

# True Anomalous Osmosis in Multi-Solute Model Membrane Systems

EUGENE GRIM and KARL SOLLNER

From the Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda. Dr. Grim's present address is Department of Physiology, University of Minnesota Medical School, Minneapolis

**ABSTRACT** The transport of liquid across charged porous membranes separating two electrolytic solutions of different composition consists of both a normal and an anomalous osmotic component. Anomalous osmosis does not occur with electroneutral membranes. Thus, with membranes which can be charged and discharged reversibly, normal osmosis can be measured with the membrane in the electroneutral state, and normal together with anomalous osmosis with the membrane in a charged state, the difference between these two effects being the true anomalous osmosis. Data are presented on the osmotic effects across an oxyhemoglobin membrane in the uncharged state at pH 6.75 and in two charged states, positive at pH 4.0 and negative at pH 10.0, in multi-solute systems with 0.2 and 0.4 osmolar solutions of a variety of electrolytes and of glucose against solutions of other solutes of the same, one-half, and twice these osmolarities. In the simpler systems the magnitude of the true anomalous osmosis can be predicted semiquantitatively by reference to appropriate single-solute systems. In isoosmolar systems with two electrolytic solutions the anomalous osmotic flow rates may reach  $300 \mu\text{l./cm.}^2 \text{ hr.}$  and more; systems with electrolytic solutions against solutions of glucose can produce twice this rate. These fluxes are of the same order of magnitude as the liquid transport rates across such living structures as the mucosa of dog gall bladder, ileum, and urinary bladder.

## INTRODUCTION

The transport of liquid across charged porous membranes separating two solutions of the same electrolyte at different concentrations has been shown to consist ordinarily of both a normal osmotic and an anomalous osmotic

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component (1). The magnitude of the anomalous osmotic component alone, that is the true anomalous osmosis, was measured with membranes that can be brought reversibly into a charged and into the uncharged state without change in geometrical structure. When such a membrane in the uncharged state separates two electrolytic solutions, any osmotic flow obtained is due to normal osmosis alone since anomalous osmosis occurs only across membranes whose pore walls are in a charged state. With the membrane in a charged state, and the same solutions, any flow of liquid across the membrane is due to both normal and anomalous osmosis. The difference in the magnitudes of the two effects, with the membrane in the uncharged and in the charged state, is the true anomalous osmosis. This method was used in the study referred to above to determine the rates of transport of liquid by true anomalous osmosis in systems with solutions of the same electrolyte at a 2:1 concentration ratio separated by an oxyhemoglobin collodion membrane which could be brought to a charged or the uncharged (isoelectric) state by adjustment of the pH of the solutions. With such membranes true anomalous osmosis was found to be of a physiologically significant magnitude in the concentration range of the mammalian body fluids (0.3 osmolar).

The purpose of the present study was to determine whether anomalous osmosis is of a significant magnitude in systems of greater physiological interest than those previously studied, namely, systems in which the membrane separates two isoosmotic solutions of different compositions.

In the case of such systems containing two or more different solutes, any attempt to predict the magnitude of anomalous osmosis on the basis of the different theories proposed in the literature to explain its basic mechanism is unlikely to be successful at present. The explanation proposed by one of us some years ago (2), and the equations given more recently by Schlögl (3), and by Kobatake (4) are concerned with single-solute systems. Even for this kind of system, they are only partially satisfactory. Although they provide reasonably accurate predictions as to the relative anomalous osmotic efficacy of different electrolytes at any particular concentration level, none is fully satisfactory for predicting in a quantitative manner the dependence of the magnitude of anomalous osmosis on concentration. For the present, predictions of magnitude and direction of anomalous osmosis in multi-solute systems can best be made on the basis of earlier, empirical observations on single-solute systems.

