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Research Article

ION ASSOCIATION. VI. INTERACTIONS BETWEEN CALCIUM, MAGNESIUM, INORGANIC PHOSPHATE, CITRATE AND PROTEIN IN NORMAL HUMAN PLASMA *

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The total measured concentration of an ionizable constituent of body fluids often fails to reveal either the varied chemical forms in which it may be present, or the portion which is present as the free ionized substance. This truism applies to most organic acids and bases and to inorganic ions which are multivalent. Univalent ions whose corresponding acids and bases are strong may be viewed as exceptions to this generalization. This exclusion is more quantitative than qualitative, since small fractions of these constituents may also be complexed or bound.

Plasma and extracellular fluid differ from other body fluids in containing predominantly univalent strong electrolytes. The plasma concentrations of organic acids and bases and of multivalent inorganic ions are relatively low. Nevertheless, the measured concentrations of these substances in plasma may fail to reflect their interactions with one another, and therefore the individual ionic species present.

By means of ultrafiltration, a protein-free fluid may be obtained which is probably identical with the protein-free phase in native plasma. This identity is supported by the observation that ultrafiltrate concentrations of calcium (1), magnesium (2) and phosphate (3) do not change during the course of ultrafiltration. In order to quantitate the interactions between nonprotein-bound constituents, one approach is to measure *1*) the free ion concentrations of all cations present, *2*) the total amount of various anions present, and *3*) calculate the amounts of each complex from reported dissociation constants. Undetermined anions will then account for the difference between the total concentration of each cation and the amount accounted for as known complexes. Calcium and magnesium ion concentrations can be determined (2). Sodium and potassium are virtually completely ionized. Therefore, this scheme offers promise of analyzing such complex mixtures as plasma, urine and intracellular fluid.

In the present report, normal plasma has been analyzed by this procedure with respect to protein, calcium, magnesium, phosphate and citrate.

METHOD

Venous blood was collected without stasis in oiled heparinized syringes from healthy volunteers (medical students, technicians and staff members). Plasma was separated by centrifuging and was stored under oil. Plasma pH was determined promptly at room temperature (23° to 26° C) using a Radiometer pH meter, model 4. No temperature correction was applied. Ultrafiltration was performed as described by Toribara, Terepka and Dewey (1) with two modifications: *1*) Visking casing $\frac{1}{4}$ inch in diameter was used, the resulting increase in area for ultrafiltration accelerating the process almost twofold; *2*) the strips of casing were soaked in distilled water for 2 days, rinsed, and then stored until used in a chamber saturated with water vapor. Drying in air caused small pinholes to form; use of wet casing, even when thoroughly wiped, led to dilution of ultrafiltrate by water remaining in the casing; if soaking was omitted entirely, impurities in the casing led to erratic results in some methods.

The ultrafiltrates were freed of heavy metals by extraction with diphenyl thiocarbazone and chloroform (2) and bubbled with 5 per cent CO_2. Two aliquots of 2.5 ml each were mixed with Tris (hydroxymethyl) aminomethane buffer at a pH within 0.1 U of the measured plasma pH. Free calcium ions and free magnesium ions were then determined spectrophotometrically (2) and the pH values of the ultrafiltrate-dye mixtures were measured. The solution remaining after determination of free calcium ions was used for analysis of citrate and phosphate. Citrate was determined by preliminary acid digestion (4), conversion to pentabromoacetone (5), and color development with thiourea (4). Phosphate was determined on ultrafiltrate and on plasma according to Fiske and Subbarow (6). Other aliquots of ultrafiltrate and of plasma were analyzed for total calcium and total magnesium (2); the succinate buffer employed in this method was modified as follows: equal parts of 0.17 M sodium succinate and 0.25 N HCl were mixed. This solution yielded protein-free filtrates

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more consistently than the mixture originally described. Plasma protein was determined by the biuret method (7).

CALCULATIONS

The general procedure consisted of calculating the amounts of various ionic species in plasma ultrafiltrate, using measured values of free calcium and magnesium ion concentrations and measured concentrations of phosphate and citrate. The proportion of each ion bound to protein was then estimated by applying approximate corrections for the Donnan factor (see below) and for plasma water content.

