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A Continuous Monitored Dialysis-Perfusion System for Biologic Application[1,2]

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The use of radioactive tracer materials in the study of compartments as related to biology, has been the subject of several reviews, exemplified by the reports of Robertson (1) and Solomon (2). To facilitate proper understanding, it is necessary to define compartments, their kinetics and the use of model systems.

Definition of a compartment for the purposes of the following study, and those projected, includes two categories. The first is a physical region with discrete boundaries and internal homogeneity for specific substances. The second is a chemical entity, or a complex of chemical entities, which may or may not be homogeneous, capable of binding a specific substance which by dissociation is related to an exchangeable pool.

A mathematical system of compartmental analysis has developed, to define the interaction of compartmentalized substances in a manner more precise than by intuitive understanding. This mathematical system has been modified in recent years to include the application of tracer materials (1, 2).

Study of model systems of biologic significance has been useful in understanding compartmental relations. Such systems allow for precise control of the conditions of observation. A preliminary description of a model system, which was a prototype of that discussed in the following text, has been previously published (3,4,5).

The current model system has been devised permitting the continuous monitoring of capture or escape of a gamma emitting radioisotope from a small dialysis bag. Specific modifications have been made for monitoring the escape of Beta emitting isotopes.

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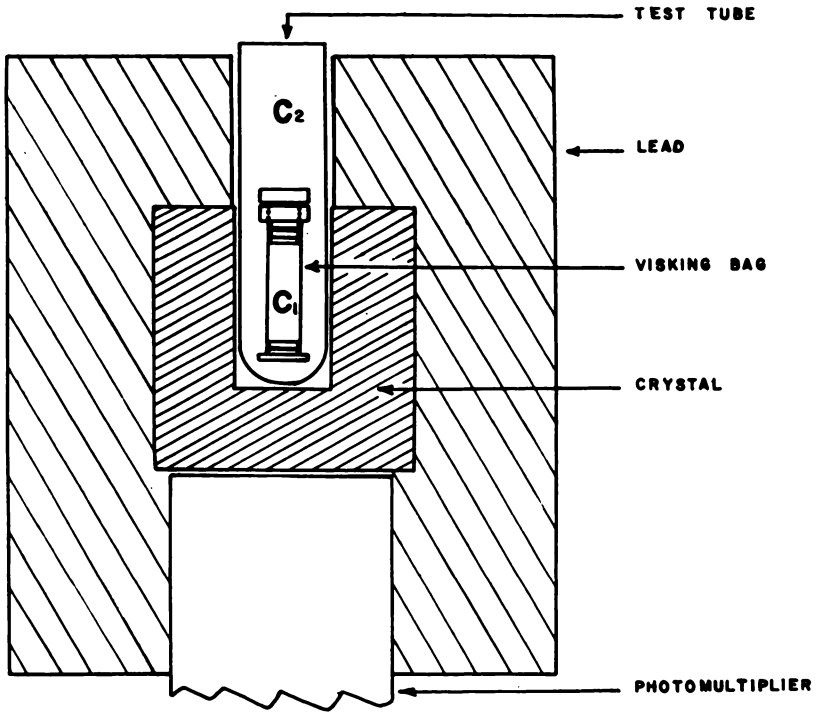

Fig. 1. Diagram of the two volumetric compartments of the Dialysis Chamber.

Evaluation of the dialysis system under the condition of a steady state and static flow, will allow development of a relatively simple equilibrium dialysis concept (Figs. 1,2a). Assuming the space within the dialysis bag is a Compartment C_1, and the space outside the bag is a second Compartment C_2, if a single permeable material P is considered equally distributed per unit volume in C_1 and C_2, a tracer portion in C_1 will distribute in C_1 and C_2 according to the kinetic formula of Solomon (2) for two compartments in the steady state.

In this instance, the detection of the tracer material by the scintillation detector will remain constant, except for minor geometric considerations (Fig. 2d).

Still assuming a steady state of the previously described system, the flow of non-labeled dialysate into and out of C_2 at an appropriate rate defined in Fig. 3 will decrease the concentration of tracer material P in C_2 effectively to zero. This will convert the closed two compartment system into an open single compartment system (Fig. 2b).

In this case, the scintillation detector will indicate the changing level of activity remaining in C_1, which is a straight line on a semi-log plot as observed, K_1 in Fig. 2d.

EQUIPMENT AND METHODS

For determining the loss of isotopes from the system, a dialysis chamber has been developed (Fig. 4).[1] The details of its operation are best seen in the

[1]Currently being processed for patent.

accompanying diagram and explained in the text below. The solution to be studied is placed in the Visking bag (1) after the distal end has been knotted or tied with nylon braided fishline to a solid cylinder of Teflon (m), and the proximal end tied with nylon braided fishline (k) to the female receptacle (j). The male insert (i) is machined to precise tolerance with the female receptacle (j) and acts as a stopper for the Visking Bag (1). The piston action of the insert reproduces the same pressure within the bag each time the same volume of solution is used for filling. Such a removable stopper allows repeated use of the same Visking bag in the dialysis experiments. The use of Teflon as the material for the cylinder, receptacle and insert, prevents significant adsorption of radioactivity on the surfaces exposed to radioactive solutions. The filled bag and plug assembly is then fastened to the thin nylon tubing (h) by threading the tubing through one set of grooves in the plug assembly. The nylon tubing (h) extends to the distal end of the Visking bag; the proximal portion of the nylon tubing has been made an integral extension of the body of the modified Swagelok heat exchanger tee (f). This fitting may be fabricated of nylon or Teflon—the latter is preferable. The bag and plug assembly suspended on the nylon tubing is inserted into the glass test tube (g) which is firmly fixed by tightening the Swagelok hex nut (c). The influent Tygon tubing (e) is secured to the tee by Swagelok hex nut (b) and the effluent Tygon tubing (d) by Swagelok hex nut (a).

The solution in the test tube external to the Visking bag is replaced at a constant rate of flow, adequate for quantitative removal of radioactivity diffusing from the bag. The influent solution enters via Tygon tubing (e) and the effluent leaves via Tygon tubing (d).

