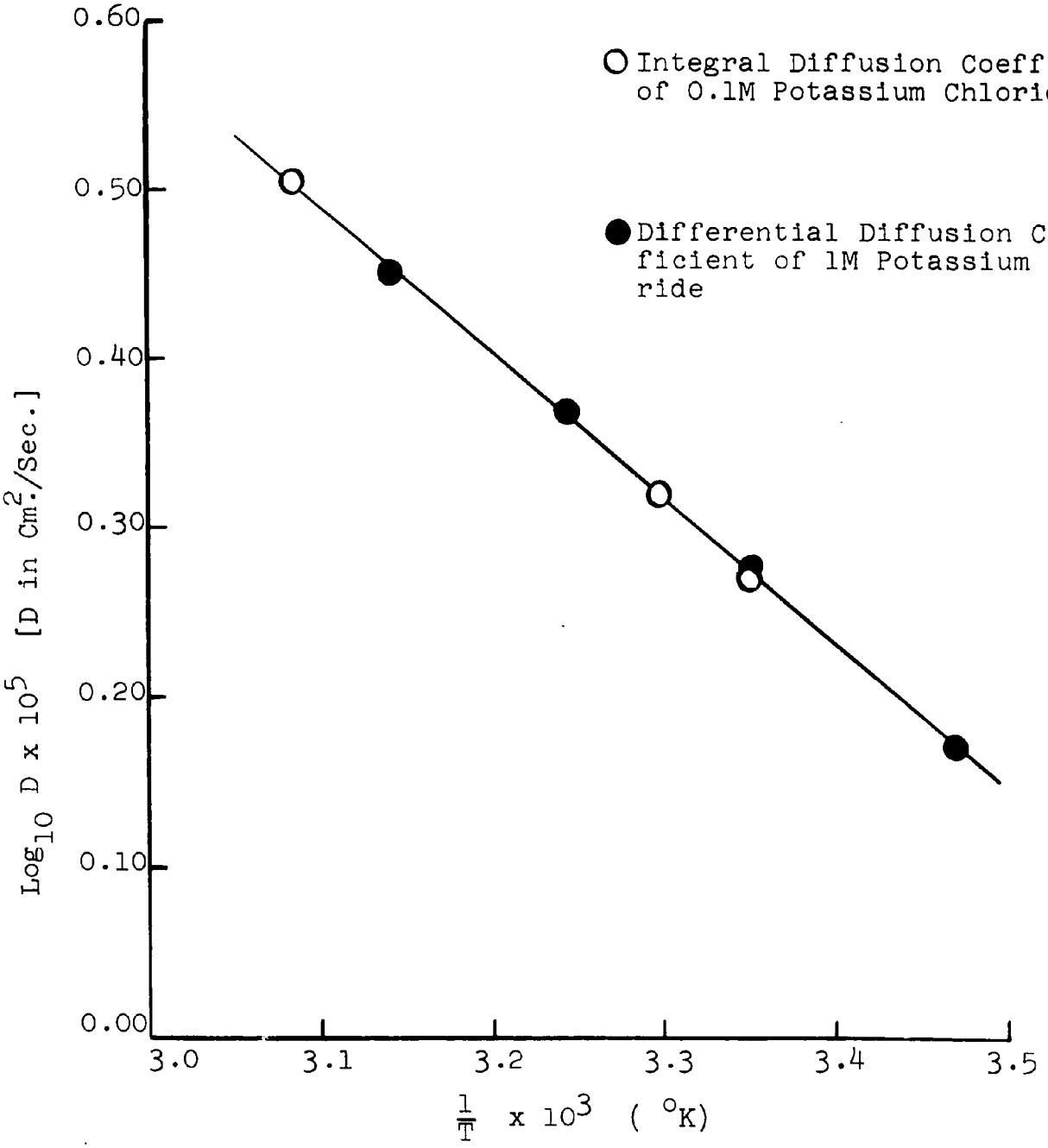


Figure 10.- Plot of differential and integral diffusion coefficients for 1M and 0.1M potassium chloride as a function of temperature. [From the data of Longsworth (66) and Chang and Wilke (39)].

where the subscripts indicate the temperature in degrees centigrade. Using the value of $\underline{D_i}$ for IM potassium chloride at 25°C. reported by Vinograd and McBain (61), the calculated value for the integral diffusion coefficient at 37°C. is 1.4356×10^{-3} cm.^{2}/min. This value was used in calculating the membrane constants listed in Table 5.

Statistical analysis of the experimentally determined membrane constants gave a standard deviation (s) of 1.62×10^{-2} cm^{-2}. The average value is 0.7264 cm^{-2}, and all values lie in a range of $\pm 2s$.

The idea of using a single membrane throughout the whole study was rejected, because of the possibilities of accidental destruction of the membrane and reduction of the effective surface area through clogging (62). However, the reporducibility of the titration technique was checked by performing five experiments using the same Millipore membrane. The results of these experiments are listed in Table 8. Calculated membrane constants from these experiments are presented in Table 9. It is apparent from these results that the assay procedure is reporducible, the maximum difference between the first four values being less than 0.7%. The average value of the membrane constant is 0.7315 cm^{-2} which is in the same range obtained for a series of different membranes. The value of the last experiment was low indicating that the permeability of the membrane was finally affected by repeated use.

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TABLE 8

RESULTS OF DIFFUSION MEASUREMENTS OF IM POTASSIUM CHLORIDE THROUGH A SINGLE MILLIPORE MEMBRANE AT 37°C.

Time	Concentration of Potassium Chloride in Sink-M./L.*				
Min.	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
30	0.0380	0.0371	0.0334	0.0321	0.0344
40	0.0482	0.0483	0.0444	0.0427	0.0449
50	0.0606	0.0588	...	0.0546	0.0565
60	0.0714	0.0703	0.0660	0.0659	0.0666
70	0.0814	0.0805	0.0789	0.0872	0.0760
80	0.0939	0.0916	0.0875	0.0872	0.0858
90	0.1030	0.1016	0.0984	0.0964	0.0959

*V_2 = 155 ml.

TABLE 9

SLOPES AND MEMBRANE CONSTANTS FOR THE DIFFUSION OF IM POTASSIUM CHLORIDE THROUGH A SINGLE MILLIPORE MEMBRANE AT 37°C.

Experiment No.	Slope Min.$^{-1}$ x 10^4	Membrane Constant Cm.$^{-2}$
1	10.6157	0.7395
2	10.6642	0.7428
3	10.6869	0.7444
4	10.6321	0.7314
5	10.0392	0.6993

Millipore membranes are cellulosic in nature (62), and swell on soaking in water. The calculated membrane thickness listed in Table 5 indicates that it may have increased to more than twice its dry thickness. This is consistent with the published data on the swelling of commercial films (67).

Diffusion Coefficient of Pentobarbital

The concentration of pentobarbital used in this study was based on a plasma level of 30 mg. of the drug per 100 ml., sufficient to produce the toxic symptoms characteristic for this hypnotic. Diffusion studies of this concentration (3×10^2 micrograms/ml.) were conducted at two different pH's, 3.7 and 11.7. At the lower pH, pentobarbital is completely undissociated while at the higher pH it is completely dissociated, for practical purposes. Experimental data are presented in Tables 10 and 11. The diffusion coefficient was calculated by dividing the calculated slope of the line resulting from a plot of equation (53) by the membrane constant obtained from the potassium chloride experiments. In some cases where V_2 is not equal to that used in potassium chloride experiments, the calculated coefficient is multiplied by the factor $\left[(V_1 + V_2)/V_1 V_2\right]_1 / \left[(V_1 + V_2) / V_1 V_2\right]_2$ where the subscripts 1 and 2 designate KCl and pentobarbital respectively. The calculated slopes, rates per unit area, and diffusion coefficients are listed in Table 12.

TABLE 10

RESULTS OF DIFFUSION MEASUREMENTS OF PENTOBARBITAL AT pH 3.7 THROUGH VARIOUS MILLIPORE MEMBRANES AT 37°C.

Time Min.	Concentration Pentobarbital in Sink-Mcg./Ml.*			
	Membrane 1	Membrane 2	Membrane 3	Membrane 4
90	12.75	12.39	11.83	11.79
120	17.38	16.64	16.19	15.75
150	21.47	20.94	19.78	19.99
180	25.34	24.63	23.81	23.72
210	29.16	28.79	26.97	27.16
240	33.14	32.81	30.33	31.03
270	36.85	36.40	34.25	24.24
300	40.13	42.38	37.27	38.39
330	43.74	...	40.87	41.26
360	47.62	46.35	44.12	44.61
	Membrane 5	Membrane 6	Membrane 7	
90	12.15	11.77	10.85	
120	16.04	...	14.72	
150	20.36	19.58	19.91	
180	24.15	23.44	22.03	
210	27.66	27.02	...	
240	31.70	31.02	29.76	
270	35.73	33.94	33.72	
300	39.00	37.38	36.74	
330	42.51	40.44	40.28	
360	46.03	42.61	...	