The simplest way to utilize the information obtained with single-solute systems is to assume that each half of a two-solute system acts as it does in a single-solute system, the net effect in the two-solute system being the algebraic sum of the flows produced by each half of the system. While such an approach cannot be expected to give quantitatively valid predictions, it can be used as a basis for a meaningful selection of systems to be studied experimentally.

The best data for this purpose are those obtained from studies of single-solute systems in which both solutions separated by the membrane contain electrolyte at relatively high concentrations rather than those acquired in the older experiments with solution *versus* water systems, because in the former cases the electrokinetic situation in the membrane is more like that in multi-electrolyte, isoosmotic systems. This kind of single-solute system with a 2:1 concentration ratio was the subject of our earlier study (1); those data which have been utilized for the selection of multi-solute systems for the present investigation are shown in Table I.

TABLE I  
TRUE ANOMALOUS OSMOSIS IN SYSTEMS IN WHICH  
AN OXYHEMOGLOBIN COLLODION MEMBRANE SEPARATES THE  
SOLUTION OF AN ELECTROLYTE FROM THE SOLUTION OF THE  
SAME ELECTROLYTE AT ONE-HALF THE CONCENTRATION

Electrolyte	Charge of membrane	True anomalous osmotic flow, $\mu\text{./cm.}^2\text{-hr.}^*$		
		0.2 osm $\ddagger$	0.4 osm $\ddagger$	0.8 osm $\ddagger$
MgCl <sub>2</sub>	Positive	137	104	83
KCl	"	52	26	10
KIO <sub>3</sub>	"	-10	-38	-53
K <sub>2</sub> SO <sub>4</sub>	"	-30	-38	-31
K <sub>2</sub> SO <sub>4</sub>	Negative	74	54	43
KCl	"	35	23	10
LiCl	"	-1	-26	-32
MgCl <sub>2</sub>	"	-34	-31	-22

\* A negative sign indicates that the direction of the flow is toward the more dilute solution (that is, negative anomalous osmosis).

$\ddagger$  Osmolarity of the more concentrated solution.

With single-electrolyte solutions, there are three types of two-solute systems; (a) one type in which both electrolytes would cause positive anomalous osmosis in single-solute systems; (b) another type in which both electrolytes would cause negative anomalous osmosis; and (c) a third type in which one of the electrolytes would cause positive and the other negative anomalous osmosis. In addition, there is a fourth type of two-solute system which is simpler than the preceding, at least from an electrochemical point of view, namely, the system in which the membrane separates a solution of an electrolyte from a solution of a non-electrolyte.

Accordingly it can be anticipated that (a) in those two-electrolyte systems in which both halves would produce positive anomalous osmosis, the direction of the anomalous osmosis would be toward the solution which shows the greater anomalous osmotic efficacy in single-solute systems. It also can be expected that (b) the magnitude of the anomalous osmotic flow in the two-electrolyte experiment will be greater the greater the difference in anomalous

osmotic efficacy of the two electrolytic solutions in single-electrolyte systems. Analogous considerations apply to systems in which the two solutions produce negative anomalous osmosis; except that the direction of the anomalous osmotic flow in the multi-solute system would be away from the solution having the greater effectiveness in single-solute systems. Finally (c) it would be anticipated that the greatest flows would be shown by systems in which one of the electrolytes produces positive anomalous osmosis and the other negative anomalous osmosis in single-solute systems; the direction of the flow in the two-solute systems would always be toward the solution containing the electrolyte which produces positive anomalous osmosis.

For those systems in which the membrane separates a solution of an electrolyte from a solution of a non-electrolyte, it is possible to predict both direction and magnitude of the true anomalous osmosis with reasonable accuracy. The electrokinetic situation is nearly identical to that in a system with the same electrolytic solution separated by the membrane from pure water; thus the direction of the anomalous osmosis will be the same in both cases. This is, of course, not necessarily true for the total osmotic flow which contains a normal as well as an anomalous osmotic component. In such two-solute systems with non-electrolytes like sucrose or glucose, the magnitude of the anomalous osmosis may be lower than in the corresponding electrolytic solution *versus* water system because of the greater viscosity of the fluid flowing through the pores of the membrane. It is difficult to estimate quantitatively the effect of the increased viscosity because the viscosity of the fluid in the pores is a complex function of the relative rates of movement of solute and solvent through the pore.

