esting to note, have been discovered or distinguished since 1910. Dr. Krober comments that "the doubling of archeological operative categories in one working life-time is a sign of the healthy growth of Peruvian archeology."

While much of the text necessarily is technical and detailed, major problems are also discussed. Perhaps the most important recent change in regard to Peruvian pre-history is that Uhle's concept of an early and primitive fishing population is no longer accepted. This culture, represented by massive incised pottery from Ancón and Supe, has been known for many years. Similar pottery, with regional variations, is now known at many other sites both on the coast and in the mountains, including the famous ruins at Chavín. Archeologists, by demonstrating the distribution and period of this culture, have a new and valuable tool for determining cultural sequence in northern Peru, comparable to the widespread Tiahuanaco and Inca cultures.

Dr. Krober has written a clear analysis of the famous Paracas culture, which he divides into two major chronological periods. These he places immediately before and after early aspects of Nasca art. He has also discussed in detail the stone carvings of Chavín and Sechín, which have been described by Drs. Bennett and Tallo in recent publications.

In regard to absolute dates of prehistoric culture in Peru, Dr. Krober is cautious in view of the lack of specific evidence. For some years it has been customary to ascribe early cultures to the early centuries of the Christian era. The author writes, "My only feeling is that many of the suggested dates have been put needlessly far back, which of course is always more impressive." The reviewer voices his agreement with this statement, especially in the light of material unearthed in Peru in 1943 and 1944. Similar downward revisions of time estimates have been necessary in regions where knowledge of absolute chronology has made great advances, such as the southwestern United States and the Maya area.

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SPECIAL ARTICLES

THE DEGRADATION OF CYSTINE PEPTIDES BY TISSUES[1]

SEVERAL years ago Bergmann, *et al.*, suggested that amino acids might be enzymatically degraded while they are yet in peptide combination, yielding by an $\alpha\beta$ dehydrogenation the corresponding "dehydropeptide."[2] Several types of dehydropeptides were synthesized, and with the discovery that extracts of swine kidney could effect the hydrolysis of such peptides the possibility that the latter were indeed biological intermediates seemed valid.[3] The enzyme responsible for this hydrolysis was named dehydropeptidase, and it was shown to be distinct from those peptidases which act upon the peptides of the naturally occurring, saturated amino acids, *i.e.*, aminopeptidase, carboxypeptidase, etc. The significant products yielded by the action of dehydropeptidase were ammonia and the corresponding α keto acid, both derived from the dehydrogenated amino-acid moiety.[3]

The concept that amino acids in peptide combination could be enzymatically attacked was a bold and discerning stroke on Bergmann's part, for it suggested that the catabolism of proteins could begin at the polypeptide stage. Bergmann did not commit himself on what the possible natural precursors of the dehydropeptides could be. We suggest that the peptides of cystine (or cysteine) serve in this capacity, yielding by desulfuration the corresponding dehydropeptide, and our evidence follows.[4]

There is present in certain mammalian tissues an enzyme which rapidly degrades cystine (aerobically or anaerobically) to pyruvic acid, ammonia and hydrogen sulfide.[5] We have found that certain peptides of cystine were degraded to the same products under similar conditions.[6] New data on such peptides, their derivatives and related substances digested with rat liver extracts are given in Table 1. The data reveal (1) that the cystine moiety of the vulnerable peptides must have either a free amino or a free carboxyl group (thus be exopeptides), since glutathione and the diketopiperazines are not attacked; (2) that the sulfur atom in an otherwise available substrate must be free since S-benzylcysteine is not attacked; (3) that the ammonia and pyruvic acid found in digests of the vulnerable peptides such as dichloracetylcystine must have been evolved from the cystine moiety subsequent to the rupture of the peptide bond; (4) that cystine

[1] Our interest in the cystine-degrading system arose from the fact that although it is found in certain normal tissues, it is the only intracellular enzyme that we have encountered which appears to be completely absent in every kind of cancerous tissue studied, *c.f.*, J. P. Greenstein, Gibson Island Conference on Cancer, 1944.

[2] M. Bergmann, V. Schmitt and A. Miekeley, *Zeits. physiol. Chem.*, 187: 264, 1930; M. Bergmann and K. Grafe, *Zeits. physiol. Chem.*, 187: 187, 1930.

[3] M. Bergmann and H. Schleich, *Zeits. physiol. Chem.*, 205: 65, 1932 and 207: 235, 1932.

[4] J. P. Greenstein and F. M. Leuthardt, *Jour. Nat. Cancer Inst.*, 5: no. 3, 1944 (in press). Cystine and cysteine, free or in peptide form, yield identical products, and to avoid repetition we shall use only the former term.

[5] C. V. Smythe, *Jour. Biol. Chem.*, 142: 387, 1942.

[6] J. P. Greenstein, *Jour. Nat. Cancer Inst.*, 3: 491, 1943.

