The maximum at 311 mμ for the more highly iodinated samples is due to the presence of diiodotyrosyl residues. The molar extinction coefficient of 3,5-diiodotyrosine at 311 mμ was found to be equal to 5815. Using this value and the absorption at 311 mμ for the various samples (Table I), the diiodotyrosyl content of the samples can then be calculated. Assuming a value of 12.3% tyrosine content in untreated insulin, we calculate values for the diiodotyrosine content of samples F-K which are lower by as much as 14% of that calculated stoichiometrically (Table I). Apparently the ultraviolet absorptivity of the 3,5-diiodotyrosyl group is slightly depressed when it is part of the protein molecule.

TABLE I

Sample	Insulin, mg.	Iodine, ml., 0.140 *N*	Optical density at λ 312 mμ for 0.77 mg./ml. initial insulin at *p*H 9.74	Calcd. % fully iodinated tyrosine from spectra	Calcd. % fully iodinated tyrosine from stochiometry	Optimum *p*H for crystallization
A	...	..	0.086	..	0	6.24
B	151.9	0.30	.320	..	10	6.21
C	159.1	.62	.598	..	20.0	6.36
D	151.1	.91	.787	..	30.0	6.72
E	147.4	1.14	1.12	..	40.0	6.92
F	154.5	1.29	1.12	41	43.1	6.94
G	150.8	1.46	1.39	46	50.0	6.87
H	148.0	1.65	1.64	54	57.5	7.40
I	161.5	2.02	1.72	57	64.5	7.49
J	151.1	2.10	1.98	65	71.7	7.51
K	151.0	2.94	2.65	87	100.0	7.58

Crystallization.—All the iodinated samples were crystallized by a modification of the method of Scott[15] as used to crystallize pure insulin. About 100 mg. of the dried dialyzed iodinated insulin was suspended in 5.0 ml. of distilled water. To this was added 135 ml. of a phosphate buffer (8.38 g. of $Na_2HPO_4 \cdot 12H_2O$ and 1.38 g. of KH_2PO_4 in 500 ml. of water) which had been adjusted to *p*H 2.32 with 1 *N* HCl to give a clear solution. Next 1.30 ml. of a 0.5% zinc chloride solution was added, followed by the addition of 13.0 ml. of acetone. The *p*H of the clear solution was in the neighborhood of *p*H 2.5. The *p*H was raised with ammonia to the appropriate value given in Table I. The optimal *p*H for crystallization of a given iodinated sample was determined by trial and error. The beaker containing the solution was scratched with a glass rod, left for 15 minutes at room temperature and then refrigerated for five days.

Crystals which fell to the bottom of the vessel were examined in the polarizing microscope. Samples which had been iodinated to 30% or less (samples A-D) had an appearance similar to that of ordinary zinc insulin, that is, they were of the trigonal system. More highly iodinated samples, on the other hand, crystallized in the form of flat plates, the edges of the plates being unequal in length and all mutually perpendicular. The crystals exhibited a feeble birefringence and appeared to belong to the orthorhombic system.

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Complexes of Alkaline Earth Cations Including Radium with Amino Acids and Related Compounds

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As a supplement to some previously reported studies[1-3] measurements have been made of the interaction of alkaline earths with additional amino acids and related compounds. Most of the work reported here has been done under approximately physiological conditions, *i.e.*, ionic strength of 0.16 and *p*H 7.2–7.3. A few measurements at higher *p*H's and lower ionic strengths are included.

Experimentally, the ion-exchange technique[2] has been employed. The mass action formation quotient, K_f, for the 1:1 complexes, refers to the reaction

$$M + A \rightleftarrows MA \qquad (1)$$

where M represents the total stoichiometric concentration of the metal uncorrected for ionization, hydrolysis, binding by other ligands except the specific ligand A, etc., and MA the resulting complex. In many cases, such as for citric and tartaric acids, the parent acid, H_nA, of the ligand A is practically completely ionized at *p*H 7.2. However, for amino acids such as glutamic acid, H_3A, where one of the amino hydrogens is considered acidic, the predominant ion in solution at *p*H 7.2 is HA^- but as the *p*H is raised to 10 and above the ion $A^=$ becomes predominant (pk_1(COOH) = 2.3, pk_2(COOH) = 4.4, $pk_3(NH_3^+)$ = 9.7).[4] The over-all molar concentration of A as a basis for calculation of K_f was deliberately chosen because of its direct applicability to physiological problems. In many cases, it is very difficult, of course, to calculate true or intrinsic values of K_f because the degree and nature of the hydrolytic and other reactions involving the metal ions and ligands are not known.