The basic premise employed herein is that determination of free cation concentrations, coupled with the relevant stability constants, permits calculation of the concentrations of each complex in a mixed electrolyte solution from these values plus total measured concentrations of individual anionic ligands. This premise may be stated algebraically as follows:

Let $[M_1^+]$, $[M_2^+]$, $[M_3^+]$, $[M_n^+]$ = free ion concentrations of cations present; let $[A_1]$, $[A_2]$, $[A_3]$, $[A_n]$ = total concentrations of anionic ligands; let $[A_1^-]$, $[A_2^-]$, $[A_3^-]$, $[A_n^-]$ = free ion concentrations of these ligands. If only 1:1 complexes are formed,

$$K_{M_nA_n} = \frac{[M_n^+] \times [A_n^-]}{[M_nA_n]} \quad [1]$$

$$[A_1] = [A_1^-] + [M_1A_1] + [M_2A_1] + [M_3A_1] \cdots + [M_nA_1]. \quad [2]$$

Substituting,

$$[A_1] = [A_1^-] + \frac{[M_1^+] \times [A_1^-]}{K_{M_1A_1}} + \frac{[M_2^+] \times [A_1^-]}{K_{M_2A_1}} + \frac{[M_3^+] \times [A_1^-]}{K_{M_3A_1}} \cdots + \frac{[M_n^+] \times [A_1^-]}{K_{M_nA_1}}. \quad [3]$$

Solving for $[A_1^-]$,

$$[A_1^-] = [A_1](1 + [M_1^+]/K_{M_1A_1} + [M_2^+]/K_{M_2A_1} + [M_3^+]/K_{M_3A_1} \cdots + [M_n^+]/K_{M_nA_1}). \quad [4]$$

Individual complexes are then calculated from Equation 1. In plasma ultrafiltrate, the only cations present which can bind anions appreciably are sodium, calcium and magnesium. Potassium concentration is too low to affect the calculations. Although sodium may bind an appreciable fraction of each anion present, the fraction of sodium bound to all anions must be very low (probably less than 1 per cent; see below). Sodium ion concentration may therefore be equated with total sodium concentration. In the present studies on normal plasma, sodium concentration in ultrafiltrate was assumed to be 0.14 M. Although individual values could differ from this estimate by several per cent, the resulting error in estimated concentrations of sodium complexes would be no greater, and in other complexes, even smaller than the error in estimating sodium concentration. Calcium and magnesium ion concentrations have been determined directly. Only two anions have been determined, phosphate and citrate. Both of these are known to complex sodium, calcium and magnesium ions (8–12). Although many other anions in plasma are doubtless bound partially to calcium and magnesium, the concentrations of these complexes must all be considerably smaller than those studied here. Bicarbonate may possibly be an exception to this generalization (see below). With this exception, no other anions are present in sufficient quantities to bind more than 0.02 mmole per L of calcium or magnesium (13).

The dissociation constants employed, all referring to 25° C, were as follows:

$$K'_{H_2PO_4^-} = \frac{a_{H^+}[HPO_4^{--}]}{[H_2PO_4^-]} = 10^{-6.9}\text{M}$$

$$K'_{NaHPO_4^-} = \frac{[Na^+][HPO_4^{--}]}{[NaHPO_4^-]} = 0.23 \text{ M}$$

$$K'_{CaHPO_4} = \frac{[Ca^{++}][HPO_4^{--}]}{[CaHPO_4]} = 0.0020 \text{ M}$$

$$K'_{MgHPO_4} = \frac{[Mg^{++}][HPO_4^{--}]}{[MgHPO_4]} = 0.0011 \text{ M}.$$

The values for $K'_{H_2PO_4^-}$ and K'_{CaHPO_4} are those given by Smith and Alberty (12) for ionic strength = 0.15 M. The values for $K'_{NaHPO_4^-}$ and K'_{MgHPO_4} given by these authors are 0.26 M (11) and 0.00132 M (12) at ionic strength = 0.2 M. In order to correct these constants to ionic strength = 0.16 M, the general activity coefficient equation of Davies (14) was employed. Although this equation is only approximate, the corrections applied are small (7 and 13 per cent, respectively).