TABLE 1

AMMONIA AND PYRUVIC ACID DERIVED FROM VARIOUS SUBSTRATES IN RAT LIVER DIGESTS*

Substrate†	Ammonia N	Pyruvic acid
	mols × 10^6	mols × 10^6
Cystine‡	11	6
Cysteine‡	7	7
S-Benzylcysteine	0	0
Cystinyldiglycine‡	10	3
Cystinyldidiglycine‡	11	4
Diglycylcystine‡	12	6
Dichloracetylcystine‡	10	6
Cystinylcystine‡	12	5
Cysteinylcysteine‡	6	4
Bisanhydrocystinylcystine	0	0
Anhydrocysteinylcysteine	0	0
Glutathione (oxidized or reduced)	0	0
Alanylglycine	0	0
Glycylalanine	0	0
Chloracetylalanine	0	0
Chloracetyldehydroalanine	19	16
Glycyldehydroalanine	21	19

* Mixtures incubated for 2 hours at 37° C consisted of 2 cc rat liver extract equivalent to 600 mgms of tissue plus 1 cc phosphate buffer at pH 7.0 plus 1 cc substrate solution at 25×10^{-6} mols concentration. Blanks subtracted from test readings. Concentration of sulfur-containing compounds was 25×10^{-6} mols S.

† Description of substrates, etc., given in footnote 4.

‡ H_2S present.

and its peptides are degraded to a nearly equal extent in the same time interval,[4] and (5) that related peptides such as alanylglycine, glycylalanine and chloracetylalanine are not degraded under these conditions.

The products found in digests of the exocystine peptides are certainly derived subsequent to the hydrolysis of the peptide bond. If it is assumed that these peptidases, using alanylglycine, triglycine and latter degraded as usual, a puzzling dilemma arises. To hydrolyze these peptides at the rate required, the presence of an active aminopeptidase for such substrates as cystinyldiglycine, cystinyldidiglycine and diglycylcystine, and the presence of a correspondingly active carboxypeptidase for dichloracetylcystine, would be expected. When however the same liver extracts were tested by the usual titrametric procedures for these peptidases, using alanylglycine, triglycine and chloracetyltyrosine as substrates, no activity at all could be noted within the two-hour incubation period. Adherence to the idea that the cystine peptides, as such, are hydrolyzed would demand the presence in liver of an aminopeptidase and a carboxypeptidase inactive toward the classical substrates for these enzymes, and specific for cystine peptides. While this possibility can not be arbitrarily denied, we have preferred to suggest another mechanism.

It is proposed that the exocystine peptides are (I) first desulfurated to yield the corresponding αβ unsaturated dehydropeptide (peptide of dehydroalanine) plus hydrogen sulfide and sulfur, followed by (II) the hydrolysis of the dehydropeptide to the principal products of pyruvic acid and ammonia. Using dichloracetylcystine as the type for all susceptible cystine peptides, the reactions may be formulated as follows:

$$\text{(I).}\quad \underset{\text{Dichloracetylcystine}}{[ClCH_2CONHCH(COOH)CH_2S-]_2} \rightarrow 2\underset{\text{Chloracetyldehydroalanine}}{[ClCH_2CONHC(COOH)=CH_2]} + H_2S + S$$

$$\text{(II.)}\quad [ClCH_2CONHC(COOH)=CH_2] \xrightarrow{H_2O} ClCH_2COOH + NH_3 + CH_3COCOOH$$

This concept is based upon the following lines of evidence: (a) the failure of S-benzylcysteine to be degraded suggests the precedence of the desulfuration reaction; (b) by the use of chloracetyl- and glycyldehydroalanine as substrates we have observed the presence of a powerful dehydropeptidase in liver (and in other tissues) which degrades both substrates equally to pyruvic acid and ammonia (Table 1); (c) we have observed that extracts of liver, kidney and pancreas (from rats, mice, rabbits and guinea pigs) which degrade cystine peptides to the products noted also degrade dehydropeptides to the same products (except sulfur, naturally); whereas extracts of spleen, brain and muscle, as well as all types of cancerous tissues, from these species, degrade neither kind of substrate, and (d) the observation by Bergmann and Schleich[3] that glycyldehydrophenylalanylglycine was not degraded by dehydropeptidase indicates that for enzymatic susceptibility both cystine peptides and dehydropeptides must possess an exo-configuration. In extracts of liver, kidney and pancreas, the rate at which the dehydropeptides were hydrolyzed was in every case much greater than that at which the cystine peptides were degraded to the same products, suggesting that the desulfuration reaction (I) is the rate-limiting process in the degradation of the latter substrates. Dialysis of liver extracts for twenty-four hours against either running tap or distilled water results in the complete loss in such extracts of the capacity to degrade the cystine peptides, although the capacity to hydrolyze the dehydropeptides is completely retained. This indicates that the systems responsible for reactions I and II are separate entities. As far as we have gone, it would appear that the capacity to degrade cystine peptides to ammonia and pyruvic acid and the capacity to degrade dehydropeptides derivable from these cystine peptides to the same products either occur together in the same tissue, or, in another kind of tissue, do not occur at all. Digests of active tissues with susceptible cystine peptides do not stop short at reaction I with desulfuration, but in every case where hydrogen sulfide appears in the digests so also do ammonia and pyruvic acid.