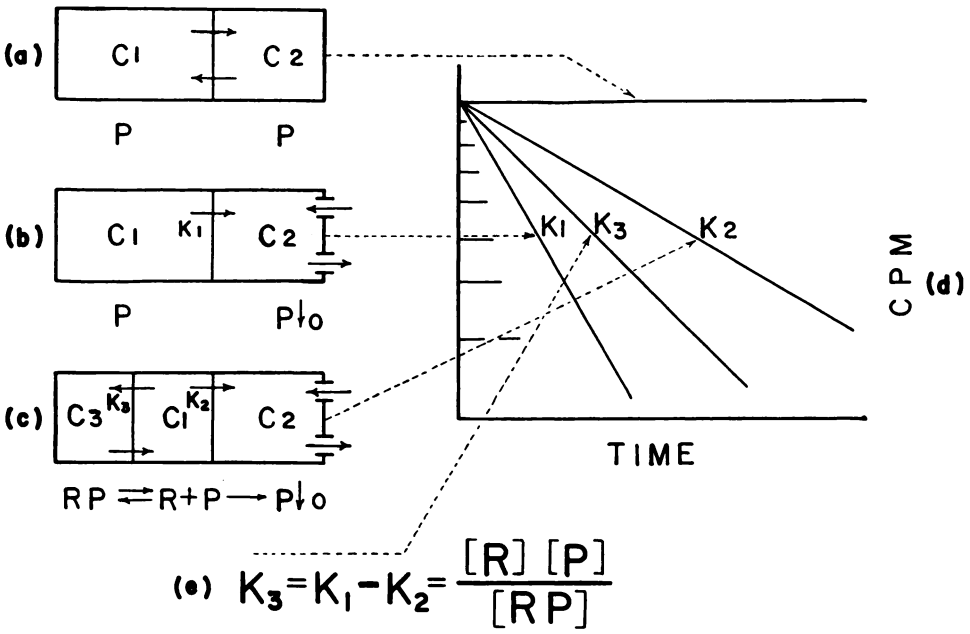

Fig. 2. Diagram of Compartmental relations.

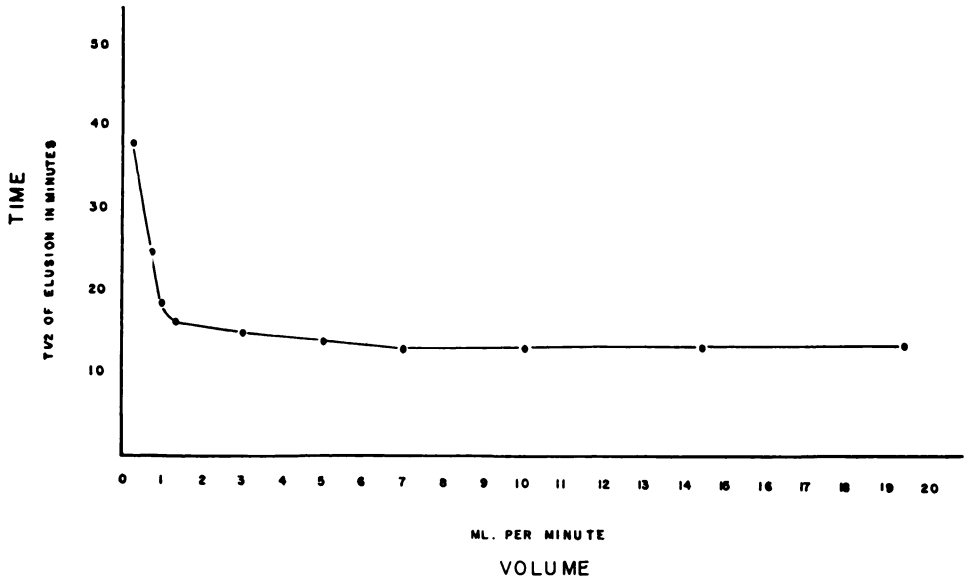

Fig. 3. Relationship of half time of elution of ^{22}Na to flow rate.

The Visking dialysis tubing is most effective with pretreatment as described by Smith (6) and modified in our laboratory. Sufficient one quarter inch Visking dialysis tubing from Visking Company, Division of Union Carbide Corporation, 6733 W. 65th Street, Chicago, Illinois, for repeated experiments is cut into 4.3 cm lengths. The pieces are heated to a gentle boil in 300 ml of distilled water in a 500 ml Erlenmeyer flask. After cooling to room temperature, the water is decanted and replaced with a clean supply. The water is replaced every day until the tubing is used or discarded after 21 days.

The dialysis chamber, as described, has several distinct advantages over an ordinary dialysis bag:

(1) The Teflon stopper allows reutilization of the same membrane for repeated dialysis experiments so that it may function as its own control.

(2) The piston action of the stopper permits precise reproduction of pressure within the bag on successive refilling when identical filling volumes are used.

(3) The notching of the Teflon plug and stopper permits attachment to the thin nylon effluent tube with exact reproduction of geometry within the test tube.

(4) The *Swagelok* fitting of inert plastic prevents significant adsorbtion of isotope by the plastic, and in addition allows easy changing of dialysis cells and chambers.

The test tube dialysis chamber is in the well of a scintillation well detector employing a 2" x 2" NaI(Tl) crystal and an EMI photomultiplier. The crystal is shielded by two inches of lead. The entire detector assembly is enclosed in an insulated chest which may be maintained at a constant temperature within a 6°C to 37°C range. A refrigerator with auxiliary heating elements and thermostat control is employed for this purpose.

The flow system is fed at a constant rate from a 13 gallon Carboy, by a

precision pump (Milton Roy mini pump). Auxiliary precision pumps running on the same shaft may feed specific proportions of substances to be added to the dialysate from the main reservoir (Fig. 5). In this way biologically active substances may be introduced into the flow system in calibrated quantity. Calibrating the output of the pumps assures extreme precision in flow rate (Fig. 6).