* V_2 = 155 ml.

TABLE 11

RESULTS OF DIFFUSION MEASUREMENTS OF PENTOBARBITAL AT pH 11.7 THROUGH VARIOUS MILLIPORE MEMBRANES AT 37°C.

Time Min.	Concentration Pentobarbital in Sink-Mcg./Ml.*		
	Membrane 1[a]	Membrane 2	Membrane 3
90	12.38	8.76	12.25
120	16.14	12.50	...
150	...	...	19.78
180	24.11	21.11	23.44
210	28.58	25.62	26.52
240	32.94	29.72	30.23
270	36.29	33.62	33.79
300	39.90	36.69	37.23
330	43.45	...	40.04
360	45.84	46.74 [b]	43.39
	Membrane 4	Membrane 5**	Membrane 6**
90	16.23	12.14	12.13
120	21.47	16.76	16.25
150	...	...	20.00
180	28.89	24.67	23.95
210	31.43	28.51	27.01
240	35.83	32.08	29.81
270	39.00	35.86	33.82
300	43.20	38.59	37.30
330	...	42.99	...
360	...	46.39	43.68

* V_2 = 150 ml.

** V_2 = 155 ml.

a- C_o = 306.44 mcg./ml.

b- Sample withdrawn after 390 minutes

TABLE 12

SLOPES, RATES PER UNIT AREA, AND DIFFUSION COEFFICIENTS OF PENTOBARBITAL FROM DIFFUSION THROUGH MILLIPORE MEMBRANES AT 37°C.

pH	Membrane	Slope $Min.^{-1} \times 10^4$	Rate/Unit Area $Cm.^{-2}$ $Min.^{-1}$ $\times 10^5$	D $Cm.^2/Min.$ $\times 10^4$
3.7	1	4.4959	8.50	6.189
	2	4.4327	8.38	6.102
	3	4.1496	7.84	5.712
	4	4.2755	8.08	5.886
	5	4.4485	8.41	6.124
	6	4.0167	7.59	5.529
	7	4.2228	7.98	5.813
11.7	1	4.4902	8.49	6.083
	2	4.5831	8.66	6.209
	3	4.5646	8.63	6.185
	4	4.0187	7.60	5.444
	5	4.4253	8.37	6.092
	6	4.0372	7.63	5.558

A representative curve for the diffusion of pentobarbital through a millipore membrane is illustrated in Figure 11. The average diffusion coefficient of pentobarbital at pH 3.7 was 5.908×10^{-4} cm.^{2}/min. with a standard deviation of 0.243×10^{-4} cm.^{2}/min; and that for pH 11.7 was 5.928×10^{4} cm.^{2}/min. with a standard deviation of 0.332×10^{-4} cm.^{2}/min.

Statistical analysis indicated that the two diffusion coefficients are not different at the 5% level of significance. Thus, it was concluded that the diffusion coefficients of ionized and unionized pentobarbital are the same within the error of experiment, and an average value (from all values of Table 12) of 5.917×10^{-4} cm.^{2}/min. with overall standard deviation of 0.277×10^{-4} cm.^{2}/min. was adopted for subsequent use.

Diffusion Coefficient of Salicylic Acid

It has been reported (70) that a dose of 10-30 Gm. of sodium salicylate caused acute poisoning and eventual death of adults. Plasma levels as high as 100 mg.% may be experienced in acute saliculism. Therefore a concentration of 10^3 micrograms/ml. was used in the diffusion studies. The diffusion coefficient of salicylic acid was determined in isotonic solution at pH 7.4, since the results of diffusion of pentobarbital indicated that no significant difference in diffusion coefficients of ionized and unionized forms of the drug. Experimental data for the diffusion of salicylic acid through millipore membranes are presented in Table 13. The calculated results are

Figure 11.- Typical diffusion curve of pentobarbital in isotonic solution at pH 3.7 through Millipore membrane at 37°C.

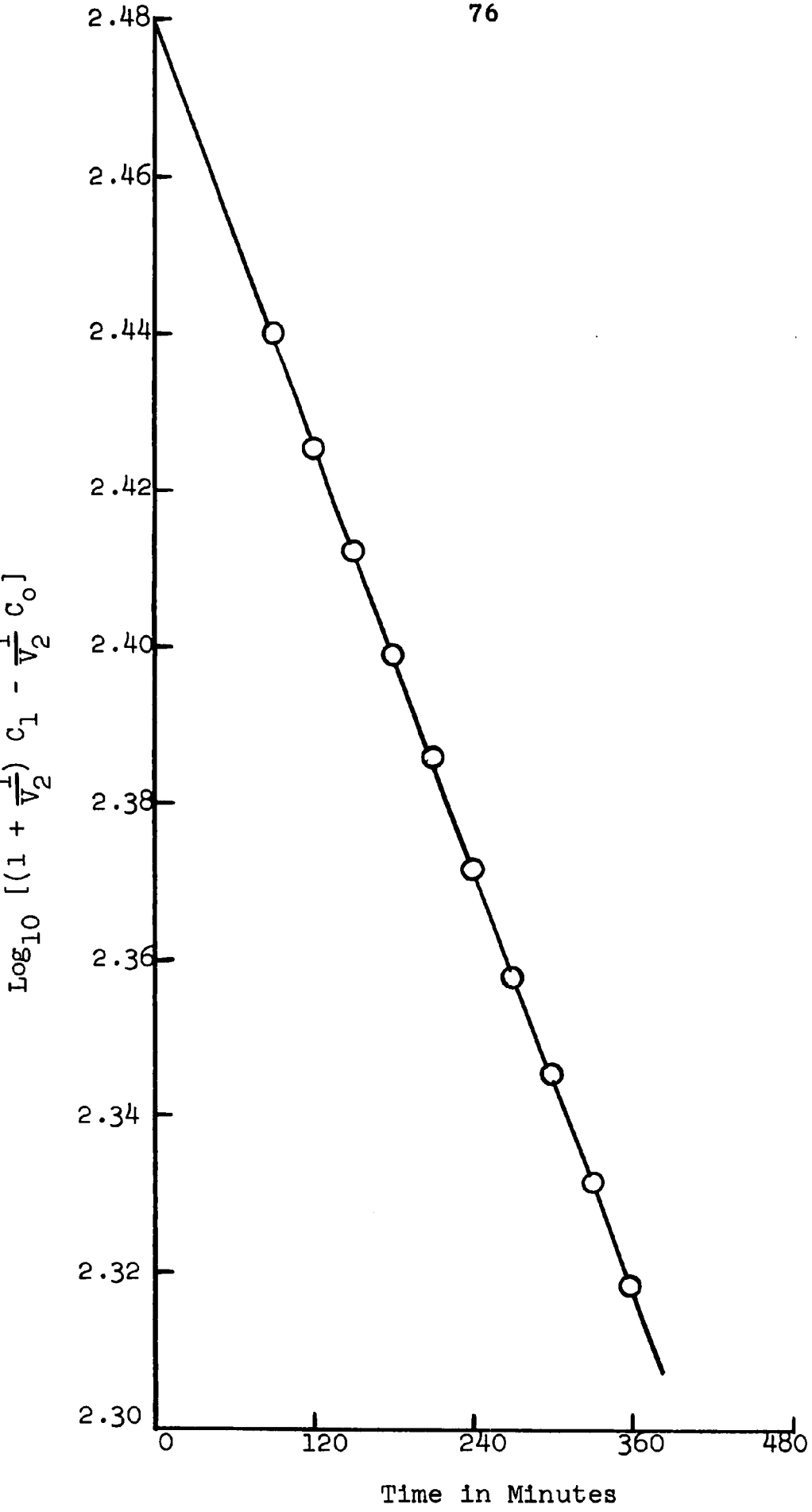

2.48
2.46
2.44
2.42
2.40
2.38
2.36
2.34
2.32
2.30
0
120
240
360
480
Time in Minutes
$\text{Log}_{10}\left[\left(1+\frac{V_1}{V_2}\right)C_1 - \frac{V_1}{V_2}C_o\right]$

TABLE 13 .

RESULTS OF DIFFUSION MEASUREMENTS OF SALICYLIC ACID AT pH 7.4 THROUGH VARIOUS MILLIPORE MEMBRANES AT 37°C.