$$K'_{NaCit^{--}} = \frac{[Na^+][Cit^{---}]}{[NaCit^{--}]} = 0.20 \text{ M}.$$

$$K'_{CaCit^-} = \frac{[Ca^{++}][Cit^{---}]}{[CaCit^-]} = 0.00070 \text{ M}.$$

$$K'_{MgCit^-} = \frac{[Mg^{++}][Cit^{---}]}{[MgCit^-]} = 0.00028 \text{ M}.$$

These values were obtained at ionic strength = 0.16 M (9). It was assumed that: *1*) total ultrafiltrable phosphate = Pi = $[H_2PO_4^-] + [HPO_4^{--}] + [NaHPO_4^-] + [CaHPO_4] + [MgHPO_4]$; and *2*) total ultrafiltrable citrate = $[Cit] = [Cit^{---}] + [NaCit^{--}] + [CaCit^-] + [MgCit^-]$. The ionic species $CaH_2PO_4^+$, $MgH_2PO_4^+$, $KHPO_4^+$, $KCit^{--}$ and $HCit^{--}$ are all present in only trace amounts at these concentrations and were therefore neglected.

Substituting the above constants in Equation 4: $[H_2PO_4^{--}] = Pi/(1 + 10^{6.9-pH} + [Na^+]/0.23 + [Ca^{++}]/0.002 + [Mg^{++}]/0.0011$. All concentrations are in moles per liter. Individual phosphate complexes are then readily calculated. Similarly, $[Cit^{---}] = [Cit]/(1 + [Na^+]/0.2 + [Ca^{++}]/0.0007 + [Mg^{++}]/0.00028)$. In this work it was assumed that $[Na^+] = 0.14$ M.

In calculating protein-binding of calcium and magnesium no corrections for plasma water or the Donnan factor were applied because in the case of divalent cations these two corrections are opposite in direction and of approximately equal magnitude. In the case of phosphate, the corrections are in the same direction. The percentage of plasma inorganic phosphate (Pi) bound to protein was therefore calculated as $100\ (1 - [Pi]_{UF}/1.175[Pi]_p)$. The factor 1.175 was derived as explained in a previous paper (3). The assumptions involved in applying this factor are: *1*) plasma water content is normal (this is borne out by the normal values for total plasma protein in this group of

subjects); and *2*) the composition of the protein-free phase of plasma water is not altered during ultrafiltration. Evidence in support of this assumption was given previously (3). Although true equilibrium cannot obtain during ultrafiltration and Donnan calculations are therefore not applicable in a strict sense, nevertheless, the restraining effect of the nondiffusible anions is evidently constant, within experimental error. Consequently, a "Donnan-like" factor must be applied; in the absence of other evidence, the most reasonable factor to use is the one which obtains in equilibrium systems.

It would have been preferable to determine both pH and free cation concentration at 37° C rather than at room temperature (22° to 26° C), and to apply dissociation constants obtained at 37° C. However, this would be considerably more difficult technically; furthermore, no *K* values at 37° C are available for most of these complexes. The results may therefore require some modification in the future, to correct for the difference between 37° C and 25° C.

RESULTS

Observed and derived data in 20 normal subjects are presented in Table I. Several aspects of these results deserve comment.

1. Sex and age differences. The mean values for 12 males are nearly identical with those for 8 females in all of the measured and derived data. Significant differences between males and females have not been found in other studies of some of these constituents (15). The age range was intentionally narrow (21 to 39 years), and no correlation between any constituent and age in this range was seen.

2. Variability. The concentrations of certain constituents, notably calcium and magnesium, exhibited little variation in comparison with other series (15–25). Especially noteworthy is the plasma calcium concentration, for which the standard deviation was only 2 per cent of the mean. Ninety-five per cent confidence limits are 2.36 and 2.60 mmoles per L (9.4 to 10.4 mg per 100 ml). The usually accepted range in normals is approximately twice as great.