A temperature equilibrating chamber is within the insulated chest. This component consists of two 3800 ml stainless steel containers filled with water

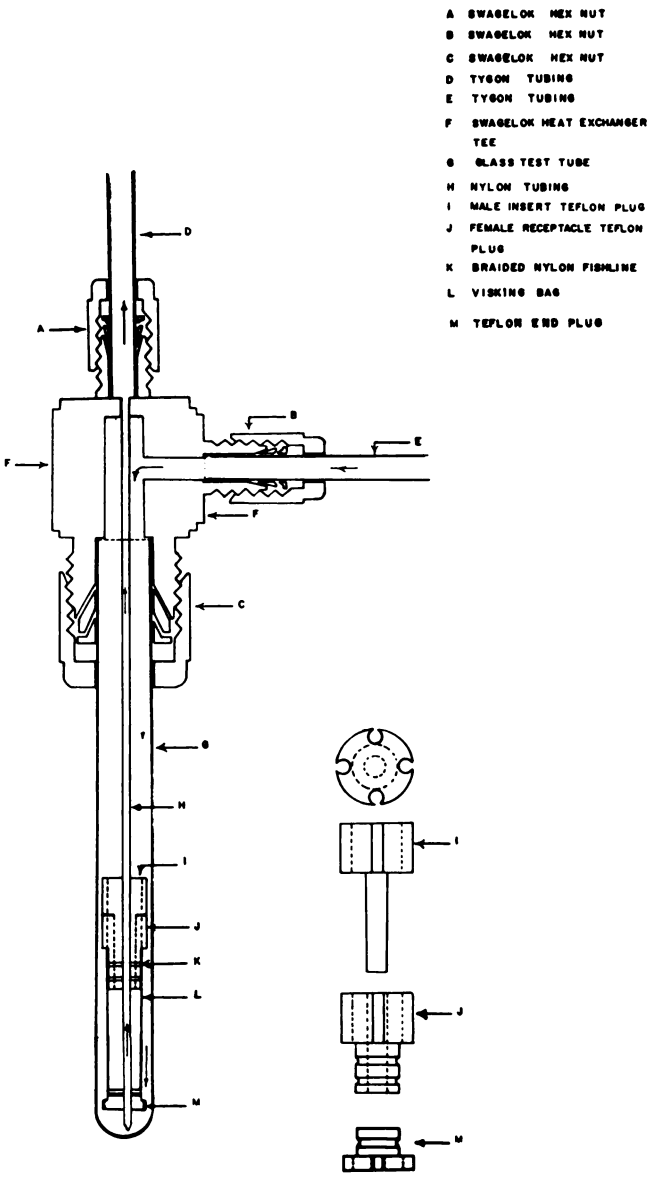

Fig. 4. Details of Dialysis Chamber.

equilibrated to the temperature of the insulated chest. Each container cover has a suspended 20-turn fixed immersible reel wound with one-quarter inch Tygon tubing (Fig. 7). At any instant each container is circulating 70 ml of dialysate. The two containers are serially connected and are inserted between the 13 gallon reservoir and the pump system (Fig. 5). Fluid from the reservoir is effectively equilibrated to the interior temperature of the chest for the flow rates employed.

The electrical output of the detector is fed from a pre-amplifier into a transistorized, modular component, digital count rate system made up of a pulse height analyzer, and a scaler-timer which accumulates counts during a preset time period. After printout the counts are again accumulated for identical intervals. The system will record simultaneously in a digital and analog manner. The analog readout is made on an X-Y point plotter, the Y axis being counts per preset interval, the X axis stepping per unit time interval. The display may be log or linear. A wide range of time intervals allows monitoring of rapid or slow phenomena (Fig. 8). The racked equipment is seen in Fig. 9. (See Table I)

An alternate mode of operation is provided to determine the kinetics of beta labeled substances in the dialysis chamber. In this mode of operation the scintillation well detector is not functional. The effluent from the dialysis cell is channeled through an anthracene crystal, beta scintillation flow detector, where appropriate electronic coupling allows readout of the interval accumulated counts by the digital and analogue recording devices (Fig. 8). A second alternate mode of function, employing a fraction collector, permits counting of aliquots in a liquid phosphor scintillation detector.

To evaluate the system several specific experiments were performed:

(1) A determination was made of the relation between the half time of

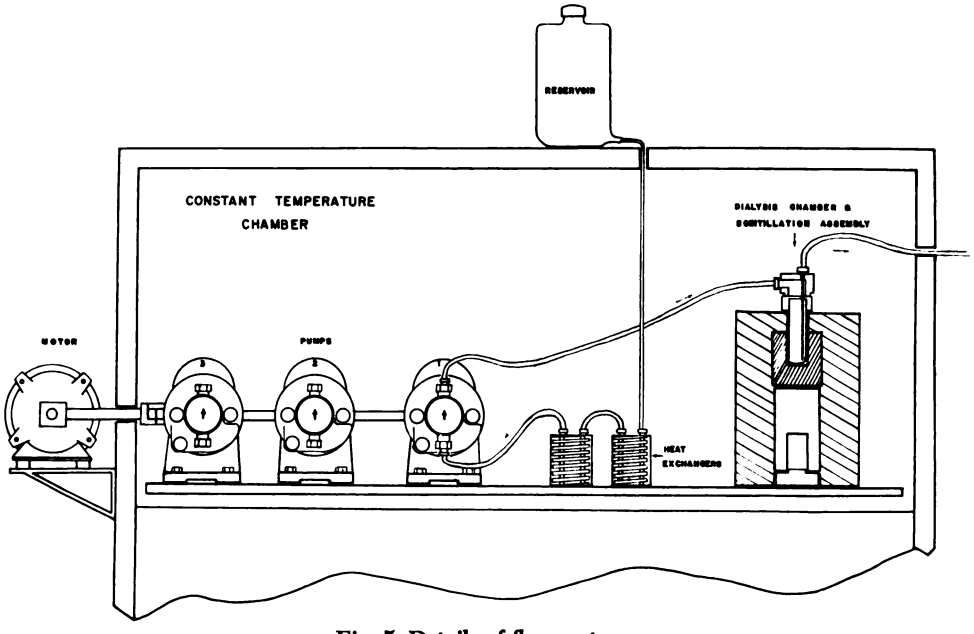

Fig. 5. Details of flow system.