Time Min.	Concentration of Salicylic Acid in Sink - Mcg./Ml.*		
	Membrane 1	Membrane 2	Membrane 3
30	16.8	20.2	15.4
60	31.3	32.2	33.0
90	48.2	45.2	49.3
120	66.4	59.2	65.8
150	83.0	73.7	83.0
180	97.5	88.0	96.0
210	112.8	103.6	110.9
240	123.1	117.0	125.0
	Membrane 4	Membrane 5	Membrane 6
30	16.6	16.5	17.4
60	32.9	32.4	33.5
90	48.8	49.2	49.1
120	65.1	63.6	63.9
150	80.2	79.6	78.1
180	94.6	93.9	92.9
210	108.7	108.6	107.8
240	...	121.4	118.0

* V_2 = 150 ml.

listed in Table 14, and a typical graph of a diffusion experiment is shown in Figure 12. In these experiments the volume of the sink, V_2, was equal to 150 ml. Therefore the calculated diffusion coefficients obtained by dividing the slope of the line by the membrane constant was multiplied by the factor $\left[(V_1 + V_2/V_1V_2)_1/(2/V)_2\right]$, where the subscripts 1 and 2 designates KCl and salicylic acid, respectively. The average diffusion coefficient is 6.901×10^{-4} $cm^2/min.$ with a calculated standard deviation(s) of 0.282×10^{-4} $cm^2/min.$ All the experimentally determined values lie in a range of $\pm 2s$.

Diffusion Coefficient of Urea

Clinical data show that blood urea levels may reach as high as 400 mg.% in acute cases of uremia, hence, a concentration of 3×10^3 micrograms/ml. of urea was selected in the determination of its diffusion coefficient. Experimental data for the diffusion of urea through Millipore membranes are presented in Table 15. The results were treated in the same manner as in the case of salicylic acid. The calculated slopes, rates per unit area, and diffusion coefficient are listed in Table 16. A representative curve for a typical diffusion experiment is shown in Figure 13. The average diffusion coefficient of urea is 1.018×10^{-3} $cm^2/min.$ and the standard deviation is 0.045×10^{-3} $cm^2/min.$ All the experimentally determined values for the diffusion coefficient of urea lie in a range of $\pm 2s$.

TABLE 14

SLOPES, RATES PER UNIT AREA, AND DIFFUSION COEFFICIENTS OF SALICYLIC ACID AT pH 7.4 THROUGH MILLIPORE MEMBRANES AT 37°C.

Membrane	Slope* $Min.^{-1}$ x 10^4	Rate/Unit Area $Cm.^{-2}$ $Min.^{-1}$ x 10^4	D $Cm.^2/Min.$ x 10^4
1	5.2960	1.005	7.205
2	4.7353	0.899	6.442
3	5.2980	1.005	7.207
4	5.1190	0.971	6.964
5	5.0992	0.968	6.937
6	4.8880	0.928	6.649

* Calculated by the method of least squares

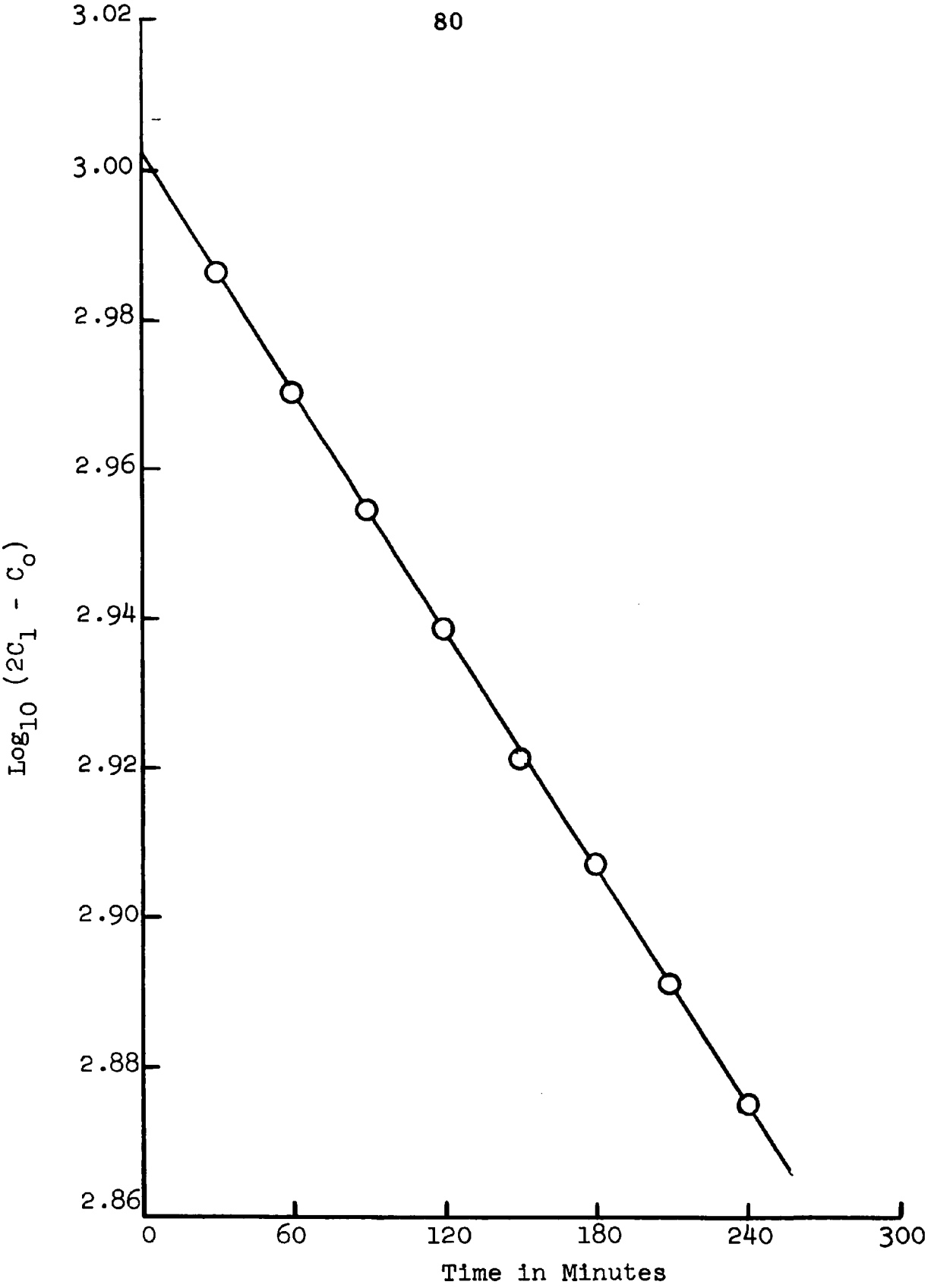


Figure 12.- Typical diffusion curve of salicylic acid in isotonic solution at pH 7.4 through Millipore membrane at 37°C.

TABLE 15

RESULTS OF DIFFUSION MEASUREMENTS OF UREA AT pH 7.4 THROUGH MILLIPORE MEMBRANES AT 37°C.

Time Min.	Concentration of Urea in Sink-Mcg./Ml.*		
	Membrane 1	Membrane 2	Membrane 3
30	82.6	66.4	65.2
45	121.6	96.5	96.4
60	160.2	128.7	130.8
75	194.5	164.4	164.3
90	227.2	195.1	196.4
105	260.3	219.0	231.0
120	291.9	255.0	268.1
135	322.6	287.1	298.4
150	355.0	313.4	328.9
	Membrane 4	Membrane 5	Membrane 6
30	87.3	86.7	70.0
45	124.9	123.7	97.9
60	163.7	157.8	143.5
75	196.6	195.2	181.6
90	231.9	227.5	212.0
105	263.7	260.4	242.4
120	292.8	298.7	273.9
135	328.3	326.0	309.3
150	360.4	356.3	339.4

* V_2 = 150 ml.

TABLE 16

SLOPES, RATES PER UNIT AREA, AND DIFFUSION COEFFICIENTS OF UREA AT pH 7.4 THROUGH MILLIPORE MEMBRANES AT 37°C.