The formation quotient, K_f, is calculated from the relation[1-3]

$$K_f = \frac{(K_d^\circ/K_d) - 1}{(A)^n} \qquad (2)$$

where K_d° and K_d are the distribution coefficients of M between the resin and solution phases in the absence and presence of the ligand A, respectively. The distribution coefficient for M is

$$K_d = \frac{\%M \text{ in resin}}{\%M \text{ in soln.}} \times \frac{\text{vol. of soln. (ml.)}}{\text{mass of resin (mg.)}} \qquad (3)$$

From plots of $1/K_d$ *vs.* A it was found that all of the complexes were of the 1:1 type, *i.e.*, $n = 1$. This assumes, as Toribara and Feldman have pointed out,[2] that the dissociated metal ion in solution is not polymerized. The results of the present study are summarized in Table I together with corresponding values of K_f reported in the literature for similar ionic strengths.[5-8]

Several points of interest present themselves. The values of K_f for the complexes of Ba and Ra are probably the most accurate available since previously reported values were preliminary ones. A good example is that of barium citrate where the literature except for reference 5 indicated a value of K_f nearly identical with that of strontium citrate an unlikely situation.

Values of log K_f for the calcium complexes of adenosine triphosphate and adenosine diphosphate

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TABLE I

INTERACTION OF ALKALINE EARTH CATIONS WITH AMINO ACIDS AND RELATED COMPOUNDS

Unless otherwise specified, measurements were made in solutions 100 ml. in volume containing 25 ml. of veronal buffer, carrier-free concentrations of one or more of the radioisotopes of the alkaline earth cations. The over-all Na^+ concentration was 0.16 *M*. *p*H 7.2–7.3, and temperature of 25°. The solutions were equilibrated with 25–100 mg. of the sodium form of a nuclear sulfonic acid cation-exchange resin (Dowex-50). Average values of K_d^3 based on *dehydrated* weight of resin were: Ca, 1.52; Sr, 2.74; Ba, 11.3; Ra, 12.6.

Complexing agent	Formation quotient, log K_f				Log K_f as reported in lit. for similar μ
	Ca^{++}	Sr^{++}	Ba^{++}	Ra^{++}	
Adenylic acid (3′-phosphate)	..	1.4	..	..	
Asparagine	0	−0.43	..	..	
Catecholdisulfonic acid	1.8	..	..	..	
Choline	<−0.8	<0.8	..	..	
Citric acid	..	..	2.54	2.36	Ba, 2.3,[5] 2.69,[6] 2.98,[7] Ra, 2.0[1]
Citric acid (μ = 0.078)	..	..	2.84	..	
Creatine	0	0	..	..	
Creatinine	0	0	..	..	
Cytidylic acid (3′-phosphate)	..	1.6	..	..	
Glutamic acid (*p*H 7.1)	0.78	0.69	..	..	
Glutamic acid (*p*H 8.1)	..	0.85	..	..	
Glutamic acid (*p*H 9.1)	..	1.06	..	..	
Glutamic acid (*p*H 10.4)	..	1.12	..	..	Sr, 1.37[4] at μ = 0.1
Glutamine	0.18	..	..	..	
Glutathione	0	..	..	..	
Hydroxyproline	0.48	0.04	..	..	
Imidazole	0.08	..	..	..	
Malic acid	..	..	1.36	0.95	Ba, 1.30[8]
Malic acid (μ = 0.078)	..	..	1.48	..	
Methionine	−0.66	..	..	..	
Salicylic acid	0.15	..	..	..	Ca, 0.14[7]
Serine	~0.5	~0.4	..	..	
Succinic acid	..	..	1.21	..	Ba, 0.97,[7] 1.03[8]
Tartaric acid	..	..	1.67	1.24	Ba, 1.62,[8] 1.95,[7] Ra, 1.2[1]
Tartaric acid (μ = 0.078)	..	..	1.76	..	