Phosphate and citrate concentrations, and therefore most of the derived data as well, were

TABLE I

Observed composition of normal plasma and plasma ultrafiltrate

Subject			Plasma				Ultrafiltrate						
Name	Sex	Age	Protein	Ca	Mg	Pi	pH*	Ca	Ca^{++}	Mg	Mg^{++}	Pi	Cit
			g%	mmoles/L	mmoles/L	mmoles/L		mmoles/L		mmoles/L		mmoles/L	
Man	M	21	7.01	2.50	1.00	0.84	7.54	1.36	1.21	0.70	0.56	0.89	0.137
Fin	M	21	6.96	2.62	0.84	1.24	7.66	1.50	1.36	0.68	0.61	1.36	0.092
Fri	M	22	6.39	2.46	0.94	1.14	7.59	1.34	1.17	0.66	0.48	1.17	0.149
Whe	M	22	6.70	2.38	1.00	1.48	7.65	1.42	1.32	0.64	0.56	1.47	0.127
Lon	M	22	6.24	2.40	1.08	1.01	7.57	1.30	1.14	0.70	0.50	1.13	0.129
Der	M	22	6.62	2.42	0.96	1.11	7.50	1.36	1.10	0.68	0.49	1.20	0.134
Pas	M	22	7.27	2.60	0.99	1.21	7.61	1.34	1.04	0.70	0.49	1.18	0.113
Sto	M	23	6.65	2.44	0.98	1.29	7.65	1.45	1.46	0.69	0.60	1.40	0.111
Hir	M	23	6.58	2.46	1.00	0.89	7.56	1.22	1.05	0.64	0.48	0.97	0.129
Wei	M	29	6.54	2.48	1.00	0.85	7.52	1.44	1.11	0.70	0.60	0.82	0.093
Wal	M	34	6.62	2.48	1.03	1.14	7.60	1.22	1.17	0.70	0.53	1.03	0.110
May	M	39	6.04	2.46	0.90	1.23	7.54	1.30	1.14	0.58	0.47	11.9	0.101
Mean of males			6.63	2.48	0.98	1.12	7.58	1.35	1.19	0.67	0.53	1.15	0.118
But	F	24	7.59	2.56	0.95	1.32	7.56	1.20	1.13	0.57	0.45	1.26	0.101
Sch	F	25	6.19	2.44	0.96	1.10	7.52	1.54	1.11	0.67	0.60	1.26	0.141
Kat	F	26	6.34	2.49	0.90	1.52	7.54	1.26	1.14	0.66	0.50	1.33	0.126
Hil	F	27	7.10	2.42	0.98	1.28	7.49	1.44	1.12	0.68	0.62	1.31	0.123
Smi	F	28	6.79	2.56	0.90	1.14	7.57	1.38	1.21	0.62	0.57	1.20	0.138
For	F	35	6.21	2.52	0.91	0.97	7.57	1.32	1.09	0.56	0.44	0.93	0.113
Pla	F	35	6.04	2.52	0.92	1.22	7.55	1.40	1.26	0.72	0.55	1.24	0.105
Kli	F	36	6.65	2.44	1.04	1.00	7.56	1.47	1.36	0.71	0.57	1.00	0.143
Mean of females			6.61	2.49	0.95	1.19	7.56	1.38	1.18	0.65	0.54	1.19	0.124
Group mean			6.63	2.48	0.96	1.15	7.57	1.36	1.18	0.66	0.53	1.17	0.121
SD			0.41	0.06	0.06	0.19		0.09	0.11	0.05	0.06	0.17	0.017
SD/mean			0.06	0.02	0.06	0.16		0.07	0.09	0.07	0.11	0.15	0.14

* At 25° C.

more variable. This may be due to the fact that the subjects were not fasting.

3. Free and complexed calcium and magnesium. The difference between ultrafiltrable calcium and free ionic calcium, representing complexed calcium, averaged 0.16 mmole per L, or 6 per cent of the plasma calcium. The variability in this quantity reflects the fact that it is obtained by difference. In only one sample (Subject Sto) did ionic calcium appear to exceed ultrafiltrable calcium. Since the error in ionic calcium determination approaches 0.1 mmole per L, this absurd result may be expected occasionally. Fanconi, Rose and Lloyd (*19, 26, 27*) have reported somewhat smaller values for complexed calcium. The use of nonlinear standard curves and the failure to remove heavy metals in their work may account for the difference between the two studies (*2*). McLean and Hastings (*28*), using the frog heart method, found 0.15 to 0.34 mmole per L of calcium which could not be accounted for by protein-binding or as ionic calcium. Similar results were obtained by Morison, McLean and Jackson (*29*) using this method.