Membrane	Slope Min.$^{-1}$ x 10^4	Rate/Unit Area Cm.$^{-2}$ Min.$^{-1}$ x 10^4	D Cm.^{2}/Sec. x 10^3
1	7.6522	1.4522	1.041
2	6.8431	1.2987	0.931
3	7.4078	1.4059	1.008
4	7.6971	1.4609	1.047
5	7.6911	1.4596	1.046
6	7.5967	1.4417	1.033

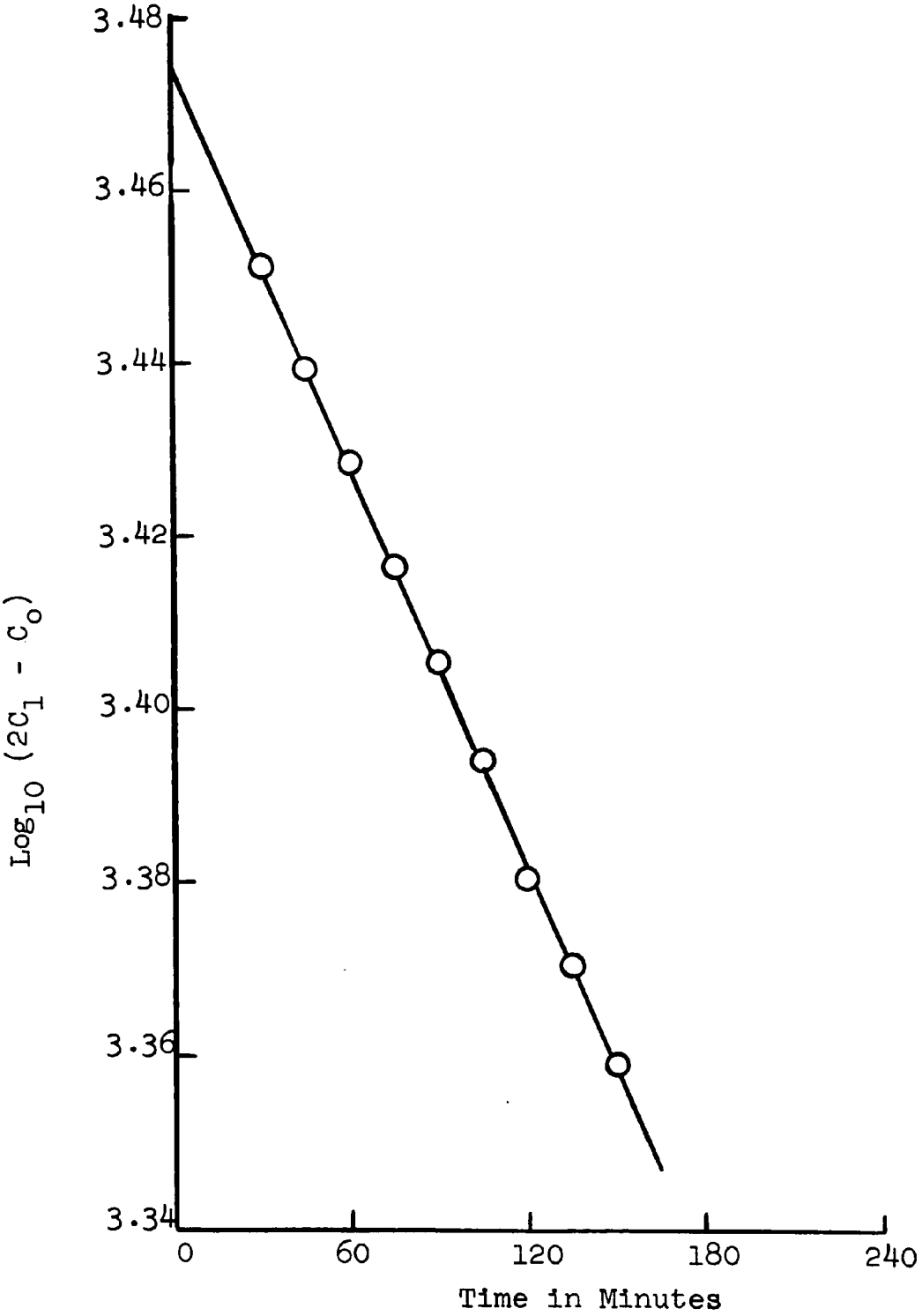


Figure 13.- Typical diffusion curve of urea in an isotonic solution at pH 7.4 through Millipore membrane at 37°C.

Diffusion Through Isolated Mesenteric Membrane

Materials and Methods

Diffusion through the isolated mesentery was studied using the three compounds whose diffusion coefficients were previously determined, these were, pentobarbital, salicylic acid, and urea. The diffusion apparatus, experimental conditions, and the concentration of solutions, as well as the method of assay were the same as was used for measurement of diffusion coefficients.

Preparation and Mounting of Membranes

Albino rabbits were sacrificed by injecting pentobarbital solution in a dose of about 50 mg./Kg. into the ear vein. Immediately after death, the abdomen was opened and the broad, fan-shaped fold of the peritoneum connecting the convolutions of the jejunum and ileum with the posterior wall of the abdomen was separated. The separated tissue was kept in normal saline solution and stored in the refrigerator for use. The saline solution was replaced every twenty-four hours to avoid putrifaction.

A piece of the mesenteric membrane freed from fat and blood vessels was mounted between a pair of teflon gaskets 1/16 in. thickness, care being taken to avoid stretching the membrane. These gaskets were designed specifically for the purpose of holding the membrane and exposing an area of membrane of 1.266 cm^2 for diffusion. This correspond-

ed to a membrane diameter of one-half inch. A diagramatic sketch of the teflon gasket is shown in Figure 14.

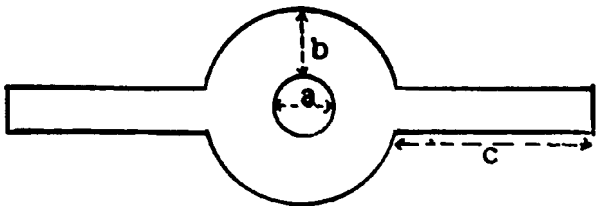


Figure 14. - Teflon gasket used in mounting the mesenteric membrane, a = 0.5", b = 7/16", c = 1-3/8".

Experimental Procedure

The mesenteric membrane mounted between the two teflon gaskets was firmly clamped in the diffusion apparatus by means of a screw type clamp. Every time a new membrane was used, the diffusion apparatus was tested for leakage. This was conducted by placing saline solution in one compartment of the apparatus and allowing the solution to rest on one side of the membrane. The remainder of the experimental procedure was the same as mentioned under the determination of diffusion coefficients with the following minor differences. Only 60 minutes was allowed before the withdrawal of the first sample in case of pentobarbital diffusion, and the sampling in case of urea diffusion was done every half-hour for a period of four hours.

Results and Treatment of Data

The experimental data for the diffusion of pentobarbital, urea, and salicylic acid through isolated rabbit mesentery are presented in Tables 17, 18, and 19, respectively. The data were plotted according to equations (53) and (55), and the slopes were calculated. From the slopes, the rates per unit area were calculated. Table 20 lists the rates per unit area of pentobarbital and urea across isolated rabbit mesentery, and Table 21 shows the rates per unit area for salicylic acid. Representative curves for diffusion of the three compounds are shown in Figures 15-17.

These data demonstrate that all the compounds tested diffuse surprisingly rapidly across the mesentery. Rates per unit area for mesentery are even faster than for the Millipore membranes, which are known to be highly porous. Furthermore, it is obvious from the data that both ionic and nonionic forms diffused rapidly.

Examination of the data presented in Table 20 for pentobarbital shows generally a decreasing trend in the rate per unit area with increase in pH. The only exception are the results in series B_{2-2} where the rate per unit area at pH 7.4 was greater than at pH 3.7 and 8.4. The decreasing trend remained the same if the order of change of pH with a given membrane was reversed or interchanged.

In order to examine the effect of pH on diffusion of pentobarbital through the mesentery, it is desirable to

TABLE 17

RESULTS OF DIFFUSION MEASUREMENTS OF PENTOBARBITAL AS A FUNCTION OF pH THROUGH ISOLATED RABBIT MESENTERY AT 37°C.

Time Min.	Concentration Pentobarbital in Sink - Mcg./Ml.*					
	Series B_{1-1}**		Series B_{2-1}			
	pH = 3.7		pH = 3.7		pH = 7.4	
60	7.44	8.61	8.45	7.87	7.80	8.27
90	11.43	12.57	11.36	11.73	10.59	11.34
120	15.13	16.29	14.32	15.27	13.61	14.50
150	18.93	19.58	16.64	18.70	16.86	...
180	22.53	23.01	19.60	22.30	19.79	20.36
210	26.13	26.78	21.45	25.89	22.27	23.52
240	29.38	29.59	23.99	29.38	25.51	26.17
270	33.28	33.17	26.52	32.23	28.01	28.86
300	36.58	36.02	28.74	34.82	31.24	32.07
330	39.34	39.55	30.65	38.57	33.63	34.81
360	41.85	42.26	33.55	41.48	36.38	36.90
	Series B_{2-1}				Series B_{2-2}	
	pH=7.4	pH = 11.7			pH = 3.7	
60	8.23	7.16	8.16	8.00	8.48	6.98
90	11.57	...	...	...	11.82	10.18
120	14.59	13.22	14.80	14.16	15.38	13.38
150	17.71	15.85	17.59	17.64	18.67	16.42
180	20.54	18.64	20.38	20.43	22.38	19.72
210	23.75	21.38	...	23.01	25.77	22.87
240	26.77	23.91	25.75	26.38	28.58	25.53
270	29.84	27.01	...	28.28	31.71	28.47
300	33.09	29.07	31.54	31.28	34.57	31.03
330	35.37	31.65	33.96	34.12	37.54	33.80
360	38.02	34.23	35.86	36.81	39.93	36.68

* V_2 = 158 ml.