have been found[9] to be 3.94 and 2.26, respectively, at an ionic strength of 0.1, *p*H 7.4 and 37°. The K_f values of 1.4 and 1.6 for the Sr complexes of adenylic and cytidylic acids given in Table I lead to the conclusion that the stability of the alkaline earth complexes of these biologically important compounds increases with an increase in the number of terminal phosphoric acid groups. Similar findings have been reported[10] in the case of complexes formed between calcium and Na_3PO_4, $Na_4P_2O_7$ and $Na_5P_3O_{10}$, the respective pK_f's at μ = 0.15, *p*H 7.4 and 37° being 1.79, 3.47 and 4.32.

In the physiological *p*H region it is apparent that none of the amino acids studied bind appreciable fractions of the alkaline earths. Similar conclusions have been reported among others by Heinz.[11] As the *p*H is raised the extent of binding would be expected to increase because of the removal of a proton from the positively charged amino group.

At *p*H 10.4 log K_f for the strontium glutamate complex is 1.12. This value is sufficiently close to that of the succinate complex[3] (log K_f = 0.9) measured under neutral conditions to suggest that in both compounds complex formation involves predominately the two carboxyl groups. This conclusion differs from that of Lumb and Martell[4] who rule out participation of the second carboxyl group in favor of a model in which the metal ion is bound as a chelate through the carboxyl group alpha to the amino nitrogen and the nitrogen of the amino group. Such a conclusion seems questionable. For example, in support of their view they state that the stability constants of the alkaline earths with the dicarboxylic amino acids were reasonably close to those listed for glycinate ion in which only one carboxyl group is present. However, in their comparison the values of log K_f cited for the glycine complexes were for μ = 0 while those for glutamic and aspartic acids were at μ = 0.1 and succinate at μ = 0.16. Actually, at μ = 0.16 the affinity of the alkaline earths for glycine is very close to that of acetic acid.[2] In addition, when the beta carboxyl group of glutamic acid is converted to the amide the binding for alkaline earths drops drastically (Table I). It seems unlikely that inductive effects would account for this result. It must be recognized, however, that unequivocal conclusions concerning the relative number of carboxyl groups and other groups involved in the chelate cannot be deduced by analogy but is best obtained by measuring the equivalents of hydrogen released per equivalent of bound cation. However, for the very weak chelates discussed here it is difficult to obtain reliable data.

In actuality, there is no one form of a complex or chelate but a mixture in which the relative proportions of the different forms are a function of the respective stability constants. It is obvious, when relative K_f values are examined, that in glutamic

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acid, for example, the predominant form involves combination of the alkaline earth cations with both carboxyl groups. In an equilibrium mixture, however, small fractions must be present in which M^{++} is combined with only one carboxyl group; with a carboxyl group and amino group; and with an amino group only. With cations of many of the transition elements, however, chelate formation involving nitrogen predominates.

The ion-exchange data are of interest in themselves and in relation to chromatographic separations. Consider the following exchange reaction of the alkaline earth cations, M^{++}, with the sodium form of the exchanger

$$M^{++} + 2\overline{Na}^{+} \rightleftharpoons \overline{M}^{++} + 2Na^{+} \qquad (4)$$

where the bar over the symbol represents the resin phase. The dependence of $K_d(M)$ on concentration changes can be deduced from Donnan membrane or mass action considerations as was done previously.[2] Under the experimental conditions in which $[\overline{Na}^{+}] >> [\overline{M}^{++}]$ and $[Na^{+}] >> [M^{++}]$ the variation of $K_d(M)$ with ionic strength of the aqueous phase should, neglecting activity coefficients, in both the external solution and resin phases, vary inversely as the square of the Na^{+} concentration. In the range $\mu = 0.16$–0.078 this is the case. Thus at $\mu = 0.16$, $K_d(Ba)$ is 9.9 while at $\mu = 0.078$, $K_d(Ba)$ is found to be 43.6 while the calculated value is $(0.16/0.078)^2 \times 9.9 = 41.5$.