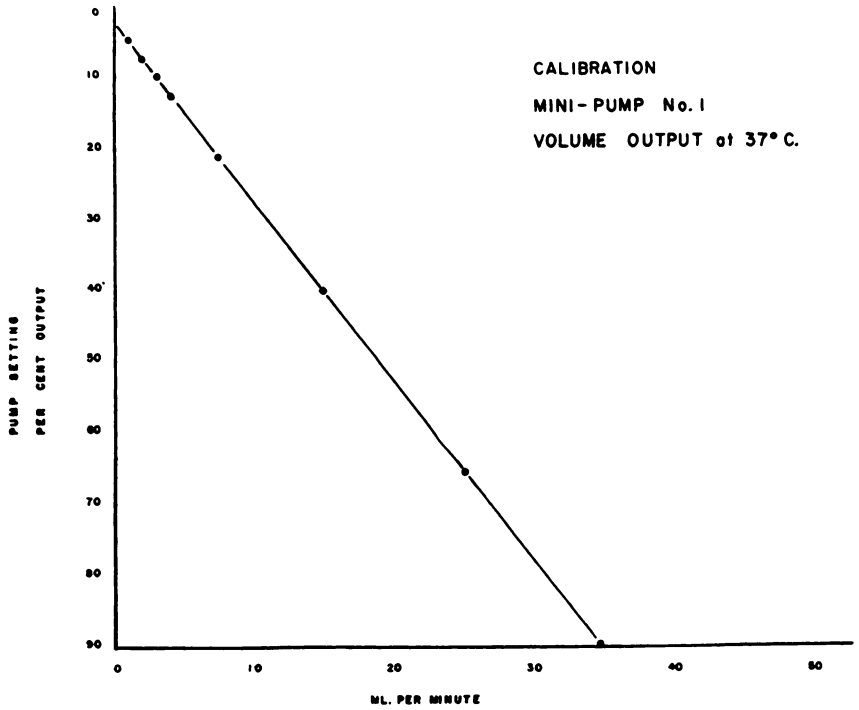

Fig. 6. Relationship of actual volumetric delivery to pump calibration.

elution of ^{22}Na from the dialysis chamber at varying flow rates, at a steady state with 0.128 M NaCl in the dialysate and within the cell.

(2) The characteristics of the elution curve of ^{22}Na were determined from the system at a flow rate of 10 ml of the above dialysate per minute through the chamber.

(3) The reproducibility of the elution curves of ^{22}Na was ascertained under the conditions in (2), using the same bag but replacing the ^{22}Na labeled solution.

(4) The reproducibility between different Visking bags of the same one-quarter inch diameter was also determined.

In all instances the ^{22}Na counts per unit time interval at zero time was approximately 10^5. The counting efficiency for ^{22}Na was 33 per cent. Counting was done in integral mode with a set threshold.

Studies were also made with ^{131}I solution using 10^{-5} NaI in 0.128 M NaCl as a carrier in the dialysis cell and in the dialysis chamber. 10^{-5} M sodium bisulfite was included as a reducing agent.

RESULTS

The rate of elution is expressed below as half time even though it may be more appropriate to use the rate constant.

(1) The half time of elution in minutes of ^{22}Na at varying flow rates between 0.2 and 19.4 ml per minute is graphically indicated (Fig. 3). When the flow rate is less than 1 ml per minute, the elution is very sensitive to flow rate. A flow rate from one to seven ml shows a slight decrease in $t_{1/2}$. Beyond seven ml per

minute the t½ of elution does not change with increased flow rate, indicating quantitative elimination of ^{22}Na from the chamber with no measurable back diffusion into the dialysis cell.

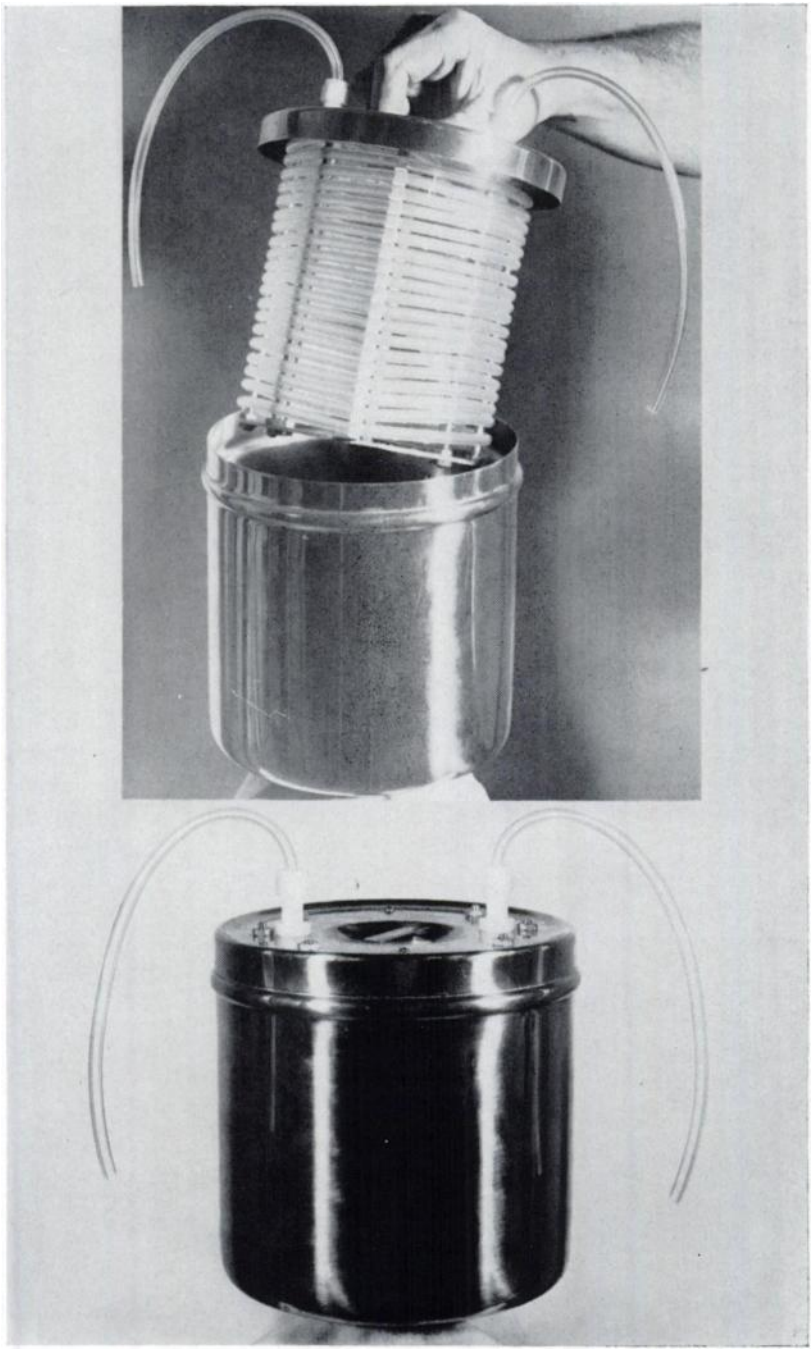

Fig. 7. Temperature equilibration.