More complex systems are those in which either one of the two solutions separated by the membrane contains more than one electrolyte or those involving three or more different electrolytes. Here it becomes completely impracticable at the present time to make predictions, and the choice of such systems for study is rather arbitrary.

#### EXPERIMENTAL

Oxyhemoglobin membranes made by adsorbing horse oxyhemoglobin on collodion membranes of proper porosity were used. Collodion membranes of the dialyzing type were made by a method similar to that used extensively in recent years in this laboratory: A 4 per cent solution of collodion (parlodion Mallinckrodt) in a mixture of equal volumes of ethyl ether and absolute ethanol was poured over rubber-coated test tubes (25 × 100 mm.) rotating at a constant speed (18 to 20 R.P.M.) in the horizontal position. The film thus formed was allowed to dry for several minutes; when thicker membranes were desired, a second layer of collodion solution was applied 3 minutes after the first. Thereafter the tubes were immersed in distilled water and washed for several hours. Finally the skins of rubber covering the test tubes together

with the membranes were slipped off the test tubes and the rubber bags removed from inside the membrane by gentle traction. The membranes were mounted on tightly fitting glass rings of about 20 to 25 mm. length, to which they were secured with linen thread. They were then proteinized by immersion for 24 hours in oxyhemoglobin solution produced by hemolyzing horse erythrocytes.

The arrangement for the measurement of the osmotic effects consisted of a rubber stopper (fitting the glass rings of the membranes) which carried a short calibrated glass tube of about 8 mm. diameter, a narrow bore glass tube with a stopcock, and two magnetic stirrers, small Alnico permanent magnets covered with lucite and mounted on glass axles held by a strip of plastic. For the experiments, the rubber stopper was inserted firmly into the ring of the membrane which had been filled with solution, and the position of the meniscus in the glass tube adjusted by means of the stopcock to a level just above the stopper. The volume of solution in the assembled apparatus was about 35 ml. This assembly was then immersed in a beaker containing the "outside" solution of 350 ml. up to a level identical with that of the meniscus in the calibrated tube. The inside solution was stirred by rotating the magnets with a regularly interrupted magnetic field supplied by a large, externally mounted electromagnet. The outside solution was stirred vigorously by a motor-driven propeller. All experiments were carried out at room temperature which remained virtually constant during any given experiment and did not vary to a significant degree during any series of experiments.

A 5 minute period was allowed to elapse before the volume change of the inside solution was read; the liquid transport rates in microliters per square centimeter and hour were calculated from such readings. As routine, each measurement was repeated with fresh solutions until succeeding results agreed within  $2\mu\text{l.}/\text{cm.}^2\text{-hr.}$ , this usually being the case with the first two readings unless a preceding experiment involved solutions of greatly different concentrations. The changes in hydrostatic pressure which accompanied the changes in volume of the inside solution were in all instances too small to cause a significant error due to filtration. The data were assumed to provide a reasonably accurate measure of the initial rates of volume change in such continuously degrading systems.

Two kinds of measurements were made with each system; one in which the solutions separated by the membrane had been adjusted to a pH of 4.0 or to a pH of 10.0 to make the membrane either positive or negative in charge, and one in which the solutions had been adjusted as closely as possible to a pH of 6.75, the isoelectric point of the oxyhemoglobin, at which the membrane is in the uncharged state. The differences between the osmotic flows measured with the membrane in the charged and the uncharged states were taken as the true anomalous osmotic flows.