Complexed magnesium averages 0.13 mmole per L, or 14 per cent of the total plasma magnesium. Free magnesium ion concentration averaged 0.53 mmole per L or 55 per cent of the total. These values are remarkably similar to those obtained in 1938 by Nordbö (30), using a different method.

Unidentified complexes, calculated by subtracting the measured complexes (with phosphate and citrate), averaged 0.08 mmole per L of calcium and 0.06 mmole per L of magnesium. Although these values are only approximate (SD = 0.10 and 0.05 mmole per L, respectively), the means differ significantly from zero ($p < 0.001$). In abnormal plasma, the amount of unidentified complexes may be much greater (31).

The only previous estimates of the amounts of individual calcium complexes in plasma are those of Neuman and Neuman (13), who postulated the amounts of calcium complexed by bicarbonate, phosphate and citrate to be 0.16, 0.06 and 0.07 mmole per L, respectively. The method by which calcium complexed by bicarbonate was calculated is not stated. Neuman and associates (32) give a value of 0.4 M for the stability constant of the complex $CaHCO_3^+$ at ionic strength 1.0 M. Subsequent studies at lower ionic strengths have failed to reveal any complex formation between calcium and bicarbonate (33); however, these results have been questioned (34). Our values for calcium complexed by phosphate and citrate are 0.036 and 0.038 mmole per L, respectively (Table II), somewhat lower than those estimated by Neuman and Neuman (13). The proportion of total plasma sodium which is bound to dibasic phosphate and citrate is calculated to be less than 0.5 per cent.

4. Free anion concentrations. The fraction of total ultrafiltrable phosphate present as free dibasic phosphate ions is only 51 per cent. If monobasic phosphate ions are included and approximate corrections made for the Donnan factor, the fraction of total plasma phosphate existing as free ions is calculated to be about 53 per cent. Thus the extent of ionization of plasma phosphate is quite similar to that of plasma calcium and magnesium.

The fraction of total ultrafiltrable citrate present as free ions is considerably lower, 19 per cent. Protein binding of citrate was not determined because of the greater degree of uncertainty in choice of a proper Donnan factor for trivalent anions. In other experiments (35), citrate concentration in whole plasma was approximately equal to citrate concentration in ultrafiltrate.

5. Ion products. The product of calcium and phosphate concentrations is relevant to the equilibrium between bone salt and plasma. The traditional procedure of multiplying whole plasma calcium in milligrams per 100 ml by whole plasma inorganic phosphorus in milligrams per 100 ml is clearly inadequate.

Levinskas (36), quoted by Neuman and Neuman (13), attempted to derive a value for free dibasic phosphate ion concentration in normal plasma, for the product $([Ca^{++}] \times [HPO_4^{--}])$, and also to apply activity coefficient corrections. However, the complexes $NaHPO_4^-$, $CaHPO_4$, and $MgHPO_4$ were ignored and the product obtained is therefore too high.

The present procedure makes possible direct calculation of the product $[Ca^{++}] \times [HPO_4^{--}]$ in plasma based upon the dissociation constants cited. No attempt has been made to correct these products to zero ionic strength. The values range from 0.47 to 1.06×10^{-6} M, averaging 0.72×10^{-6} M. There was no inverse correlation found

TABLE II

Interactions between calcium, magnesium, phosphate, citrate and protein in normal plasma; derived data