(2) A typical elution curve of ^{22}Na from the system at a flow rate of 10 ml per minute and a temperature of 37°C is illustrated (Fig. 10). Counts of residual activity in the chamber were determined at 0.5 minute intervals for 99 intervals. Estimation of elution rate was made by the fitting of least squares on an IBM 1620 computer using the formula:

$$\ln Y = A + bT$$

where Y = the ordinate (in cts/time interval)
A = the intercept
b = the slope
T = the half time of elution

The calculation was made for nine successive groups of 10 time intervals plus one final group of nine intervals (Table II).

The mean value for elution half time was 16.48 minutes with a 95 per cent confidence level of the values of any of the groups falling between 16.10 minutes and 16.88 minutes.

The reproducibility of successive groups of 10 time intervals between the 100th and 198th time interval, indicated progressive increase in variability with a trend toward increased half time of elution (Table II, Fig. 11).

The mean value for elution half time was 17.43 minutes with a 95 per cent confidence level of the values falling between 16.49 and 18.49 minutes.

(3) The reproducibility of the elution half time of ^{22}Na was determined for the same dialysis tubing, with successive changes of ^{22}Na labeled NaCl solution.

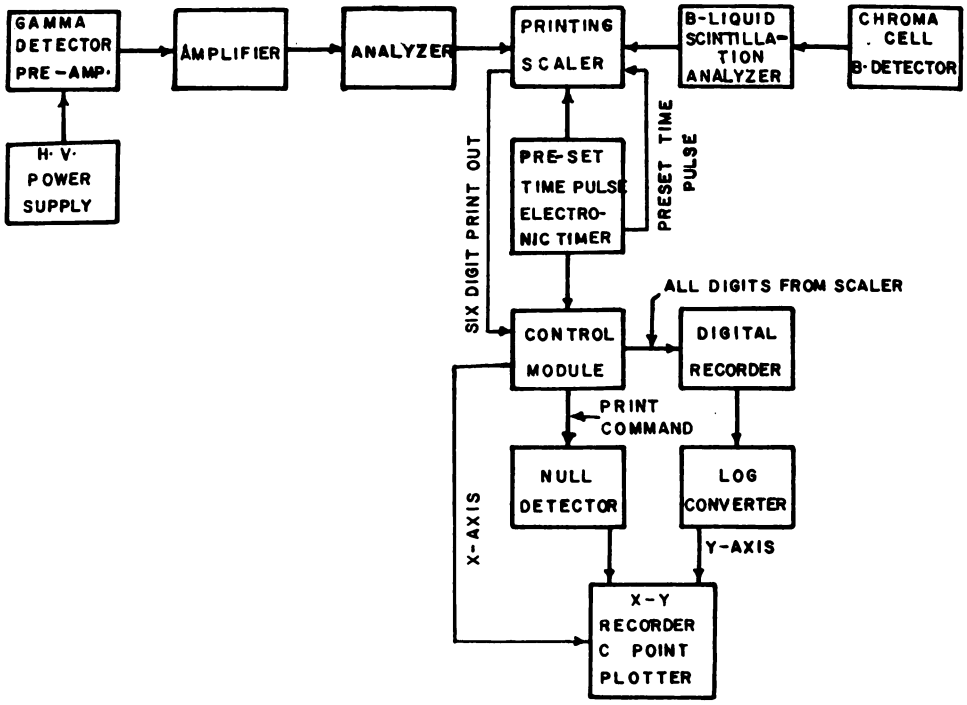

Fig. 8. Block diagram of electronic system.

Employing the least squares method described above, the time interval of observation was 0.5 minutes. Half life estimations were made on three successive blocks of 10 0.5 minute intervals for each curve. Five changes of solution for each sample of tubing were accomplished on an individual day. Observations were made on three bags on three different days (Table IV). The difference of half time of any of the three blocks of ten within a single run should not differ from the mean value of all the three blocks by more than 0.70 minutes or 4.77 per cent within a confidence level of 95 per cent.

The difference of estimated half time of elution of any block of 10 from the mean value of all the five runs on a specific day should not differ by more than 0.75 minutes or 5.12 per cent within a confidence level of 95 per cent.

Estimates of half time of elution of ^{22}Na made as indicated above with exception of a temperature of 9°C was made on three different days with intervals of three and eight days from the initial observation. The time interval between individual values on the curves was 2.0 minutes. The estimates of half time in each instance were made on the block represented by the 11th to 25th 2.0 minute interval. For the total of eight different curves on three different days, the mean value was 28.2 minutes half elution time with a 27.9 to 28.6 minute range and a 95 per cent confidence level or ± 1.24 per cent.

The individual sections of Visking tubing gave adequate and reproducible results between the 4th and 21st day of use. A longer period of use is possible if adequate precautions are taken to prevent growth of contaminating micro organisms.

TABLE I (See Fig. 7,8)

ELECTRONIC COMPONENTS OF MONITORED DIALYSIS SYSTEM

Component	*Manufacturer*	*Model No.*
Gamma Detector		
Photomultiplier Tube	EMI	9536S
NaI(T1)2″ x 2″ Well Crystal	Harshaw	——
Scintillation Preamplifier	RIDL	31–15
High Voltage Power Supply	RIDL	40–9B
Linear Amplifier and Discriminator	RIDL	30–19
Anti-Walk Single Channel Analyzer	RIDL	30–10
Six Decade Printing Scaler	RIDL	49–44 Modification W/O 5130
Pre-Set Electronic Timer	RIDL	54–8
Control Module	RIDL	Special
Digital Recorder	Hewlett-Packard	H43–562A
Null Detector	Moseley	G–2
Log Converter	Moseley	60D
X-Y Recorder	Moseley	2DR–2
Chromacell B Detector	Nuclear-Chicago	——
Liquid Scintillation Flow System	Nuclear-Chicago	6770

[1]Assembled by RIDL, Division of Nuclear-Chicago Corp.