The representative experiments reported below were all performed with the same oxyhemoglobin membrane having a surface area of  $54\text{ cm.}^2$  and a filtration rate of  $12\mu\text{l.}/\text{cm.}^2\text{-hr.}$  at 10 cm. water pressure. To test whether the membrane had changed in the course of this study, several experiments were repeated at the end of the series. The results agreed within the limits of experimental error with those obtained during the series, proving that the membrane did not deteriorate. All the experiments are, therefore, strictly comparable.

The experimental systems were chosen on the basis described in the introduction to include electrolytes which in single-solute systems yield both positive and negative anomalous osmoses, with the membrane in both the positively and the negatively charged states. In order that the solute concentrations in the systems should have the closest possible analogy to living systems, six experiments were performed with each multi-solute system, three with the osmolarity of the inside solution at 0.4 and three at 0.2 (osmolarity is defined here as the product of the molar concentration and the number of ions formed upon complete dissociation). For each set of three, the osmolarity of the outside solution was twice, equal to, and one-half that of the inside solution.

### RESULTS AND DISCUSSION

In Tables II to IV, the compositions of the two solutions are given in columns 1 and 2; the sign of charge of the membrane in the charged state is shown in column 3. The osmotic flows obtained with the membrane in the charged state are given in column 4 and those obtained with the membrane in the isoelectric state, in column 5. Arbitrarily a negative sign is given to the flow if it is directed toward the outside solution; and the absence of a sign indicates the opposite direction of flow. The difference between the observed osmotic effects shown in columns 4 and 5, that is the true anomalous osmotic flow, is given in column 6, the sign indicating the direction of flow as stated above.

Table II shows the results of experiments in which the membrane separated a solution of a single electrolyte from a solution of another single electrolyte. In this, as in the two subsequent tables, the numerical data referring to systems of equal osmolarity are printed in bold face type.

Part A of Table II contains data for systems in which each of the two electrolytes used produces positive anomalous osmosis in single-electrolyte systems. In the isoosmotic systems, the direction of the true anomalous osmotic flow was toward that solution which as shown in Table I produces the greater anomalous osmosis in single-solute systems; the magnitude of the flows was in the range of 75 to 148  $\mu\text{l./cm.}^2\text{-hr.}$  In the non-isoosmotic systems, the magnitude of the flow was smaller with a higher concentration of the outside solution, and larger with a lower concentration.

Part B shows data obtained with systems in which both electrolytes cause negative anomalous osmosis in single-solute systems with the membrane charged as indicated. For the isoosmotic systems, the rates of anomalous osmotic flows were in the range  $-14$  to  $-57 \mu\text{l./cm.}^2\text{-hr.}$ , the direction of the flow being, as predicted, away from the solution which produces the greater negative anomalous osmosis in single-solute systems.

Part C contains data from systems in which the electrolyte of the inside solution in single-electrolyte systems causes positive anomalous osmosis and

that of the outside solution, negative anomalous osmosis with the membrane charged as indicated. As predicted on the basis of the data in Table I, the greatest anomalous osmotic flows for isoosmotic systems were obtained with these systems, being in the range, 149 to 320  $\mu\text{l./cm.}^2\text{-hr.}$  The anomalous

TABLE II  
ANOMALOUS AND NORMAL OSMOSIS IN SYSTEMS IN WHICH  
AN OXYHEMOGLOBIN COLLODION MEMBRANE SEPARATES THE  
SOLUTION OF ONE ELECTROLYTE FROM THE  
SOLUTION OF A SECOND ELECTROLYTE