Subject	Percentage protein-bound			Complexes						Free anions		Ion products	
	Ca	Mg	P	$NaHPO_4^-$	$CaHPO_4$	$MgHPO_4$	$NaCit^{--}$	$CaCit^-$	$MgCit^-$	HPO_4^{--}	Cit^{---}	$Ca^{++} \times HPO_4^{--}$	$Mg^{++} \times HPO_4^{--}$
	%	%	%	*mmoles/L*		*mmoles/L*		*mmoles/L*		*mmoles/L*		$M \times 10^{-6}$	
Man	46	30	10	0.28	0.028	0.023	0.018	0.043	0.051	0.46	0.025	0.56	0.26
Fin	43	19	7	0.44	0.048	0.040	0.011	0.030	0.034	0.71	0.016	0.96	0.43
Fri	46	30	12	0.37	0.036	0.027	0.020	0.049	0.050	0.61	0.029	0.71	0.29
Whe	40	36	15	0.47	0.051	0.039	0.016	0.043	0.046	0.77	0.023	1.01	0.43
Lon	46	36	5	0.36	0.033	0.027	0.018	0.041	0.045	0.59	0.025	0.67	0.28
Der	44	29	8	0.37	0.034	0.027	0.019	0.042	0.047	0.61	0.027	0.67	0.30
Pas	48	29	17	0.38	0.032	0.028	0.016	0.034	0.040	0.62	0.023	0.64	0.30
Sto	41	30	8	0.45	0.053	0.040	0.013	0.039	0.040	0.73	0.019	1.06	0.44
Hir	50	36	7	0.31	0.026	0.022	0.018	0.039	0.045	0.50	0.026	0.52	0.24
Wei	42	30	17	0.26	0.023	0.023	0.012	0.027	0.037	0.42	0.017	0.47	0.25
Wal	50	34	23	0.33	0.032	0.026	0.015	0.035	0.040	0.54	0.021	0.63	0.29
May	46	38	18	0.37	0.035	0.026	0.014	0.033	0.034	0.61	0.020	0.70	0.29
Mean of males	45	31	12	0.36	0.036	0.029	0.016	0.038	0.042	0.60	0.022	0.72	0.32
But	52	42	19	0.40	0.037	0.027	0.014	0.033	0.033	0.65	0.020	0.73	0.29
Sch	37	30	2	0.39	0.036	0.035	0.018	0.041	0.056	0.64	0.026	0.71	0.38
Kat	49	30	25	0.42	0.039	0.031	0.017	0.040	0.044	0.68	0.025	0.78	0.34
Hil	54	30	13	0.40	0.037	0.037	0.016	0.036	0.050	0.66	0.022	0.74	0.41
Smi	46	31	11	0.38	0.038	0.032	0.018	0.044	0.052	0.62	0.025	0.75	0.35
For	46	41	19	0.30	0.026	0.019	0.016	0.036	0.037	0.48	0.023	0.52	0.21
Pla	44	22	13	0.39	0.040	0.032	0.013	0.035	0.038	0.64	0.019	0.81	0.35
Kli	40	32	15	0.31	0.035	0.027	0.018	0.049	0.051	0.51	0.025	0.69	0.29
Mean of females	46	32	15	0.37	0.036	0.030	0.016	0.039	0.045	0.61	0.023	0.72	0.33
Group mean	46	32	13	0.37	0.036	0.029	0.016	0.038	0.044	0.60	0.023	0.72	0.32
SD	4	5	6	0.06	0.008	0.006	0.002	0.006	0.006	0.09	0.003	0.15	0.07
SD/mean	0.09	0.17	0.44	0.15	0.21	0.20	0.15	0.14	0.14	0.15	0.15	0.21	0.21

TABLE III

Forms of calcium, magnesium, phosphate and citrate in normal plasma

	mmoles/L	Percentage of total
Calcium		
Free ions	1.18	47.5
Protein-bound	1.14	46.0
$CaHPO_4$	0.04	1.6
$CaCit^-$	0.04	1.7
Unidentified complexes	0.08	3.2
Total	2.48	100
Magnesium		
Free ions	0.53	55
Protein-bound	0.30	32
$MgHPO_4$	0.03	3
$MgCit^-$	0.04	4
Unidentified complexes	0.06	6
Total	0.96	100
Phosphate		
Free HPO_4^{--}	0.50	43
Free $H_2PO_4^-$	0.11	10
Protein-bound	0.14	12
$NaHPO_4^-$	0.33	29
$CaHPO_4$	0.04	3
$MgHPO_4$	0.03	3
Total	1.15	100
Citrate (in ultrafiltrate)		
Free ions	0.023	19
$NaCit^{--}$	0.016	13
$CaCit^-$	0.038	32
$MgCit^-$	0.044	36
Total	0.121	100

between $[Ca^{++}]$ and $[HPO_4^{--}]$; in fact, there was a suggestive, though statistically insignificant, positive correlation.

