crystallized (Å. Åkeson and G. Lundquist, to be published) and purified in greater amounts. Tryptic digests of ^{14}C-carboxymethylated EE and SS isoenzymes have now been extensively compared by multidimensional "fingerprinting" techniques similar to those used with the ES isoenzyme[2]. The peptide differences can be seen more distinctly with the two homogeneous isoenzymes than with the EE and hybrid ES forms previously compared.

More differences between the two types of chains have been found so that six mutations are now known (Table 1). The first three mutations have been detected previously[2]. Their numerical positions and those of the fourth and fifth mutations are now exactly known from the amino-acid sequence of the E-chain (H. J., submitted for publication). The position of the sixth mutation is still somewhat uncertain, for the structure of this part of the protein chain is not definitely established. The peptide from the S-chain containing the fifth mutation (a basic heptapeptide) is difficult to purify; only a small yield was available. Although sufficient material for complete sequence analysis was not obtained, it was possible to determine the total composition. From this and the electrophoretic charge of the peptide, the mutation in Table 1 is deduced on the assumption that the peptide contains only one substitution. All other mutations have been proved by analyses of both compositions and complete amino-acid sequences of differing peptides. Although five of the mutations are compatible with one-base mutations, the fifth requires a change of two bases.

Table 1. MUTATION SITES

Mutation number	Position along the protein chain	Residue in E-chain	Residue in S-chain
1	17	Glu	Gln
2	94	Thr	Ile
3	101	Arg	Ser
4	110	Phe	Leu
5*	115	Asp	Ser*
6	366†	Glu	Lys

* Based on total composition only (see text).
† Preliminary number.

The six mutations now known render the S-chain more positive than the E-chain by three charged units around neutral pH. This explains the electrophoretic properties of the isoenzymes[1] and is in keeping with the differences in pI[2] between them. Before, when only the three first mutations were known[2], these facts could not be accounted for.

Of the six mutations, four are closely situated at the region around position number 100 of the chains. In addition, these four substitutions are for both smaller and more hydrophobic residues in the S-chain when compared with the similar portions of the E-chain. This fact and the known difference in substrate specificity between the chains (the S-chain is active towards steroid substrates but the E-chain is not[1]) might suggest that the mutation-rich region around position 100 is close to the substrate binding site. The need for more space and hydrophobicity at this site of the S-chain, as compared with that of the E-chain, might then possibly be explained by these mutations, although changes in the three-dimensional folding of the chains are, of course, alternative explanations.

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Size-specific Metal Complexing Sites in Native Collagen

THERE is evidence that metal ions are involved, directly and indirectly, in the structure and function of the collagens[1–11]. The direct interactions between collagen proteins and metal ions must be elucidated in order to understand the roles of these ions in connective tissues. One approach is to study the metal binding properties of relatively intact collagen matrices in model systems consisting of water solutions of several ionic salts. Previous experiments have involved the binding of a few ions to purified reconstituted calf-skin collagen[12], egg shell matrix[13], gelatine[14] and bivalent amino-acids[15].

We report here the results of experiments in which whole sections of decalcified bone matrix and tendon were exposed to twenty-four metallic ions.

Table 1. EXPERIMENTAL CONDITIONS DURING EXPOSURE TO IONIC SOLUTIONS

	Experiment 1	Experiment 2
pH	1^+ 6·3, 2^+ 6·4, 3^+ 3·5; Adjusted NH_4OH	5·4; Adjusted NaOH
Ionic strength	0·011–0·016 M	0·16 M
Indiv. ion conc.	0·3–3·0 mM	4·0 mM
Sample/solution (w/v)	0·05 g/100 ml.	0·2 g/100 ml.
Exposure	3 days	2 days
Ions 1^+	Li, Na, Ag, K, Rb, Cs	Li, Na, Ag, K, Rb, Cs
2^+	Be, Mg, Sr, Ba, Mn, Zn, Cu, Cd, Hg, Pb	Be, Mg, Ca, Sr, Ba, Zn, Cu, Cd, Co, Ni, Pb
3^+	Al, Ga, Cr, Fe, Bi	