1 Inside solution	2 Outside solution	3 Charge of membrane	4 5 6 Rates of osmotic flow, $\mu\text{l./cm.}^2\text{-hr.}^*$		
			Membrane charged	Membrane isoelectric	$\Delta$ , true anoma- lous osmosis
<b>A. Systems with electrolytes which in single-solute systems cause positive anomalous osmosis</b>					
0.4 osm $\text{MgCl}_2$	0.8 osm $\text{KCl}$	Positive	76	-5	81
" " "	0.4 " "	"	173	34	139
" " "	0.2 " "	"	291	75	216
0.2 osm $\text{MgCl}_2$	0.4 osm $\text{KCl}$	Positive	70	-2	72
" " "	0.2 " "	"	174	26	148
" " "	0.1 " "	"	314	48	266
0.4 osm $\text{MgCl}_2$	0.8 osm $\text{MgSO}_4$	Positive	-50	-90	40
" " "	0.4 " "	"	62	-19	81
" " "	0.2 " "	"	161	27	134
0.2 osm $\text{MgCl}_2$	0.4 osm $\text{MgSO}_4$	Positive	-1	-42	41
" " "	0.2 " "	"	68	-7	75
" " "	0.1 " "	"	142	19	123
0.4 osm $\text{K}_2\text{SO}_4$	0.8 osm $\text{KCl}$	Negative	57	-1	58
" " "	0.4 " "	"	137	31	106
" " "	0.2 " "	"	237	47	190
0.2 osm $\text{K}_2\text{SO}_4$	0.4 osm $\text{KCl}$	Negative	51	-1	52
" " "	0.2 " "	"	134	20	114
" " "	0.1 " "	"	258	33	225
<b>B. Systems with electrolytes which in single-solute systems cause negative anomalous osmosis</b>					
0.4 osm $\text{K}_2\text{SO}_4$	0.8 osm $\text{KIO}_3$	Positive	-7	-47	40
" " "	0.4 " "	"	-5	9	-14
" " "	0.2 " "	"	-21	43	-64
0.2 osm $\text{K}_2\text{SO}_4$	0.4 osm $\text{KIO}_3$	Positive	8	-28	36
" " "	0.2 " "	"	-9	6	-15
" " "	0.1 " "	"	-20	30	-50
0.4 osm $\text{MgCl}_2$	0.8 osm $\text{LiCl}$	Negative	-44	-44	0
" " "	0.4 " "	"	-52	5	-57
" " "	0.2 " "	"	-68	45	-113
0.2 osm $\text{MgCl}_2$	0.4 osm $\text{LiCl}$	Negative	-27	-25	-2
" " "	0.2 " "	"	-52	2	-54
" " "	0.1 " "	"	-76	29	-105

\* In this table a negative sign indicates that the osmotic flow is directed toward the outside solution, the absence of a sign indicates the converse.

TABLE II (concluded)

1 Inside solution	2 Outside solution	3 Charge of membrane	4 5 6 Rates of osmotic flow, $\mu\text{l./cm}^2\text{-hr.}^*$		
			Membrane charged	Membrane isoelectric	$\Delta$ , true anomalous osmosis
C. Systems with electrolytes one of which in single-solute systems causes positive anomalous osmosis and the other, negative anomalous osmosis					
0.4 osm $\text{MgCl}_2$	0.8 osm $\text{KIO}_3$	Positive	209	-44	253
" " "	0.4 " "	"	312	14	298
" " "	0.2 " "	"	419	55	364
0.2 osm $\text{MgCl}_2$	0.4 osm $\text{KIO}_3$	Positive	242	-25	267
" " "	0.2 " "	"	332	12	320
" " "	0.1 " "	"	444	36	408
0.4 osm $\text{MgCl}_2$	0.8 osm $\text{K}_2\text{SO}_4$	Positive	68	-77	145
" " "	0.4 " "	"	154	-18	172
" " "	0.2 " "	"	221	23	198
0.2 osm $\text{MgCl}_2$	0.4 osm $\text{K}_2\text{SO}_4$	Positive	100	-47	147
" " "	0.2 " "	"	140	-9	149
" " "	0.1 " "	"	188	18	170
0.4 osm $\text{K}_2\text{SO}_4$	0.8 osm $\text{LiCl}$	Negative	202	10	192
" " "	0.4 " "	"	272	48	224
" " "	0.2 " "	"	350	74	276
0.2 osm $\text{K}_2\text{SO}_4$	0.4 osm $\text{LiCl}$	Negative	218	18	200
" " "	0.2 " "	"	286	35	251
" " "	0.1 " "	"	384	59	325

osmotic flows produced, in a sense, by each of the two solutions were both directed toward the solution which caused positive anomalous osmosis in single-solute systems and, hence, were additive. In the cases of parts A and B, the effects of the two solutions were antagonistic.