The ion product of $[Mg^{++}] \times [HPO_4^{--}]$ averaged 0.32×10^{-6} M. The solubility product of this salt is given as 70×10^{-6} M by Tabor and Hastings (37).

6. Protein-binding. The fraction of plasma calcium bound to protein in these samples averaged 46 per cent. This is somewhat greater than the values obtained by Toribara and co-workers (1) or by Hopkins, Howard and Eisenberg (38), but comparable to those found by Prasad and Flink (22). One reason for larger values in the present work is the use of the dry (or nearly dry) casing; under certain circumstances the error due to use of wet casing may be as great as 10 per cent, even after thorough wiping. It should be noted, however, that the total weight of casing employed in the present work is greater than that used by other investigators.

Protein-binding of magnesium averaged 32 per cent, which is consistent with other reports (17, 18, 20, 21, 23, 25). In every subject, protein-binding of magnesium was less than that of calcium. However, there was no correlation between the percentage of calcium bound and the percentage of magnesium bound.

Protein-binding of phosphate averaged 13 per cent. In a previous series of normal subjects (3), an average value of 25 per cent was bound. In azotemic subjects without phosphate retention, an average of 14 per cent was bound. The lower values obtained in the present work are probably attributable chiefly to the use of drier casing.

DISCUSSION

The results with respect to the physicochemical state in normal plasma of the four ions studied are shown in Table III. Here the observed values in ultrafiltrate have been corrected (approximately) for the Donnan factor and plasma water content in order to obtain estimates of the concentrations in native plasma. The same qualifications described above apply to these Donnan factors as to the estimates of protein-binding.

The data presented in this study are offered chiefly as a basis for further investigation. At present, the significance of the complexes and ion products described is unknown. It is reasonably certain, however, that the free ion concentrations of calcium, magnesium, dibasic phosphate and citrate are those upon which the known physiological function of these ions depends. Complexed calcium, for example, is known to be ineffective in promoting the clotting of blood, or the contraction of muscle (39). In the formation of bone salt, on the other hand, the complexes $CaHPO_4$ and $MgHPO_4$ may play a role as may the complexes formed with citrate.

Bianchi and Shanes (40) have postulated that ion transport across cell membranes may occur as ion-pairs. It is possible that some of the ionic complexes determined here may be involved.

Of greater immediate interest is an extension of these studies to abnormal plasma and to other body fluids. To cite one example, the hypercitremia seen following administration of parathyroid

extract or vitamin D may represent an increase in free citrate ion concentration or simply a secondary rise in complexed citrate associated with hypercalcemia. This problem is currently under investigation. In a preliminary report (31), we have described the changes seen in uremic plasma. The most striking alterations observed were a diminution in the percentage of plasma calcium and magnesium present as free ions, often in association with the appearance of large amounts of unidentified complexes, and an increase in the product $[Ca^{++}][HPO_4^{--}]$.

SUMMARY

The physicochemical state of calcium, magnesium, phosphate and citrate in normal human plasma has been studied by ultrafiltration followed by determination of free calcium and magnesium ion concentrations in ultrafiltrate. Employing known stability constants, the concentrations of the complexes $NaHPO_4^-$, $CaHPO_4$, $MgHPO_4$, $NaCit^{--}$, $CaCit^-$ and $MgCit^-$ have been calculated. Free ion concentrations of citrate and phosphate are obtained by difference. The fractions present as free ions are: calcium, 47.5 per cent; magnesium, 55 per cent; phosphate, 53 per cent; citrate, 19 per cent. The product of calcium ion concentration by free dibasic phosphate ion concentration averages 0.72×10^{-6} M.

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