** First subscript represents rabbit number, and the second subscript represents membrane number.

TABLE 17 - Continued

Time Min.	Concentration Pentobarbital in Sink - Mcg./Ml.*					
	Series B_{2-2}					Series B_{3-1}
	pH = 7.4			pH = 8.4		pH = 3.7
60	10.42	8.26	8.88	8.91	9.38	7.99
90	14.32	12.01	12.47	...	...	10.17
120	18.33	15.50	16.27	14.55	16.06	12.45
150	22.18	19.35	19.92	17.36	18.98	14.72
180	26.54	23.15	23.31	20.39	22.37	16.90
210	29.93	26.54	26.54	23.41	25.08	19.81
240	33.32	29.98	30.19	26.17	28.52	21.75
270	36.86	33.37	32.85	28.62	31.65	24.46
300	39.74	36.45	36.14	31.07	34.05	26.25
330	43.43	39.27	39.74	33.89	37.12	27.95
360	46.87	42.20	42.41	36.65	40.46	30.37

	Series B_{3-1}		Series B_{3-2}		
	pH=7.4	pH=8.4	pH=3.7	pH=7.4	pH=8.4
60	6.51	7.28	7.98	8.12	6.87
90	8.89	...	10.98	11.08	...
120	11.32	11.98	13.78	14.00	12.19
150	13.41	13.97	16.78	16.96	14.80
180	15.84	16.25	19.69	19.44	17.56
210	17.79	18.49	22.49	22.06	19.98
240	20.46	20.81	24.96	24.44	22.30
270	22.60	23.14	27.67	26.83	25.01
300	24.74	24.55	30.47	29.65	27.28
330	26.34	26.93	32.60	31.05	...
360	28.67	28.53	36.03	34.16	33.86***

* V_2 = 158 ml.

** First subscript represents rabbit number, and second subscript represents membrane number.

*** Sample withdrawn after 390 minutes

TABLE 18

RESULTS OF DIFFUSION MEASUREMENTS OF UREA AS A FUNCTION OF pH THROUGH ISOLATED RABBIT MESENTERY AT 37°C.

Time Min.	Concentration of Urea in Sink - Mcg./Ml.*						
	Series B_{2-2}**		Series B_{3-1}	Series B_{3-2}	Series B_{3-3}		
	pH 7.4				pH 3.7	pH 7.4	pH 8.4
30	79.5	78.3	51.9	54.7	103.5	131.3	93.3
60	134.9	130.9	88.6	106.5	150.6	175.1	142.4
90	189.8	181.2	125.8	153.7	198.4	223.9	189.9
120	239.0	229.8	159.1	199.5	242.2	262.6	237.4
150	289.9	277.9	...	241.7	285.4	296.8	284.9
180	336.2	317.9	225.7	283.6	321.8	340.6	318.3
210	392.2	367.6	264.0	326.1	373.5	382.1	355.6
240	428.8	412.8	299.0	367.7	409.9	420.3	401.3

* V_2 = 158 ml.
** First subscript represents rabbit number, and second subscript represents membrane number.

TABLE 19

RESULTS OF DIFFUSION MEASUREMENTS OF SALICYLIC ACID AS A FUNCTION OF pH THROUGH ISOLATED RABBIT MESENTERY AT 37°C.

Time Min.	Concentration of Salicylic Acid in Sink - Mcg./Ml.*			
	Series B_{4-1}**		Series B_{4-2}	
	pH 3.0	pH 7.4	pH 3.0	pH 7.4
30	12.6	14.7	23.5	11.1
60	25.6	29.7	40.1	23.1
90	40.3	44.4	55.9	36.4
120	55.0	59.3	70.0	49.7
150	67.9	73.1	83.3	62.6
180	79.2	88.5	...	75.3
210	92.7	101.2	105.3	87.9
240	105.4	116.2	116.2	99.1
	Series B_{5-1}		Series B_{5-2}	
	pH 3.0	pH 7.4	pH 3.0	pH 7.4
30	16.2	14.2	9.1	22.6
60	33.3	30.2	18.8	34.7
90	48.7	44.4	31.0	46.7
120	66.8	60.5	43.9	60.4
150	81.2	75.3	55.8	72.1
180	96.0	91.7	68.5	82.9
210	107.9	103.4	80.3	94.4
240	124.1	118.0	93.2	108.7

* V_2 = 150 ml.

** First subscript represents rabbit number, and the second designates membrane number.

RATES OF DIFFUSION PER UNIT AREA OF PENTOBARBITAL AND UREA THROUGH ISOLATED RABBIT MESENTERY AS A FUNCTION OF pH AT 37°C.

Series*	pH	Rate of Diffusion of Pentobarbital Per Unit Area Cm.$^{-2}$ Min.$^{-1}$ x 10^4			Rate of Diffusion of Urea Per Unit Cm.$^{-2}$ Min.$^{-1}$ x 10^4	
		EXPERIMENTS				
		1	2	3	1	2
B_{1-1}	3.7	3.1494	3.0394	...	...	...
B_{2-1}	3.7	2.1090**	2.9817	...	...	...
	7.4	2.4919	2.5335	2.6396	...	...
	11.7	2.2983	2.4244	2.4949	...	...
B_{2-2}	3.7	2.8266	2.5774	...	...	...
	7.4	3.3129	3.0250	3.0051	4.5095	4.2162
	8.4	2.4256	2.7375	...	...	...
B_{3-1}	3.7	1.9204	...	...	...	...
	7.4	1.8637	...	...	...	2.9537
	8.4	1.8075	...	...	...	...
B_{3-2}	3.7	2.3976	...	...	...	...
	7.4	2.2117	...	...	...	3.8294
	8.4	2.1152	...	...	...	...

*The first subscript represents rabbit number, and second subscript represents membrane number

** Low value due to formation of air bubbles on the surface of membrane.

TABLE 21

RATES OF DIFFUSION PER UNIT AREA OF SALICYLIC ACID ACROSS ISOLATED RABBIT MESENTERY AS A FUNCTION OF pH AT 37°C.

Series*	pH	Rate of Diffusion of Salicylic Acid per Unit Area $Cm.^{-2}$ $Min.^{-1}$ x 10^4
B_{4-1}	3.0	3.4351
	7.4	3.8200
B_{4-2}	3.0	3.5069
	7.4	3.2814
B_{5-1}	3.0	4.1175
	7.4	3.9373
B_{5-2}	3.0	3.1009
	7.4	3.2075

* First subscript represents rabbit number, and the second designates membrane number.