TABLE II

ANALYSIS OF 198 POINT ELUTION CURVE OF ^{22}Na FIRST 99 POINTS

Serial Time Intervals 0.5 Minutes	*Zero Intercept Counts/Time Interval*	*Slope %/Minute*	*Half Time in Minutes*
10	92,251	4.12	16.84
10	92,990	4.31	16.08
10	92,945	4.26	16.29
10	94,055	4.34	15.96
10	94,079	4.32	16.03
10	89,398	4.13	16.79
10	89,977	4.5	16.69
10	90,844	4.16	16.65
10	89,533	4.13	16.78
9	88,923	4.11	16.83
Combined Slope		4.21	
Average Half Time			16.48 ± 2.43%(2S.D.)

(4) The differences observed between different Visking bags was significant and variable despite the use of sections of tubing from the same roll. This variability did not influence the reproducibility of a single sample which is accurately evaluated above. Rate of elution is dependent and proportional to temperature (Fig. 12). Between 9° and 33°C for each degree increase in temperature the half time of elution of ^{22}Na at a steady state decreases by .58 minutes. Between the range of 9° to 33°C the response is linear. It is assumed that as freezing

TABLE III

ANALYSIS OF 198 POINT ELUTION CURVE OF ^{22}Na SECOND 99 POINTS

Serial Time Intervals 0.5 Minutes	*Zero Intercept Counts/Time Interval*	*Slope %/Minute*	*Half Time Minutes*
10	11,433	4.39	15.80
10	11,776	4.44	15.61
10	11,482	4.07	17.03
10	12,256	4.45	15.56
10	11,723	4.15	16.70
10	10,856	3.82	18.15
10	10,325	3.70	18.75
10	11,499	4.01	17.30
10	9,295	3.48	19.92
9	7,332	2.99	23.20
Combined Slope		3.98	
Average Half Time			17.43 ± 5.14%(2S.D.)

temperature is approached, the rate of elution would be more significantly diminished, and with increased temperature the rate of decrease of half time of elution would be somewhat less temperature dependent. This latter trend is indicated and should be further studied. The membrane is not altered by the experiment since the initial and final experimental determination at 9°C was reproducible. The rate of change of elution is much more rapid than could be attributed to change in absolute temperature. It may be assumed that transference of $^{22}Na^{+}$ is related to energy transferred from water and that velocity of water molecules is decreased at lower temperature by water to water binding resulting in a clathrate structure.

The surface contamination of the glass, plastic and Visking components within the well, is not significant for either ^{22}Na or ^{131}I. This contamination did not exceed 300 counts per time interval, using a dialysis cell containing activity of 10^5 counts of the isotope per time interval at zero intercept. Background counts were less than 300 counts per time interval at intervals of 2.5 minutes or less. Total background plus contamination usually did not exceed a total of 350 counts per minute.

TABLE IV

REPRODUCIBILITY OF ELUTION TIME OF ^{22}Na WITH SERIAL REFILLING OF DIALYSIS CELL

Serial Refilling of Dialysis Cell	*0.5 Min. Time Intervals*	*Bag 1 Half Time*	*Bag 2 Half Time*	*Bag 3 Half Time*
1	0–10	16.66	12.93	14.05
	11–20	16.08	12.54	14.21
	21–30	15.93	13.37	13.59
2	0–10	16.28	13.53	13.35
	11–20	16.51	14.04	14.11
	21–30	16.05	13.95	14.84
3	0–10	17.56	13.09	14.10
	11–20	16.51	13.35	14.17
	21–30	16.80	13.89	14.45
4	0–10	17.17	13.25	13.02
	11–20	16.24	13.54	13.91
	21–30	15.35	12.88	13.94
5	0–10	17.68	13.51	13.55
	11–20	16.96	13.30	14.19
	21–30	16.48	14.32	14.60

The elution time of ^{131}I in a steady state system was comparable with that of ^{22}Na. The rate differed from that of ^{22}Na in being continually variable with a decrease in slope with time. This could not be eliminated with adequate reduction by bisulfite, and was not accounted for by adsorption of isotope to surfaces (Fig. 13). A tentative explanation of this phenomen is based upon the probable

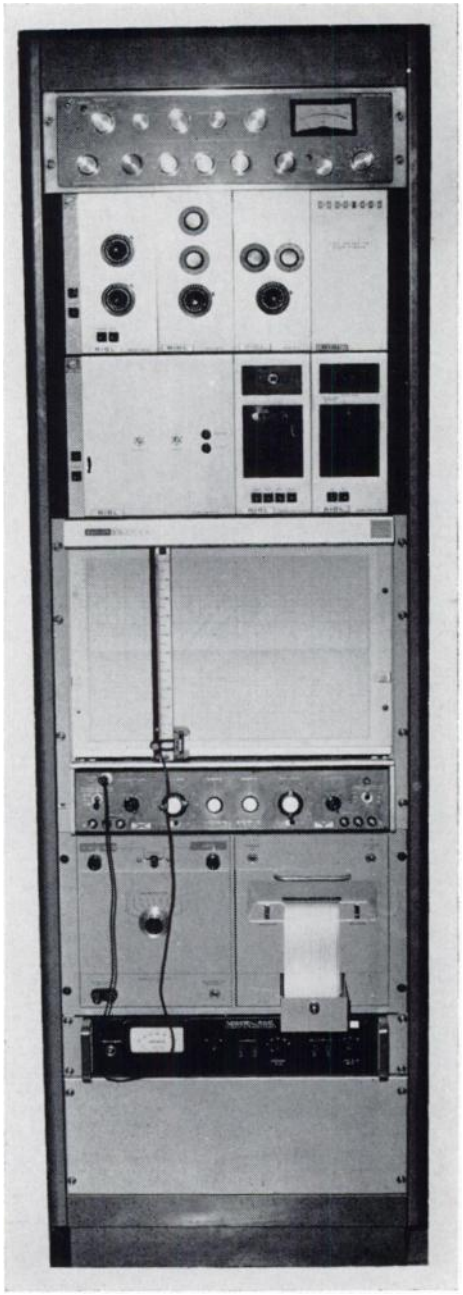

Fig. 9. Racking of electronic system.

presence of a group of complexes and oxidation products of iodine normally associated with iodide ion in solution.