Small sections of normal human tibial cortex and human Achilles tendon were demineralized for 5 days (5 per cent formic acid, 25° C), washed and stored under distilled deionized water until use. Other tendon sections were only washed. The samples were immersed in compound equimolar nitrate salt solutions at room temperature (Table 1), and washed in three changes of water for 1 h. In the first experiment, demineralized bone and washed tendon (in the same vessel) were exposed to compound solutions of either 1^+, 2^+ or 3^+ ions. Demineralized tendon was included in the divalent ion solution. In experiment 2, demineralized bone and demineralized tendon (in separate vessels) were exposed to solutions containing many 1^+ and 2^+ ions mixed together. Relative uptakes were determined by measuring the resulting metal concentrations in the substrates, using d.c. arc emission spectroscopy[8,9]. Control concentrations in unexposed tissues were generally well below induced concentrations. Comparison of preliminary uptake with time showed that equilibrium was reached within 12 h of exposure.

Table 2. CONCENTRATIONS OF IONS BOUND TO COLLAGEN SAMPLES

		Experiment 1			Experiment 2	
		Demin. bone	Demin. tendon	Washed tendon	Demin. bone	Demin. tendon
1^+	Li	–		–	?	?
	Na	–		–	–	+
	Ag	+ + + +		+ + + +	+ + +	+ + + +
	K	–		–	–	+
	Rb	–		–	–	+
	Cs	–		–	–	+
2^+	Be	+	+	+	+ + +	+ +
	Mg	+	+	–	+	+
	Cu	+ +	+ +	+ +	+ + + +	+ + +
	Zn	+ +	+	+ +	+ + + +	+
	Mn	+	+	+		
	Cd	+	–	–	+	+
	Hg	–	–	–		
	Sr	–	–	–	+	+ +
	Pb	+ + + +	+	+	+ + + +	+ + + +
	Ba	–	–	–	+	+
	Ca				+	+
	Co				+	+
	Ni				+	+
3^+	Al	+		+ +		
	Ga	+		+ + +		
	Fe	+		+ + + +		
	Cr	+		+ + +		
	Bi	+		+ + + +		

– Indicates less than 0·1 μmoles/g of metal in dry tissue.
+ Indicates 0·1–10 μmoles/g of metal in dry tissue.
+ + Indicates 10–30 μmoles/g of metal in dry tissue.
+ + + Indicates 30–50 μmoles/g of metal in dry tissue.
+ + + + Indicates 50 or more μmoles/g of metal in dry tissue.

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The concentrations of metal ions bound to the substrates are outlined in Table 2. Differences in Pb^{2+} and 3^{+} ion uptakes in the first experiment may reflect structural or conformational differences between the bone and tendon collagen, as prepared here. When plotted as a function of the ionic radii of the metal ions[16], the preferentially bound ions fell into at least two size categories, 0·65–0·75 Å and 1·2–1·3 Å (Fig. 1). These categories were to a certain extent independent of the specific ions involved, the charges on the ions, and the experimental conditions used. It may also be significant that the "preferred sizes" differed approximately by a multiple of two. To our knowledge, these are the first such size-specific interactions observed for collagen matrices.