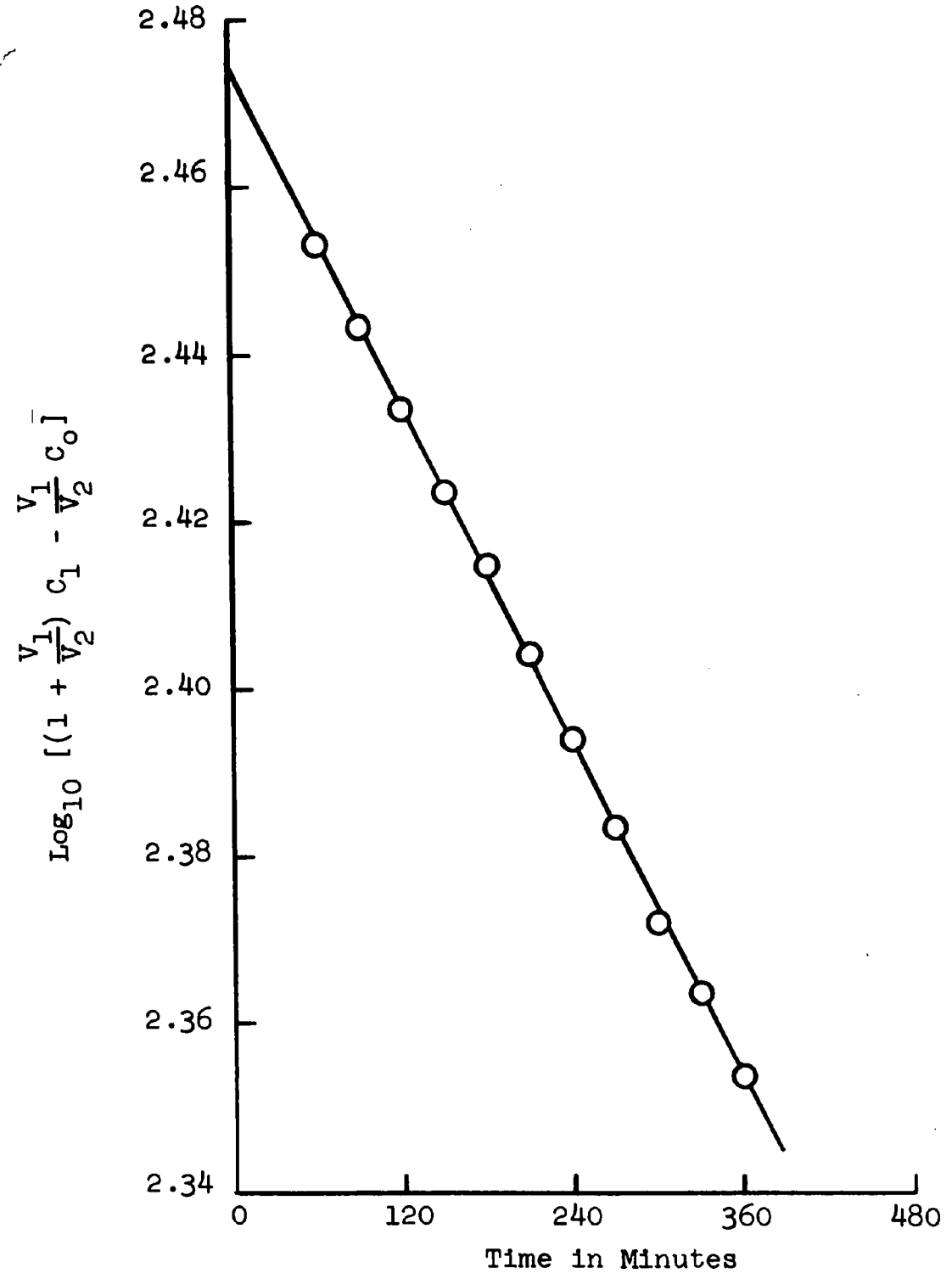


Figure 15.- Typical diffusion curve of pentobarbital in isotonic solution of pH 7.4 across isolated rabbit mesentery at 37°C. Series B_2-1.

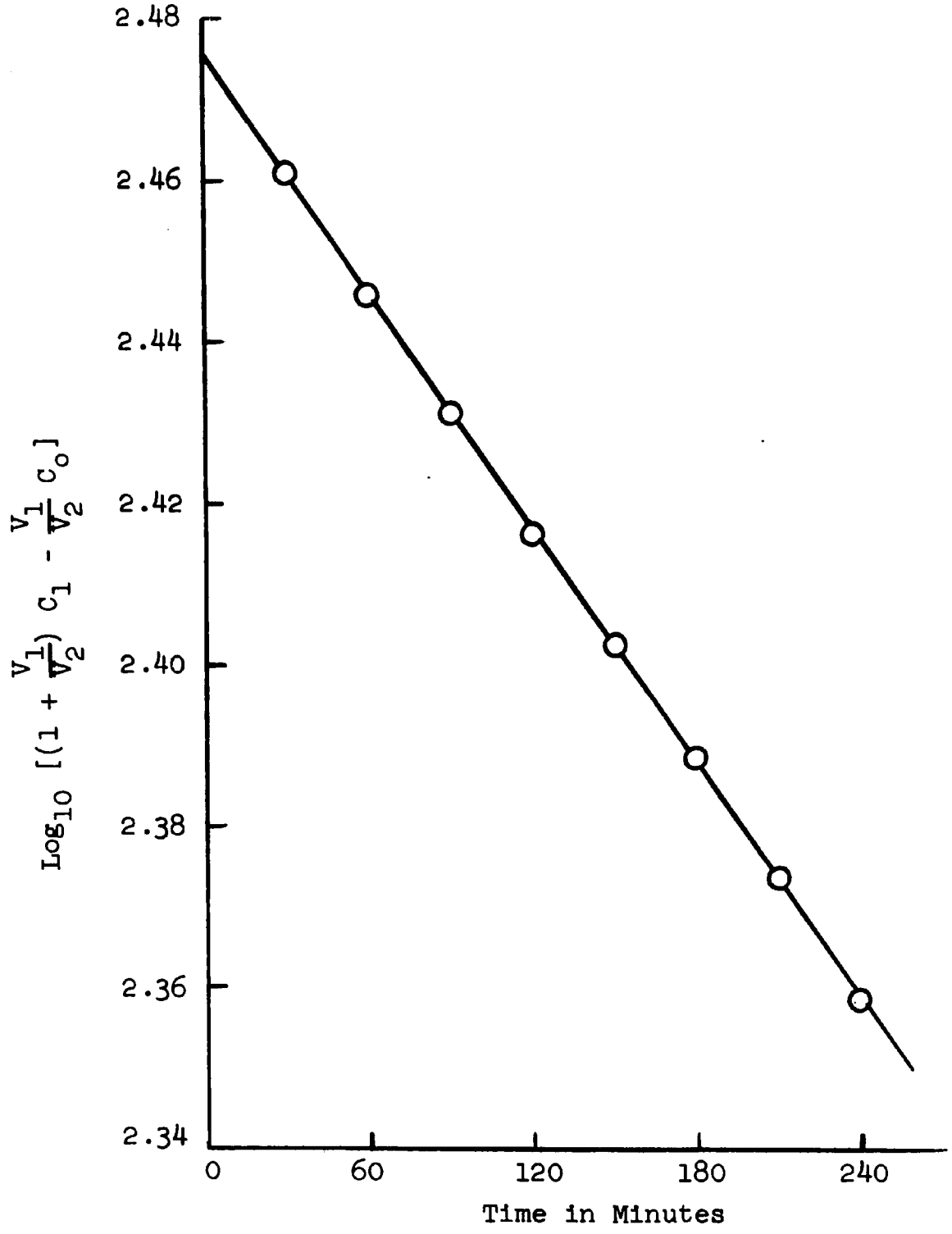


Figure 16.- Typical diffusion curve of urea in isotonic solution at pH 7.4 across isolated rabbit mesentery at 37°C. Series B_3-2.