The simple approach to prediction of results with multi-solute systems which was suggested in the introduction seems to be justified by the experimental results. In general, the results in Table II are in agreement with expectations based upon the assumption that each half of the two-solute systems acted as it would have in a single-solute system. There is, however, one exception which demonstrates that this simple approach is not completely adequate. In Table I, the negative anomalous osmotic efficacies of  $\text{K}_2\text{SO}_4$  and  $\text{KIO}_3$  are shown to be equal in the 0.4 osm systems. As expected, the anomalous osmosis produced in the isoosmotic two-solute systems with  $\text{K}_2\text{SO}_4$  and  $\text{KIO}_3$  (as shown in part B of Table II) was very small. On the basis of both these observations, it would have been predicted that the isoosmotic systems with  $\text{MgCl}_2$  solution *versus*  $\text{KIO}_3$  solution would produce an anomalous osmotic flow of nearly the same magnitude as that with  $\text{MgCl}_2$  *versus*  $\text{K}_2\text{SO}_4$ . This, however, was not the case; as can be seen in part C, the former systems produced nearly twice the flow of the latter.



Table III contains the data from experiments with systems in which the membrane separated a solution containing a single electrolyte from the solution of a non-electrolyte. The electrolytes used were such as to produce both positive and negative anomalous osmosis in single-solute systems. As expected, these systems produced a greater anomalous osmotic flow than those which contained electrolyte in both solutions, the flow rates being in the range,  $-170$  to  $678 \mu\text{l./cm.}^2\text{-hr.}$ , for isoosmotic systems. The direction of the flow was in all cases the same as in the single electrolyte systems. The magnitude of the flows was large, but nevertheless smaller than for comparable electrolyte solution *versus* water systems. The experiments in part A of Table III with  $0.2 \text{ osm MgCl}_2$  show that anomalous osmosis became smaller when water was replaced with glucose solution, the effect being more pronounced the greater the concentration of the glucose. This was most probably a consequence of the increased viscosity of the fluid in the membrane pores.

The data of Table IV refer to systems in which one or both of the two solutions separated by the membrane contained two different solutes. In all cases, the inside solution was an equiosmolar mixture of  $\text{MgCl}_2$  and  $\text{KCl}$ . In the first group of systems, the outside solute was a non-electrolyte. In the second group, the outside solute was an electrolyte which with the membrane positively charged as indicated produced positive anomalous osmosis. In the third and fourth groups, the electrolytes in the outside solution produced negative anomalous osmosis under the indicated conditions. For the isoosmotic systems, the anomalous osmotic flows were in the range,  $87$  to  $516 \mu\text{l./cm.}^2\text{-hr.}$

Although the emphasis of this study was on anomalous osmosis, two comments concerning the normal osmotic flows observed with the membrane in the isoelectric state seem of interest. First, the normal osmotic flow in systems with isoosmotic solutions of two different electrolytes may be quite large; values as high as  $70 \mu\text{l./cm.}^2\text{-hr.}$  were observed (see Table II). Second, the direction of the osmotic flow between the isoosmotic solutions of two different solutes with the membrane in the isoelectric state indicates the relative permittivity of the membrane for the solutes in the two solutions, the direction of the flow being toward the solution of that solute which has the lower permeation coefficient in the membrane (see Tables II and III). For example, the membrane used in this study had a greater permittivity for glucose than for  $\text{MgCl}_2$  and for  $\text{KCl}$  than for glucose, etc.