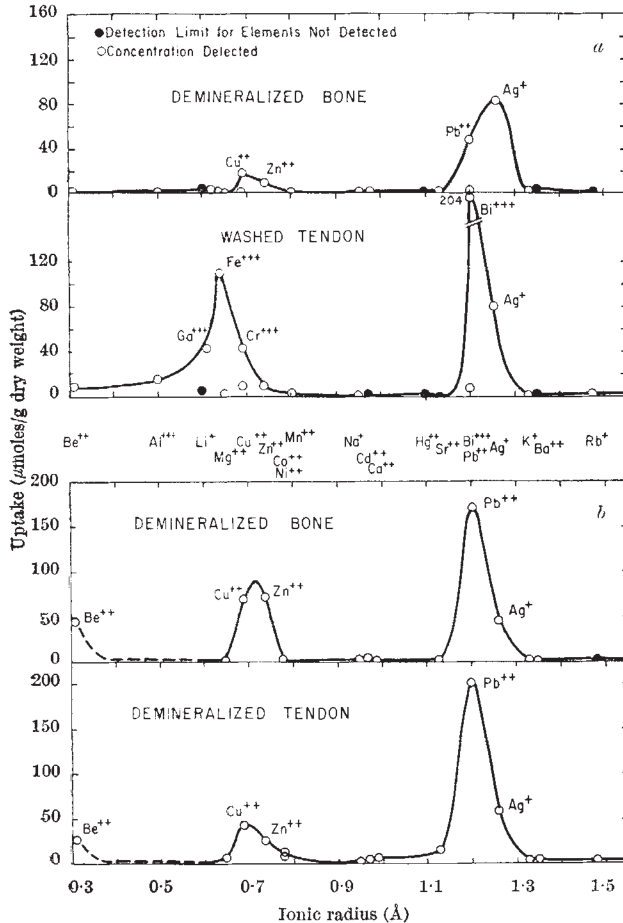

Fig. 1. The ion uptake (μmoles/g dry weight of tissue) as a function of ionic radius for (*a*) demineralized bone and washed tendon in experiment 1, and (*b*) demineralized bone and demineralized tendon in experiment 2. The uptake of divalent ions in demineralized tendon in experiment 1 (not shown) was identical to the results for washed tendon shown above. Trivalent ions were omitted in experiment 2 to maintain the *p*H of the hetero-ionic solutions above 5. The two preferred size regions are evident as are differences between the ion selectivities of bone and tendon matrices in experiment 1.

For chelate complexes involving a given donor and several different metal ions, it has been shown that the logs of the formation constants are a linear function of the total ionization potentials of the ions[17]. Furthermore, in the conditions of a large excess of several metal ions in equimolar concentrations, the log of the metal ion concentration measured in the substrate should also be proportional to the ionization potential[18] if a coordination complex is formed.

Thus the general linearity in Figs. 2 and 3 is strong evidence for coordination bonding (or chelation) in these collagen matrices, and that most of the ions were bound to a single type of donor group. Many

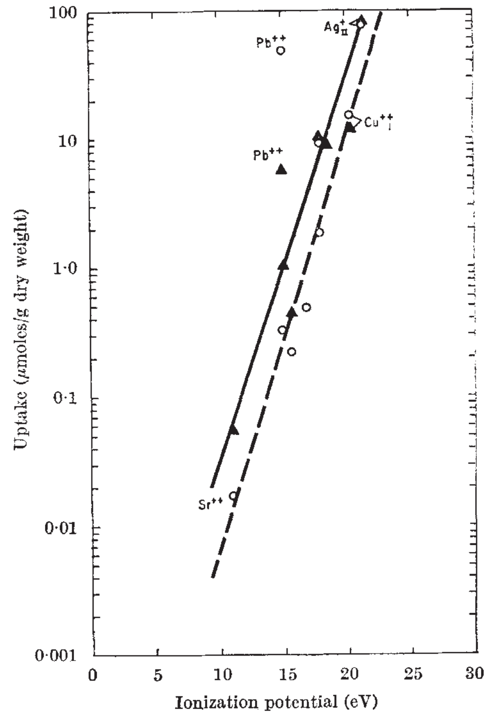

Fig. 2. The ion uptakes in experiment 1 (μmoles/g dry weight of tissue) plotted against the total ionization potentials of the ions on a semi-log scale. The trivalent data are omitted because of the extremely different experimental conditions obtaining in that part of the experiment. The divalent uptakes in decalcified tendon (not shown) were similar to those for washed tendon. The second ionization potential of Ag was used in this plot. The concentrations bound to the tendon matrix were higher than that for demineralized bone matrix, although the slopes were nearly identical. The Pb concentrations clearly stray from the rest. ○, Decalcified bones; ▲, washed tendon.

