to develop a characteristic picture of long-term effects. Certainly, other factors than mere storage in the fat determine the toxicity of compounds ingested over long periods of time. However, we believe that here we have an example of a parallelism between gross signs of chronic toxicity and tissue accumulation.

Summary. 1. The alpha, beta, gamma and delta isomers of benzene hexachloride are preferentially stored in the adipose tissue of rats and dogs. 2. Some storage of benzene hexachloride is found in kidneys, brain, liver and muscle tissue of rats. 3. Dogs show considerable storage of the alpha, beta and gamma isomers in the adrenals. 4. The degree of accumulation in the adipose tissue bears a direct relationship to the chronic toxicity of the isomers. 5. The relative rates of accumulation in rat adipose tissue show that the approximate maximum storage is accomplished within 6 weeks. 6. Upon removal of the benzene hexachloride diet, alpha, gamma and delta isomers disappear from rat fat depots within 3 weeks; the beta still persists in small amounts after 14 weeks.

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Amino Acid Composition of Dentin Protein.* (18632)

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The nature of the protein in the enamel of human teeth has been studied by Losee and Hess(1), Losee, Neidig, and Hess(2), and Block, Horwitt, and Bolling(3). From these investigations it appears that enamel protein is an eukeratin according to the criteria of Block. Block *et al.*(3) have also investigated the nature of human dentin protein by the technic of paper chromatography applied to the hydrolysate of dentin protein prepared by us and, although their results were qualitative, they have classified it as a collagen. Using quantitative colorimetric, chromatographic, and microbiological methods we have determined a number of the amino acids present in dentin protein.

Experimental. The protein was prepared from dentin ground from the fully erupted, permanent, non-carious first and second human molars used for the isolation of enamel protein(1). The teeth were cut at the cementoenamel junction and only the crown was employed. The ground dentin was dried in a desiccator, over anhydrous calcium chloride, for 48 hours and then demineralized by the method used to prepare enamel protein(1). The isolated protein was dried in the same manner as the original dentin. The yield of dentin protein obtained in several separate experiments was between 10 and 11 percent based upon the dry weight of the dentin. The sample of dentin protein used for the various determinations of the amino acids contained 2.06% ash and 16.09% nitrogen calculated upon the weight of the desiccator dried sample. Three methods of analysis were employed: colorimetric procedures applied directly to an acid hydrolysate of the protein, colorimetric procedures upon eluates resulting from chromatographic separation of the amino acids from the hydrolysate

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by the method of Bergdoll and Doty(4) as modified by us(2) using a mixture of Lloyd's reagent and Hyflo Super-Cel and also microbiological assay. The protein was hydrolyzed by heating with 20% hydrochloric acid for 6 hours in a wax bath maintained at 125°. The hydrolysate was evaporated to dryness under reduced pressure to remove the hydrochloric acid and made to a definite volume with distilled water.

Phenylalanine, cystine, methionine, and tryptophane were determined by the method of Hess and Sullivan(5-7). For the estimation of hydroxyproline the procedure of McFarlane and Guest(8) was employed. After the determinations by this method had been made the modification of Neuman and Logan (9) appeared and it was applied to one hydrolysate with the same result as originally obtained. Histidine, arginine, and lysine, the basic amino acids, were determined by the procedures of Macpherson(10), Thomas, Ingalls, and Luck(11), and Bergdoll and Doty(4) respectively. The periodic acid methods of Nicolet and Shinn(12) and Shinn and Nicolet(13) for the determination of serine and threonine were employed. The value for serine has been corrected for the presence of 1.1% hydroxylysine based upon the value obtained in collagen by Rees(14). Glycine and alanine were estimated by the methods of Alexander, Landwehr, and Seligman(15) and Alexander and Seligman(16) respectively. Tyrosine was determined by the method of Folin and Marenzi(17). The microbiological assays for arginine, histidine, and lysine were carried out at the National Institutes of Health by the methods of Stokes, Gunness, Dwyer, and Caswell(18). The results are given in Table I together with a comparison of some literature values upon gelatin and collagen. The values are based upon the weight of the desiccator dried sample and are not corrected for moisture or ash. Each value represents the average of at least duplicate determinations upon two different hydrolysates.

Discussion. The data indicate that the 13 amino acids thus far determined in dentin protein conform, in general, to the pattern of these same amino acids in collagen and gelatin. In addition to the literature values cited in Table I, which were selected because they represent series of determinations upon the same proteins by the several investigators, other values for individual amino acids may be mentioned. Macpherson(10) found 8.57% arginine in collagen by colorimetric methods and 4.47% lysine determined by the difference between the total basic nitrogen and the nitrogen of the combined arginine and histidine. Salo(22) found that the arginine content of collagens from various sources varied from 8.61 to 9.55% and the histidine content from 0.70 to 1.09%.

The large amount of hydroxyproline, 13%, is in accord with the value of 14.4% found by Bergmann(23) in a sample of gelatin by

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TABLE I. Amino Acid Content of Dentin Protein.

	Method of determination			Literature values			
Amino acid	Color, %	Bergdoll and Doty chromat., %	Microbiol., %	Bowes and Kenten(19) collagen, %	Graham *et al.*(20) Gelatin, %	Collagen, %	Neuman(21) collegan, %
Lysine		2.4	3.34	4.5	4.1	3.5	4.9 – 5.7
Arginine	7.4	7.5	6.91	8.8	8.0	7.0	8.6 – 9.4
Histidine	.7	.8	.8	.8	.8	.9	.56– .94
Hydroxyproline	12.9*	13.1		14.0			
Phenylalanine	1.9			4.2	2.2	2.5	2.3 – 2.6
Methionine	.5			.8	1.0	.9	.69– 2.3
Cystine	.1			.0	.07	.3	.05– .12
Tryptophan	.0			.0			.0
Serine	3.1			3.4			2.8 – 3.2
Threonine	1.9			2.4	1.9	2.5	
Glycine	19.0			26.2			23.3 –29
Alanine	8.5			9.5			8.7 –10
Tyrosine	1.1			1.4	.44	.9	.86– 1.06
Nitrogen	16.09			18.6	17.6	16.9	17.8 –18.4

* 13.0 by the method of Neuman and Logan(9).

isolation methods. Similar values were obtained by McFarlane and Guest(8) and more recently by Neuman and Logan(9), although no values for this amino acid in collagen itself were found in the literature. No other protein thus far analyzed has shown this high content of hydroxyproline.

Our values are upon desiccator dried samples of dentin protein, the literature values are, in most cases, upon moisture free, ash free proteins. We have observed definite signs of decomposition on drying dentin protein in an oven at 105° and have preferred to analyze the desiccator dried samples. The nitrogen value of 16.07%, uncorrected for moisture and ash, is lower than the literature values upon collagen and gelatin which are corrected for moisture and ash and range from 16.9 to 18.6%. These differences may account for our finding of 19% glycine which is lower than the cited values but, on the other hand, our value for tyrosine of 1.1% is higher than the literature values which range from 0.44 to 1.06% in gelatin and in collagen. However it is apparent that in general the pattern of amino acid composition, particularly the high concentration of hydroxyproline and glycine and also the absence of tryptophan, resembles that of collagen.

Summary. Protein prepared from the dentin removed from the crowns of noncarious, fully erupted, permanent, first and second human molars has been analyzed for lysine, arginine, histidine, hydroxyproline, phenylalanine, methionine, cystine, tryptophan, serine, threonine, glycine, alanine, and tyrosine. The amounts found of many of these 13 amino acids, particularly the values for glycine, hydroxyproline, and the absence of tryptophan suggest that dentin protein resembles collagen.

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