An estimate of whether or not the magnitude of true anomalous osmotic flow rates obtained in this study is of potential physiological significance can be made by comparing them with liquid transport rates observed in living systems. For the comparison, recently published data on water flux rates per unit area of the mucosa of dog gall bladder, ileum, and urinary bladder can be used (5). The net absorption of fluid when isotonic saline is placed in these

TABLE III  
ANOMALOUS AND NORMAL OSMOSIS IN SYSTEMS  
IN WHICH AN OXYHEMOGLOBIN COLLODION MEMBRANE  
SEPARATES AN ELECTROLYTIC SOLUTION FROM  
THE SOLUTION OF A NON-ELECTROLYTE

1 Inside solution	2 Outside solution	3 Charge of membrane	4 5 6 Rates of osmotic flow, $\mu\text{l./cm}^2\text{-hr.}^*$		
			Membrane charged	Membrane isoelectric	$\Delta$ , true anomalous osmosis
<b>A. Systems with an electrolyte which in single-solute systems causes positive anomalous osmosis</b>					
0.4 osm KCl	0.8 osm glucose	Positive	-22	-178	156
" " "	0.4 " "	"	132	-70	202
" " "	0.2 " "	"	212	-20	232
0.2 osm KCl	0.4 osm glucose	Positive	210	-82	292
" " "	0.2 " "	"	284	-33	317
" " "	0.1 " "	"	332	-8	340
0.4 osm MgCl <sub>2</sub>	0.8 osm glucose	Positive	340	-98	438
" " "	0.4 " "	"	531	17	514
" " "	0.2 " "	"	643	67	576
0.2 osm MgCl <sub>2</sub>	0.8 osm glucose	Positive	353	-178	531
" " "	0.4 " "	"	596	-28	624
" " "	0.2 " "	"	700	22	678
" " "	0.1 " "	"	751	46	705
" " "	H <sub>2</sub> O	"	816	78	738
0.4 osm (MgCl <sub>2</sub> + glucose) †	0.8 osm glucose	Positive	399	-109	508
" " "	0.4 " "	"	585	2	583
" " "	0.2 " "	"	685	67	618
0.2 osm (MgCl <sub>2</sub> + glucose) †	0.4 osm glucose	Positive	529	-50	579
" " "	0.2 " "	"	625	3	622
" " "	0.1 " "	"	683	28	655
0.4 osm K <sub>2</sub> SO <sub>4</sub>	0.8 osm glucose	Negative	212	-111	323
" " "	0.4 " "	"	422	8	414
" " "	0.2 " "	"	564	67	497
0.2 osm K <sub>2</sub> SO <sub>4</sub>	0.4 osm glucose	Negative	487	-29	516
" " "	0.2 " "	"	636	29	607
" " "	0.1 " "	"	714	63	651
<b>B. Systems with an electrolyte which in single-solute systems causes negative anomalous osmosis</b>					
0.4 osm K <sub>2</sub> SO <sub>4</sub>	0.8 osm glucose	Positive	-264	-111	-153
" " "	0.4 " "	"	-162	8	-170
" " "	0.2 " "	"	-112	67	-179
0.2 osm K <sub>2</sub> SO <sub>4</sub>	0.4 osm glucose	Positive	-156	-29	-127
" " "	0.2 " "	"	-101	29	-130
" " "	0.1 " "	"	-72	63	-135

\* In this table a negative sign indicates that the osmotic flow is directed toward the outside solutions; the absence of a sign indicates the converse.