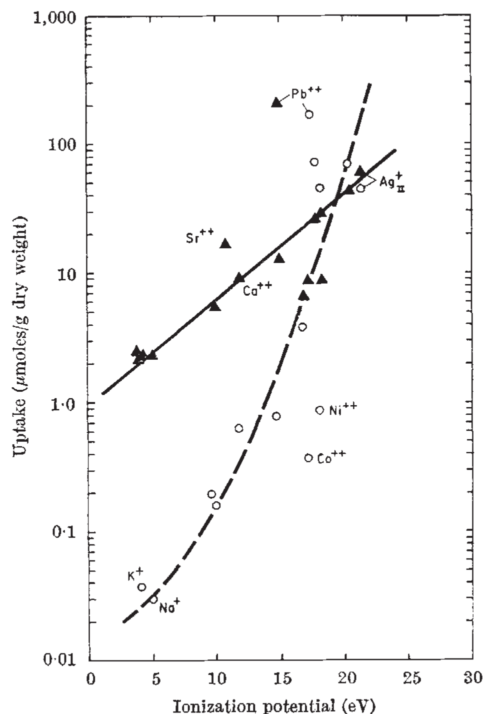

Fig. 3. The ion uptakes in experiment 2 (μmoles/g dry weight of tissue) plotted against the total ionization potentials on a semi-log scale. Ag is plotted using its second ionization potential. As in Fig. 2, the tendon matrix bound many ions more avidly than demineralized bone, but with a considerably smaller slope. The slope for decalcified bone is generally similar to that in the previous experiment; the considerable scatter and curvature in this experiment probably reflects the higher ionic strength and lower *p*H used. The Pb, Co and Ni uptakes stray from the bulk of the data. ○, Decalcified bone; ▲, decalcified tendon.

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of the strongly bound ions (1+ and 2+) readily coordinate to donor nitrogens[19,20], so the bonding may be primarily to uncharged amino, imidazole or guanidino groups in the substrates, and may be largely ion-polar or covalent in character[20]. Although titratable, the charged carboxyls are only partially available for binding in native collagen due to internal charge compensation at medium pH[21]. The binding of trivalent ions in experiment 1 at low pH, however, may have been to the latter site[22].

Others have suggested that metallic coordination complex formation in cartilage[23], elastin[11,24] and egg shell matrix[13] is important in the primary calcification process. Similar complexes may be involved in the calcification (or lack of it) in bone and tendon matrices.

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Genetic Control of Deficiencies in γG Subclasses observed among Families with Hypogammaglobulinaemia

SEVERAL research units have studied the genetic mechanisms controlling the synthesis of immunoglobulins and its alteration in hypogammaglobulinaemia. Fudenberg *et al.*[1] investigated Gm and Inv groups in families with agammaglobulinaemia, and suggested the involvement of two genes in the synthesis of an immunoglobulin: one controlling the synthesis of the heavy chains, the other that of the light chains. Burtin *et al.*[2] applied the same principle in their studies on "non sex-linked atypic hypogammaglobulinaemia", and postulated the existence of abnormal alleles leading to an impaired synthesis of the heavy chains of one or several immunoglobulins.

Since then, the heavy chains of γG have been subdivided into four subclasses: γG1, γG2, γG3 and γG4[3,4]. The Gm factors were located on these subclasses as follows: the γG1 carry Gm factors 1, 2, 4, 8, 9, 17, 18 and 22; the γG2 carry factors Gm 8 and 23. The Gm factors 5, 6, 10, 11, 14, 15, 16 and 21 are located on the heavy γG3 chains. So far the γG4 have not been assigned a genetic marker. Independent genetic control of the synthesis of each γG subclass could be determined by a study of these Gm groups in families with one or several cases of immunoglobulin deficiency. Previously[5] we have shown a selective lack of some subclasses of γG in apparently healthy members of a family with two cases of