The chromatographic separation of Ra^{++}, Ba^{++} and Sr^{++} by elution with ammonium citrate from columns of Dowex-50 has been reported by Tompkins.[12] From the plate theory[13] the peaks of the published elution curves can be shown to be directly related to the distribution coefficients, K_d, of the elements involved. Thus, by definition, the separation factor, α, is given by the relation[14]

$$\alpha = \frac{K_d(M_1)}{K_d(M_2)} \times \frac{K_f(M_2)}{K_f(M_1)} \qquad (5)$$

From the data given in Table I, the separation factor for Ra–Ba is, where $M_1 = Ra^{++}$ and $M_2 = Ba^{++}$

$$\alpha = \frac{12.6}{11.3} \times \frac{350}{229} = 1.11_5 \times 1.52_8 = 1.7 \qquad (6)$$

From the ratio of the peaks of the elution curve shown in reference 12 the separation factor found is 2.3. This approximate agreement between the calculated and observed values is actually better when activity coefficient corrections are made since the solutions employed in the column experiments were 0.5 M ammonium citrate ($\mu = 3.0$).

Under the experimental conditions the separation factor α', corrected for activity coefficients in the external solution phase only, is

$$\alpha' = \alpha \times \frac{\gamma_{M_1}\gamma_{M_1}\gamma_{M_2A}}{\gamma_{M_2}\gamma_{M_2}\gamma_{M_1A}} = \alpha \times \left(\frac{\gamma_{M_1}}{\gamma_{M_2}}\right)^2 \frac{\gamma_{M_2A}}{\gamma_{M_1A}} \qquad (7)$$

It is easily shown that the calculated value of α' made at $\mu = 0.16$ is too low when approximate activity coefficient corrections are made. Similar calculations have been made for Ba–Sr, and Ra–Sr separations. The corrections are all in the proper direction and order of magnitude since[15] $\gamma_{Sr^{++}} > \gamma_{Ra^{++}}$ and presumably $\gamma_{Ba^{++}} > \gamma_{Ra^{++}}$ while M_1A and M_2A, *e.g.*, $(SrCit)^-$ can be considered[2] to behave as the monovalent ion, $(H_2Cit)^-$.

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Ion Exchange as a Separations Method. VIII. Relative Elution Positions of Lanthanide and Actinide Elements with Lactic Acid Eluant at 87°

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Because lactic acid has been shown to be a more selective eluant than citric acid for the ion-exchange separation of lanthanides,[1] its selectivity for the trivalent actinide elements appeared to merit investigation. Consequently, a number of column studies have been made of the elution of Am, Cm, Cf and element 99 from Dowex-50 cation-exchange resin with pH 3 lactate solutions at a temperature of 87°.

Since β-emitting isotopes of the lanthanides and α-emitting isotopes of Am, Cm, Cf and 99 were available, it was possible to obtain data on any desired combination of elements from a single run. Thus, the position of any given actinide peak could be readily determined, even though it were superimposed upon a background of lanthanide activities.

Finally, a simple correlation between the relative elution positions of homologs in the lanthanide and actinide series has been determined.

Experimental

Radioactive tracers were used to determine the relative elution positions of all elements except Dy, which was obtained from Dy_2O_3 of >98% purity.[2] All other apparatus and materials have been previously described.[3] The procedure used to make these separations and to determine the distribution of β-emitting isotopes was essentially the same as that of Freiling and Bunney.[3] However, advantage was taken of the insensitivity of C ratios obtained with lactic acid[1] in order to adjust the eluting conditions to obtain either more accurate data or more rapid elutions. The α-activity in each actinide fraction was most conveniently and rapidly determined by collecting small samples of eluate on Pt discs, drying on a hot plate, destroying the organic residue with successive additions and evaporations of fuming HNO_3, and counting in an alpha scintillation counter. By this technique the appearance of microgram amounts of Dy in the eluate could also be detected.

Results and Discussion

A quantitative summary of the results is made in Table I by listing, for each element studied, the C ratio[1,4] to Eu. As in the case of 0.25 M citric

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