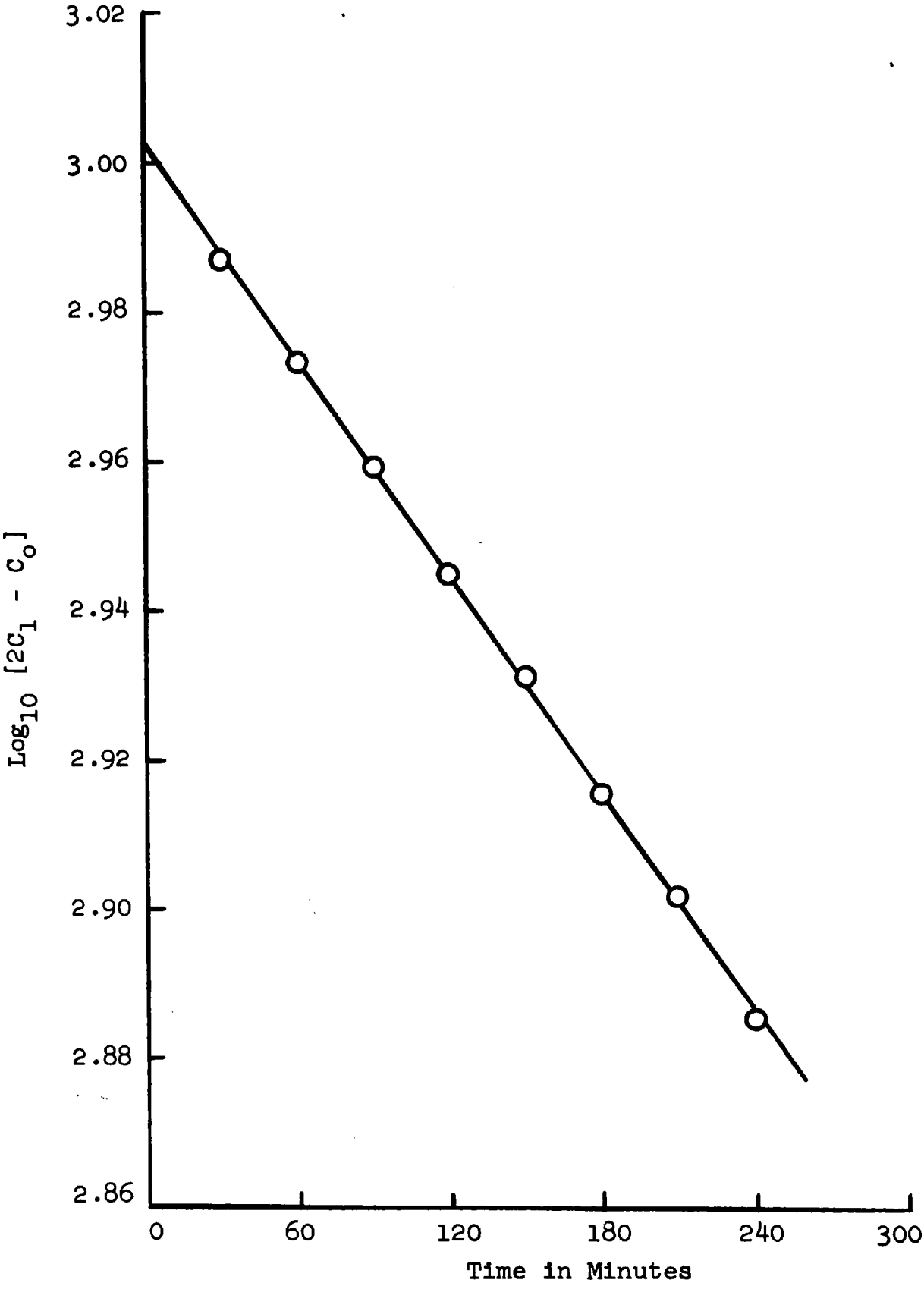


Figure 17.- Typical diffusion curve of salicylic acid in isotonic solution at pH 7.4 across isolated rabbit mesentery at 37°C. Series B_4-1.

estimate the proportions of ionized and unionized drug present at the four pH levels studied. The pka of pentobarbital in water at 25°C. has been reported as 8.11 (71). At the ionic strength of 0.16 the apparent dissociation constant of this drug is 7.96 at 25°C. The change in the apparent dissociation constant in a narrow range of ionic strengths is so small that it can be considered insignificant in this consideration. The ionic strengths of the solutions used in this study were in a range of 0.15 - 0.17. The calculated apparent dissociation constants of pentobarbital at 37°C. according to Krahl (71) for these two ionic strengths are, respectively, 7.66 and 7.65. Hence a value of 7.66 was adopted for the purpose of discussion. At pH 3.7 pentobarbital is almost exclusively undissociated and at pH 11.7 almost exclusively dissociated. The percentage of undissociated pentobarbital at pH 7.4 and 8.4 are approximately 64.5% and 15.4%, respectively.

From the above estimates of percentage of drug ionized, one should expect practically no drug to diffuse at pH 11.7, if solubility of unionized drug in lipids of the membrane were the major diffusion pathway. At pH 8.4 very little would be expected to diffuse and at pH 7.4 and 3.7 large increases in diffusion rate would be expected. In contrast to this, high rates of diffusion were obtained at all pH levels. This demonstrates that both ionized and unionized drug diffuse rapidly through the membrane and that lipid solubility of unionized drug is not the major pathway of diffusion in the case of pentobarbital.

The data contained in the first column of Table 20 were used for an analysis of variance testing the hypothesis that there was no difference in diffusion rate with change in membrane or with change in pH. The analysis of variance is summarized in Table 22. It is seen that a significant effect of membranes is found but the effect of pH is not significant. According to this analysis, then, the trend observed, slightly decreased rate with increase in pH, is not proven. If one examines the data used for this analysis in detail, it is found that a single membrane, B_{2-2}, showed a different pH effect. This resulted in a high interaction term in the analysis. Were it not for this one membrane the pH effect may have been significant. Thus, it seems likely that if a larger number of experiments were run to reduce the error term a small but significant pH effect might be found.

It was thought that the trend of decreased diffusion rate of pentobarbital with increase in pH might be an effect of pH on the membrane itself. To see if pH alone affected the permeability of the membrane it was decided to measure the diffusion of a compound which is equally undissociated over a wide range of pH. Urea was selected for this purpose. Urea is a weak base with pKa of 0.18 at 25°C.(72). The temperature coefficient of a weak base with a pKa of 3.3 is 0.011/degree (73), and the smaller the pKa the smaller the temperature coefficient. Therefore, if the temperature is raised one degree, the pKa of that base is reduced by 0.011 unit. Under the

TABLE 22

ANALYSIS OF VARIANCE FOR THE RATE OF DIFFUSION OF PENTOBARBITAL PER UNIT AREA FOR DIFFERENT pH AND MEMBRANES

Source	Sum of squares x 10^{16}	Degrees of Freedom	Mean Square x 10^{16}	$F = \frac{\text{Mean Square}}{\text{Residual}}$
Between pH	19,723,148	2	9,861,574	1.62[a]
Between membranes	150,144,144	2	75,072,072	12.33[b]
Residual	24,521,204	4	6,086,867	...
Total	194,388,496	..	...	...

a-Not significant; $F_{0.05;2.4} = 6.94$

b-Significant

conditions used in the present study, urea would occur almost exclusively in its undissociated form at the three pH's studied. Furthermore, urea is highly polar and its diffusion across the rabbit mesentery cannot be attributed to lipid solubility. Thus, the diffusion of urea at pH 3.7, 7.4, and 8.4 was studied, using a single segment of mesentery.

Results are shown in Table 23

TABLE 23

RATES OF DIFFUSION OF UREA PER UNIT AREA ACROSS AN ISOLATED RABBIT MESENTERY IN ISOTONIC SOLUTION OF DIFFERENT pH'S AT 37°C.

Series	pH	Rate of Diffusion of Urea per Unit Area $Cm.^{-2}$ $Min.^{-1}$ x 10^4
B_{3-3}	3.7	3.9219
	7.4	3.7225
	8.4	3.8711

The results in Table 23 illustrate that pH has no direct effect on the permeability of the membrane, the differences observed being within the usual experimental error.

Both urea and pentobarbital were run on three individual membranes in order to obtain a direct comparison of their rates of diffusion. Since urea is expected to be lipid

insoluble the ratios of diffusion rates of pentobarbital to that of urea should be constant at all pH's if lipid solubility of the former is not a major channel of diffusion. Furthermore, this ratio should be equal to the ratio of their diffusion coefficients, which is 0.581. On the other hand, if lipid solubility has a major role, the ratios of diffusion rates should vary, becoming larger at lower pH. Thus ratios of diffusion were calculated from the data and are presented in Table 24. The high ratio obtained for B_{2-2} with pentobarbital at pH 7.4 was excluded from the table.

TABLE 24

RATIOS OF THE RATES OF DIFFUSION OF PENTOBARBITAL/ UREA-RABBIT MESENTERY AT 37°C.

Series	Diffusion Ratio - Pentobarbital/Urea		
	pH 3.7	pH 7.4	pH 8.4
B_{2-2}	0.619	...	0.592
B_{3-1}	0.650	0.630	0.612
B_{3-2}	0.626	0.577	0.552
Average Ratio	0.632	0.603	0.585

Table 24 indicates a trend of increase in ratio with decrease in pH, though it can be seen that the change is small and is no greater than the variation between membranes. Thus, one must conclude that no significant lipid solubility effect is evident, though a small effect is suspected.

On examination of the salicylic acid data it is seen that diffusion occurred equally rapidly at pH 3.0 and 7.4. A _t_ test of these data showed no significant difference.

The published values for partition coefficients of unionized pentobarbital and salicylic acid are 0.05 and 0.001 (74). These were determined from a heptane-water system. If these values represent the true _in vivo_ partitioning into lipid membranes, the rate at which these two drugs would be expected to diffuse by lipid solubility would be quite low.

From these data and considerations, it thus seems clear that both ionic and nonionic forms of pentobarbital and salicylic acid pass through the mesenteric membrane mainly by passive diffusion through pores.