Table 1. Gm PHENOTYPES, INHIBITION TITRES AND IMMUNOGLOBULIN LEVELS IN MEMBERS OF THE B 492 FAMILY

Family relationship	γG1 Gm (1,	γG1 2,	γG1 17)	Gm (4)	γG2 γG1 Gm (8)	γG3 Gm (5)	γG3 Gm (21)	Immunoglobulin levels (mg/ml.) γG	γA	γM
Control	1/512	1/512	1/2,048	–	–	–	1/512			
Control	–	–	–	1/128	1/64	1/512	–			
II 3 Pat. aunt	+	+	+	+	1/4	1/2	1/8	1,030	251	35
II 4 Pat. aunt	+	–	+	+	1/4	1/32	1/16	823	123	109
II 5 Pat. aunt	+	–	+	+	1/8	1/8	1/4	1,030	303	159
II a Mother	–	–	–	1/4	1/8	1/64	–	167	23	31
II b Mat. uncle	–	–	–	+	1/8	1/2	–	978	209	86
II c Mat. uncle	–	–	–	+	1/2	1/2	–	1,103	251	95
II d Mat. uncle	–	–	–	+	1/4	1/32	–	843	211	99
II e Mat. aunt	–	–	–	+	+	+	–	930	204	113
III 1 Pat. cousin	–	–	–	+	+	+	–	797	207	54
III 2 Pat. cousin	+	+	+	+	+	+	+	1,200	266	77
III 5 Pat. cousin	+	+	+	–	–	–	+	1,210	229	83
III 6 Brother	–	–	–	1/16	+	1/8	–	1,066	210	92
III 8 Proband	1/16	–	1/64	1/8	–	1/8	1/4	29	0	0
III a Mat. cousin	–	–	–	+	1/4	1/4	–	533	40	35
III b Mat. cousin	–	–	–	+	+	+	–	1,007	90	85
III d Mat. cousin	–	–	–	+	1/4	1/32	–	741	142	86
IV 1 Pat. sec. cousin	+	–	+	+	+	+	+	1,323	106	15
IV 2 Pat. sec. cousin	–	–	–	+	+	+	–	1,040	134	21
IV 4 Pat. sec. cousin	–	–	–	+	–	+	–	853	149	41
IV 5 Pat. sec. cousin	+	+	+	–	–	–	+	1,250	181	88

–, No inhibition. +, The inhibiting power is similar to that of the control serum.

Table 2. Gm PHENOTYPES, PROBABLE Gm STRUCTURE GENES AND REGULATORY GENES IN FAMILY U 204

Family member	Gm phenotypes	Unusual structure; gene alternative	Regulatory gene alternative: Structural genes	Regulatory genes
Father 2,392	Gm (1, –2, 4, 5, 8, 17, 21)	$Gm^{1,17,21}/Gm^{4,5,8}$	$Gm^{1,17,21}/Gm^{4,5,8}$	*G1, G2, G3*
Mother 2,393	Gm (1, 2, –4, 5*, 8*, 17, –21)	$Gm^{1,2}/Gm^{5,8}$	$Gm^{1,2,17,21}/Gm^{4,5,8}$	*G1, g1, G2, g2, G3, g3*
Children 2,394 II–1	Gm (1*, 2*, 4*, 5*, 8*, 17*, –21)	$Gm^{1,2,17}/Gm^{4,5,8}$	$Gm^{1,2,17,21}/Gm^{4,5,8}$	*G1, g1, G2, g2, G3, g3*
2,395 II–2	Gm (1, 2, 4*, 5*, 8*, 17, –21)	$Gm^{1,2,17}/Gm^{4,5,8}$	$Gm^{1,2,17,21}/Gm^{4,5,8}$	*G1, g1, G2, g2, G3, g3*
2,396 II–3	Gm (1, –2, 4, 5, 8, 17, 21)	$Gm^{1,17,21}/Gm^{4,5,8}$	$Gm^{1,17,21}/Gm^{4,5,8}$	*G1, G2, G3*

* Weakened inhibiting power as compared with the control serum.

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