DISCUSSION

The evaluation of the electronic and mechanical component of the continuous monitored dialysis system demonstrates a stable device, capable of a high degree of precision and reproducibility in determining the rate constant of diffusion of a permeable substance, out of a defined compartment.

Predicated upon this precision will be the capacity to resolve an altered rate of diffusion resulting from interaction of a permeable labeled substance with a non-permeable substance. Assuming steady state conditions, addition of a non-permeable substance R, to C_1 which may reversibly bind a permeable substance P, introduces a chemical compartment C_3 as an integral component of C_1. Thus, converting the open one compartment system (Fig. 2b) into a catenary system with reversible and nonreversible components (Fig. 2c). Examination of the reversible component $C_1 \overset{K_3}{\rightleftharpoons} C_3$ indicates that the constant K_3 is similar to the dissociation constant of RP (Fig. 2e). Since K_3 is not directly measurable it must be derived by subtracting the rate constant K_2 in Fig. 2c from the rate constant of the two compartment system K_1 in Fig. 2b. Where the interaction between R and P is binding, the rate of the nonreversible component will be limited by the availability of P from the dissociation of RP in the reversible component. K_2

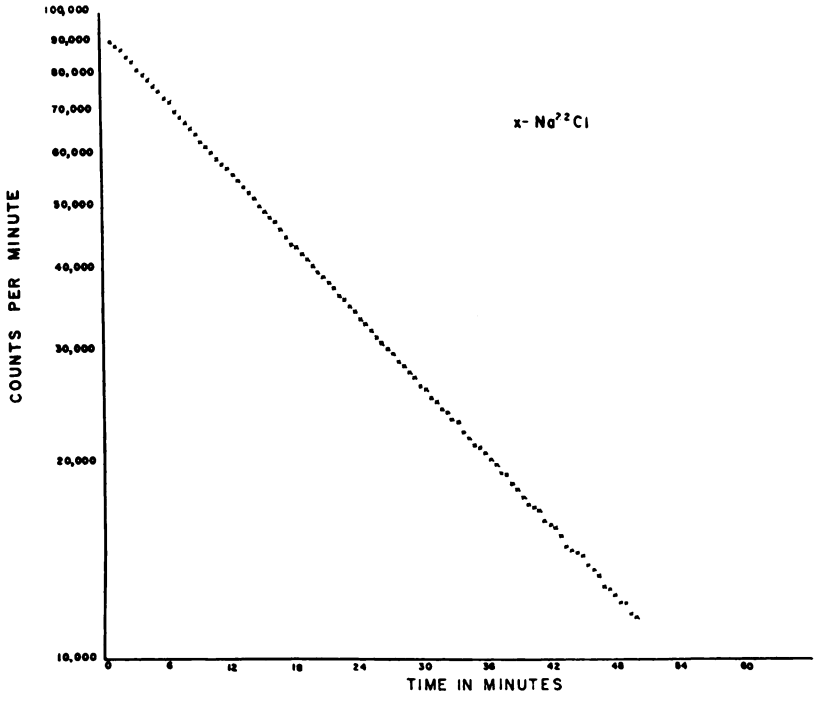

Fig. 10. Curve of elution of ^{22}Na from dialysis cell first 99 points of 198-point curve.

will always be less than K_1. The steady state and chemical concentrations are not altered as the indicated changes occur in the tracer components only.

It may not be assumed that C_3 within C_1 represents a pure dissociation in dealing with such complex nonpermeable systems as cells, tissues, protein mixtures, etc. The altered kinetics of a permeable component leaving C_1 will only represent the total resultant of interaction within C_1.

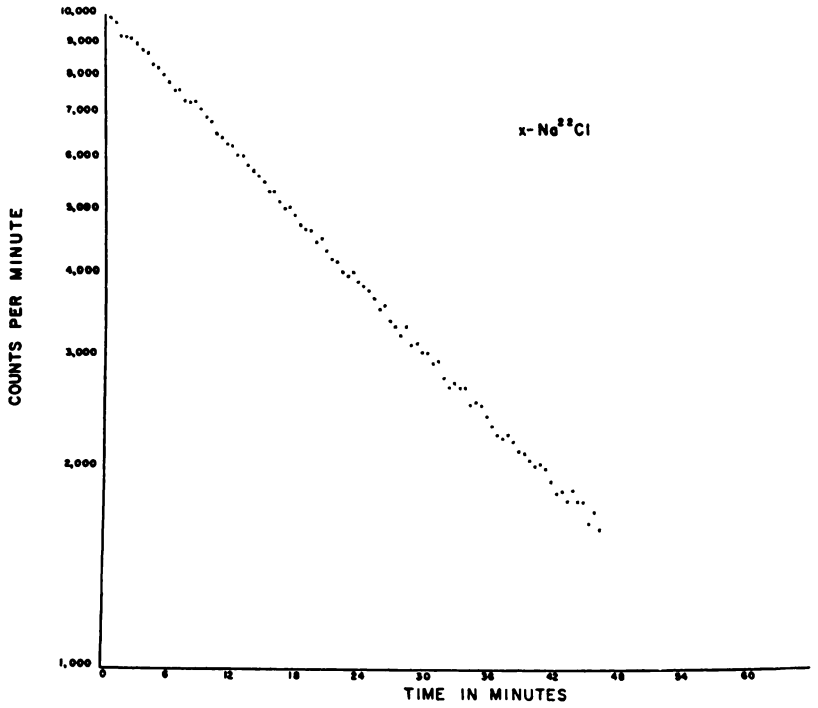

Fig. 11. Second 99 points of curve in Fig. 10.