† Equiosmolar mixtures with the total osmolarity as indicated.

organs is in the range of 12 to 66  $\mu\text{l./cm.}^2\text{-hr.}$  Also, it may be pertinent to recall that according to the concept of a proposed "fluid circuit" mechanism of intestinal absorption (6) such net fluxes are the differences between two much larger fluxes, one inward and one outward, across different parts of the mucosa of these organs. While no quantitative estimate can be made of these larger fluxes, they obviously cannot exceed the one-way water fluxes as determined by isotope studies. For the organs named, these one-way fluxes were in the range of 66 to 396  $\mu\text{l./cm.}^2\text{-hr.}$  In this study the anomalous osmotic flows

TABLE IV  
ANOMALOUS AND NORMAL OSMOSIS IN SYSTEMS IN WHICH AN  
XYHEMOGLOBIN COLLODION MEMBRANE SEPARATES SOLUTIONS,  
ONE OR BOTH OF WHICH CONTAIN TWO ELECTROLYTES

1 Inside solution	2 Outside solution	3 Charge of membrane	4 5 6 Rates of osmotic flow, $\mu\text{l./cm.}^2\text{-hr.}^*$		
			Mem- brane charged	Mem- brane isoelectric	$\Delta$ , true anomalous osmosis
0.4 osm (MgCl <sub>2</sub> + KCl) ‡	0.8 osm glucose	Positive	156	-187	343
" " " " "	0.4 " "	"	337	-69	406
" " " " "	0.2 " "	"	421	-14	435
0.2 osm (MgCl <sub>2</sub> + KCl) ‡	0.4 osm glucose	Positive	390	-91	481
" " " " "	0.2 " "	"	480	-36	516
" " " " "	0.1 " "	"	529	-8	537
0.4 osm (MgCl <sub>2</sub> + KCl) ‡	0.8 osm KCl	Positive	16	-28	44
" " " " "	0.4 " "	"	87	0	87
" " " " "	0.2 " "	"	177	19	158
0.2 osm (MgCl <sub>2</sub> + KCl) ‡	0.4 osm KCl	Positive	13	-18	31
" " " " "	0.2 " "	"	87	-4	91
" " " " "	0.1 " "	"	196	7	189
0.4 osm (MgCl <sub>2</sub> + KCl) ‡	0.8 osm KIO <sub>3</sub>	Positive	137	-67	204
" " " " "	0.4 " "	"	214	-17	231
" " " " "	0.2 " "	"	284	19	265
0.2 osm (MgCl <sub>2</sub> + KCl) ‡	0.4 osm KIO <sub>3</sub>	Positive	172	-39	211
" " " " "	0.2 " "	"	228	-7	235
" " " " "	0.1 " "	"	312	12	300
0.4 osm (MgCl <sub>2</sub> + KCl) ‡	0.8 osm (K <sub>2</sub> SO <sub>4</sub> + KIO <sub>3</sub> ) ‡	Positive	62	-95	157
" " " " "	0.4 " " " "	"	121	-37	158
" " " " "	0.2 " " " "	"	176	-1	177
0.2 osm (MgCl <sub>2</sub> + KCl) ‡	0.4 osm (K <sub>2</sub> SO <sub>4</sub> + KIO <sub>3</sub> ) ‡	Positive	92	-58	150
" " " " "	0.2 " " " "	"	123	-23	146
" " " " "	0.1 " " " "	"	171	-3	174

\* In this table a negative sign indicates that the osmotic flow is directed toward the outside solution, the absence of a sign indicates the converse.

‡ Equiosmolar mixtures with the total osmolarity as indicated.

for isoosmotic systems containing electrolytes only were in the range 12 to 320  $\mu\text{l./cm.}^2\text{-hr.}$  In the systems with non-electrolytic solutions, the range was extended to 678  $\mu\text{l./cm.}^2\text{-hr.}$  Thus, the true anomalous osmotic transport rates obtained with the various multi-solute, isoosmotic systems studied here are of the same order of magnitude as liquid transport rates in living structures. Of course, the question of whether or not anomalous osmosis is actually involved in water transport in living mammals is outside the scope of this paper; nevertheless, it has been demonstrated that contrary to earlier beliefs, anomalous osmosis can operate in isoosmotic systems to a physiologically significant extent at the electrolyte concentration levels of mammalian body fluids.

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