Our findings may be considered to support Bergmann's original concept of peptide dehydrogenation; we have simply suggested the possible mechanism whereby this metabolic step may be effected.[7] Because

[7] The interesting observations by B. H. Nicolet, L. A. Shinn and L. J. Saidel (*Jour. Biol. Chem.*, 142: 609, 1942), that treatment of silk with alkali leads to the formation of dehydroalanine at positions in the peptide chain formerly occupied by serine, raises the question as

of the structural requirements of the cystine peptide substrates, it is suggested that the enzyme responsible for reaction I be designated "exocystine desulfurase." It may be that the exocystine desulfurase-dehydropeptidase system is involved in a detoxification mechanism. Free cystine in excess is known to produce hepatic and renal damage.[8] Peptides of cystine are more soluble than the free amino acid, and it may be advantageous for the tissues to destroy the cystine while it is in susceptible peptide form before it can accumulate to the free, largely insoluble and certainly toxic amino acid.[9]

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THE DIFFUSION CONSTANT OF PENICILLIN

USING the sintered glass membrane technique, the diffusion constant of penicillin has been measured over the pH range 4.0–8.0. To minimize errors due to the decomposition of the active material, the experiments were conducted at 0.5° C. Preliminary experiments indicated that at low temperatures the rate of decomposition of penicillin was not significantly affected by concentration at pH 7; this fact has been verified for the entire pH range covered.

The diffusion cells were similar to those described by Mouquin and Cathcart.[1] They were calibrated with 2.0M sodium chloride solutions, the value 1.27 cm² per day being taken for the diffusion constant of this substance at 25.0°.[2] Lederle penicillin (sodium salt, potency 250–300 Oxford units per mg) was employed. At pH 4.0 and 5.0, Na_2HPO_4-citric acid buffer was the solvent; for the other experiments KH_2PO_4-Na_2HPO_4 mixtures were used. The diffusion constants were calculated by means of the equation:

$$D = \frac{V_2 C_2}{tKC_1}$$

where V_2 is the volume of the cell compartment into which diffusion occurs, K is the cell constant, and C_2 and C_1 are the penicillin concentrations on the "low" and "high" sides of the membrane, respectively, at the end of time t. For experiments of short duration ($C_2 < 0.02\ C_1$) this calculation gives results nearly identical with those obtained using the more elaborate expression derived from the integration of Fick's first law.

Penicillin concentrations were estimated by a turbidimetric microbiological assay procedure, using *Staphylococcus aureus* as the test organism. This method will be described in detail in a subsequent publication.

The experimental results are summarized in Table 1. Included are the values of C_1; essentially, this figure represents the concentration difference across the membrane throughout the run. Each value for D presented is the average of at least two determinations made with different cells. The average deviation was about 2 per cent.

TABLE 1

pH	C_1 (Oxford units per ml.)	$D_{0.5°}$ (Cm² per day)
4.0	50	0.192
5.0	59	0.165
6.0	46	0.180
7.0	45	0.176
8.0	38	0.178
7.0	608	0.165

The change in D observed as the pH is decreased is probably a reflection of the conversion of the salt to the acid form. Abraham and Chain[3] have reported that penicillin appears to be a dibasic acid, characterized by "titration constants" of 2.43 and 3.5.

The last value in Table 1 is the result of an experiment performed to observe the effect of concentration upon the diffusion constant. The significance of this datum as compared with the corresponding one at lower concentration is uncertain.

Friedman and Carpenter[4] have demonstrated that the Stokes-Einstein equation (relating D to molecular radius) is valid for molecules as small as the hexoses. Applying this equation to the data at pH 7 results in the value 5.37 Å for the radius of the penicillin molecule. Assuming the density to be 1.25, the calculated molecular weight is 490. This figure is consistent with the value 510 suggested by Abraham and Chain.[3]

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to whether dehydropeptides might not also be derived under natural conditions from peptides of serine. This possibility must be borne in mind, although there is as yet no enzymatic evidence relating to the possible degradation of serine peptides. We have confirmed Smythe's findings that free serine, unlike free cystine, is not enzymatically attacked in rat liver extracts.[5]

[8] A. C. Curtis and L. H. Newburgh, *Arch. Int. Med.*, 39: 828, 1927; D. P. Earle and J. Victor, *Jour. Exp. Med.*, 73: 161, 1941; P. György and H. Goldblatt, *Jour. Exp. Med.*, 75: 355, 1942.

[9] Since this communication was submitted for publication, Dr. Max Bergmann died, untimely, at the height of his productivity and usefulness. It is my hope that this brief note may serve, even if inadequately, as a tribute to the memory of a man who was a great chemist, an unassuming personality and a warmhearted friend.—J.P.G.

[1] H. Mouquin and W. H. Cathcart, *Jour. Amer. Chem. Soc.*, 57: 1791, 1935.

[2] M. L. Anson and J. H. Northrop, *Jour. Gen. Physiol.*, 20: 575, 1937.

[3] E. P. Abraham and E. Chain, *Brit. Jour. Exp. Path.*, 23: 103, 1942.

[4] L. Friedman and P. G. Carpenter, *Jour. Am. Chem. Soc.*, 61: 1745, 1939.