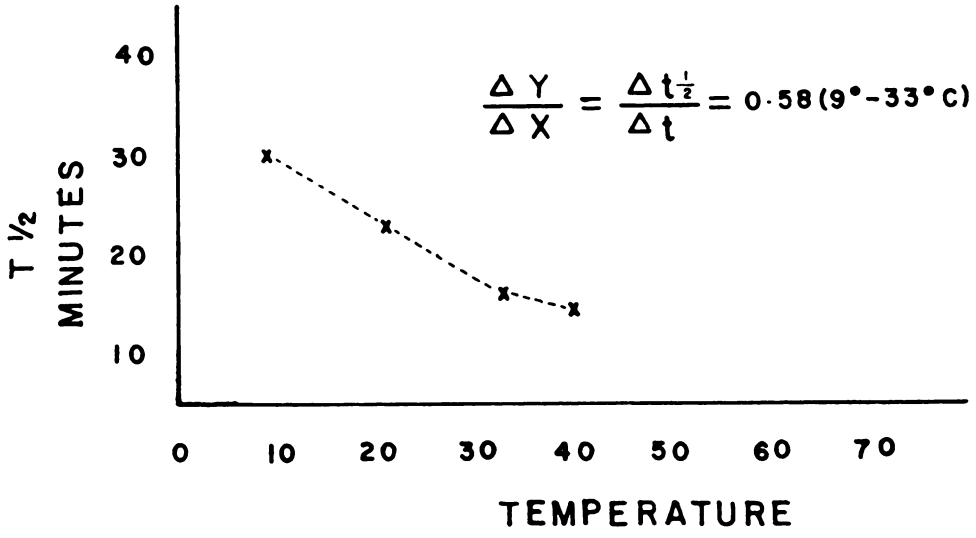

Fig. 12. Relationship of half time of elution of ^{22}Na as related to temperature.

The kinetics of perfusion may be derived for the system described when it is perfused by low concentration of tracer capable of accumulating in a concentrating biologic reactant. This derivation may be made using known published formula (1) (2). If this statement may be assumed lengthy, discussion may be avoided.

If the above theoretical discussion is valid in the steady state and the dissociation constant may be determined, the generalized formula $\triangle F = -R T \ln K$ would yield the free energy of dissociation.

Several types of application have been undertaken and others are proposed, all of which may be accomplished with the system in its current state. Observation of a rigidly controlled microcosm may be undertaken wherein the conditions of observation do not effect the subject concerned. It then is possible to express the interaction of permeable labeled substances with nonpermeable nonlabeled substances, including modifying nonlabeled environmental chemicals and express this relationship in terms of mathematical constants. A means is provided for veryifying hypothetical concepts of compartmental kinetics which may be useful in the interpretation of non-steady state phenomena, which are so common and so frustrating in biologic systems.

The interaction of specific ions with protein may be studied. These are exemplified by Fe, Ca, Cu and Mg which are bound by proteins and in the case of serum often by specific proteins. A preliminary study on binding of thyroxine to serum protein was published from this laboratory prior to the development

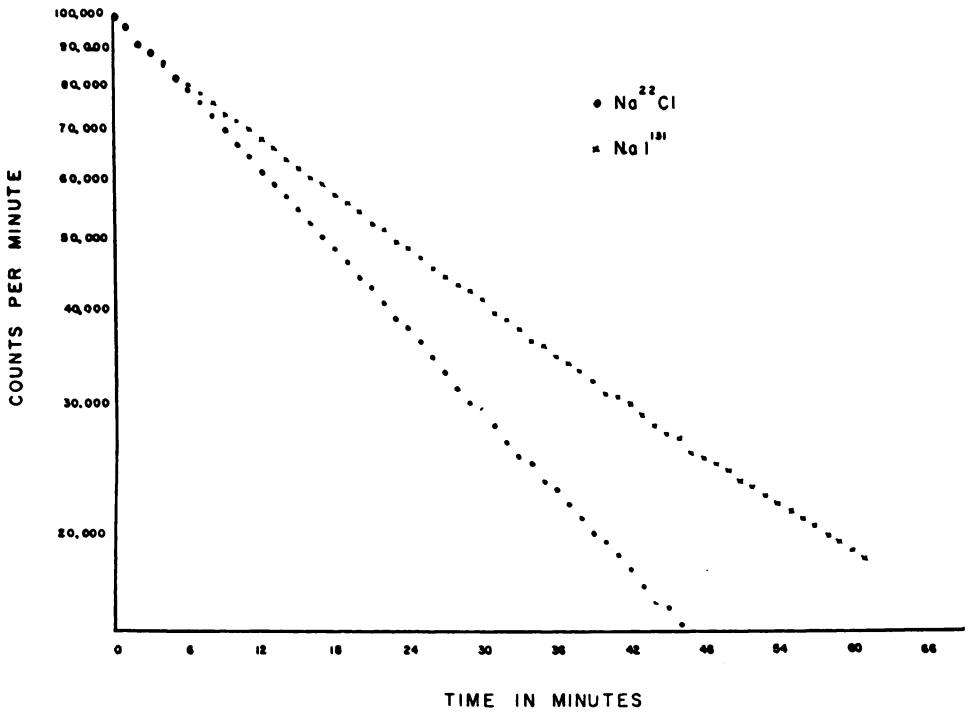

Fig. 13. Comparison of elution curves of ^{22}Na and ^{131}I.

of the physical system and kinetics described in this paper (7). Utilizing the described system, it should be possible to quantitate the dissociation constant as influenced by temperature, concentration gradient, pH, etc. The interaction of protein with freely soluble ions such as Na, K, Cl, etc., is of a more subtle nature. Work is currently underway which indicates that the minimal altered diffusion rates may be determined. These soluble ions may not represent chemical binding but may be related to sorption, charge effect, ionic radius, and reaction with spheres of hydration. The effects on interaction of steroids, diuretics and pathologic states of altered salt and water metabolism may be studied. The interaction of protein with water may be studied using tritiated water.

The interaction of labeled permeable substances with formed elements such as organelles, cells, tissue fragments and small organisms is contemplated with this system. The nature of membranes is amenable to quantitative study under the conditions described.

SUMMARY

A physical system is described which allows the monitoring of unidirectional diffusion of a radioisotope from a dialysis cell, this unidirectionality is verified. In addition, the characteristics of the elution curve for ^{22}Na from the dialysis cell are described, as is the reproducibility of the curve employing the same membrane, with serial changes of ^{22}Na and with changes of membrane. Applicability of the system is discussed, including the kinetics permitting the quantitation in terms of interaction constants of permeable labeled substances with nonlabeled substances.

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