It is of interest to calculate the porosities of the membranes used. This was done using the average diffusion rates and the thickness of 38.5 microns (17). Average rates and calculated porosities are presented in Table 25. It is seen that porosities varied from 0.193 to 0.388, with an average value of 0.293. The average value is

TABLE 25

AVERAGE RATES PER UNIT AREA OF PENTOBARBITAL, UREA, AND SALICYLIC ACID ACROSS ISOLATED RABBIT MESENTERY AND CALCULATED POROSITIES OF THE MEMBRANES STUDIED

Series	Average Rate per Unit Area Cm^{-2} $Min.^{-1}$ x 10^4			Porosity of Membrane
	Pentobarbital	Urea	Salicylic Acid	
B_{1-1}	3.0944	...	...	0.357
B_{2-1}	2.5520	...	...	0.294
B_{2-2}	2.8443	4.3628	...	0.328[a] 0.293[b]
B_{3-1}	1.8639	2.9537	...	0.215[a] 0.198[b]
B_{3-2}	2.2415	3.8294	...	0.258[a] 0.257[b]
B_{3-3}	2.2415	3.8385	...	0.349
B_{4-1}	...	...	3.6275	0.327
B_{4-2}	...	...	3.3941	0.386
B_{5-1}	...	...	4.0274	0.304
B_{5-2}	...	...	3.1542	0.293
Average	2.5192	3.7461	3.5508	0.293

a– Calculated from pentobarbital value
b– Calculated from urea value

almost three times as great as calculated by Gosselin and Berndt (17). The reason for this difference may be in the segments of membrane tested, since it is reasonable to expect a wide variation in porosity from one part of the peritoneal membrane to another. In this study all sections were taken from the area thought to be most permeable, yet a wide variation between membranes was found.

DISCUSSION

One of the advantages of experimenting with isolated mesenteric membrane is that rates of diffusion may be evaluated under ideal and carefully controlled conditions. This enables one to eliminate the variables of an *in vivo* dialysis which may be mechanical in nature and in some manner may affect the apparent rates. One of the most important results of this work was the finding that substances employed in this investigation have diffused through the mesentery at surprisingly high rates. This indicates that peritoneal dialysis has the potential of being far more effective than it presently is in the clinic.

It was found that about 4-6% of the absorbed dose of pentobaribtal could be removed in a three-hour period by three exchanges of fluid in the peritoneal cavity (30,59). Whether this amount removed is sufficient to justify the treatment is questionable (54,59). Using the average diffusion rates obtained in this study and assuming ideal conditions, that is, perfect mixing in the abdominal cavity and blood stream, no protein binding, and no removal of drugs by other channels, one may predict the ultimate amount removed by intermittent dialysis. For this purpose assume a 60 Kg. adult with plasma volume of 5% body weight, volume of distribution 60%, and assume a pentobarbital plasma level of 30 mg./100 ml. Using equation (55) and assuming a value of 2,200 cm.2 for the effective surface area of the peritoneum, the amount of pentobarbital that

could be removed during a one-hour period of intermittent dialysis was calculated. This value is equivalent to 4% of the total amount present in the body. Considering the fact that pentobarbital is protein-bound to the extent of about 40%., this calculated result agrees rather closely to clinical experience. This indicates that intermittent dialysis for one-hour periods achieves nearly maximum results.

The predicted amounts for salicylic acid and urea removed, calculated in the same manner but using a plasma level of 100 mg./100ml. for the former and 300 mg./100 ml. for the latter, are shown along with pentobarbital calculations in Table 26.

TABLE 26

PREDICTED AMOUNTS OF SUBSTANCES REMOVED BY THE INTERMITTENT DIALYSIS USING THE IN VITRO DATA

Substance	Concentration in the Dialysis Fluid after one Hour Mg./Ml.	Amount of Subs. removed per Hour Gm.	% dialyzed
Pentobarbital	0.147	0.441	4.1
Salicylic Acid	0.498	1.496	4.1
Urea	1.496	4.487	4.1

From the above calculations it is seen that equilibrium between the blood and dialysis fluid is nearly reached within one hour and that extension of dwell time of the fluid in the abdomen would be of no benefit.

If one makes the same basic assumptions as above for the case of continuous dialysis, a very different result is obtained. In this case, the volume of the dialyzing fluid is much greater than that of the plasma, that is, $V_2 \gg V_1$; hence $\underline{(V_1/V_2)C_o}$ in equation (53) is very small compared to $\underline{C_1}$, therefore equation (53) reduces to

$$\log_{10} \frac{C_1}{C_o} = \frac{-\underline{KDA\epsilon}}{2.303\ V_1 h}\, t \qquad (60)$$

where $\underline{V_1}$ is the volume of the plasma, and $\underline{K}$, the partition coefficient, is eliminated assuming no partitioning across the peritoneum. Calculations, employing experimental values of rates of diffusion of these chemicals for the same blood levels, show that 94.6, 93.3, and 72.2 per cent of the total amount of urea, salicylic acid, and pentobarbital present in the body should be removed in one hour of continuous dialysis, respectively, again neglecting protein binding. Thus, with continuous peritoneal dialysis under optimum conditions, practically all of the drug should be removed in less than two hours, with no protein binding. If the drug is approximately 40% protein-bound, as in the case of pentobarbital, it should still be almost completely removed in three hours.

The predicted amount of a substance removed by continuous dialysis is far greater than is actually achieved in practice. The failure in practice seems most likely to be due to inefficient mixing of the dialysis fluid within the abdomen. Furthermore, there is a tendency for the fluid to form a direct channel of flow between the inlet and outlet and thus leaves a stagnant fluid volume throughout most of the cavity. It is doubtful that sufficient mixing may be attained to prevent the existence of these stagnant layers of fluid at the surface of the membrane. Nevertheless, it seems that a great improvement may be made by intensifying the mixing by mechanical means.

In case of both intermittent and continuous dialysis a reduction or elimination of protein binding would greatly accelerate the dialysis rate for pentobarbital and salicylic acid and probably all other drugs which are highly protein-bound.

The results of this study clearly show that both ionic and nonionic forms of the drugs studied diffused rapidly through the mesentery. This refutes the interpretations by Knochel et al.(45) that THAM and bicarbonate buffers improve the dialysis rates of phenobarbital by converting the unionized form of the drug to its ionic form and thus maintaining a high concentration gradient of the unionized form. The effects observed by these authors may have been due to reduction in protein binding, and possibly, in the case of THAM, to a direct action on the membrane. It should be

recognized, however, that other drugs with higher lipid solubility might diffuse, in addition to passive diffusion through pores, *via* the solubility of the unionized form in the membrane lipids, and in such cases the use of alkaline buffer might be highly effective in promoting dialysis.

This study illustrates the value of an *in vitro* evaluation of drug diffusion across the mesentery. In this manner one might determine the optimum conditions under which a variety of poisons, endogenous or exogenous, will diffuse. Also, the knowledge of the maximum rate attainable should be most helpful, since on this basis one can predict whether *in vivo* dialysis can be fruitful and recognize when improvements in the clinical technique are required.

The results of this work demonstrate that peritoneal dialysis can be greatly improved and that the published clinical results are far from optimum in many instances. It is hoped that this important method for the removal of poisons will thus be improved rather than be dropped from practice due to the limitations involved in the existing techniques of dialysis.

SUMMARY AND CONCLUSIONS

1. The diffusion coefficients of pentobarbital, salicylic acid, and urea in isotonic solutions were determined using Millipore filters. Their respective values were 5.917×10^{-4}, 6.901×10^{-4}, and 1.018×10^{-3} $Cm.^2/Min.$

2. The diffusion of these chemicals across isolated rabbit mesentery was studied as a function of pH. The results of these investigations indicated that both ionized and unionized forms of the drug diffused across this membrane with comparable rates.

3. It was indicated that the major channel of transference of these drugs across the mesenteric membrane was passive diffusion through pores.

4. The rate of diffusion of pentobarbital across the mesentery showed a slight increasing trend with a drop in pH. It was thought that this increase was due to partitioning of the unionized form in acid pH. Analysis of variance proved that the difference in rates of diffusion of pentobarbital at various pH's was insignificant at the 5% level. Under the experimental conditions of these studies, the contribution of lipid solubility to the overall rate was not proven.

5. The average experimentally determined rates of diffusion of pentobarbital, salicylic acid, and urea were used in calculating the amount of each chemical that could be removed, under optimum conditions, by both types of

dialysis. Calculations have shown that amount of the chemical that could be removed by intermittent dialysis was very small which rendered it an inefficient technique for the removal of poisons. This was due to the limitations of equilibrium conditions which were achieved in a short time. According to predictions, continuous dialysis, on the other hand, proved to be quite promising. Therefore, it was believed that the inefficiency of the present technique of continuous dialysis was due to poor mixing inside the peritoneal cavity. Therefore, it was felt that if the mechanics of continuous dialysis were improved, better efficiency would have been achieved. Consequently, a large portion of the administered toxic dose could be removed in one hour, thus providing the best known means for the removal of poisons from the blood stream.

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