LXXXVI. QUALITATIVE TESTS ON HUMAN ENAMEL PROTEIN

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IN a previous communication [Pincus, 1936] it was pointed out that enamel protein, which is of epithelial origin and is usually classed as a keratin, failed to conform to certain of the criteria for keratins. The sodium nitroprusside test for cysteine was applied to enamel protein after hydrolysis with 5 and 25 % KOH, and after hydrolysis with HCl. (Enamel protein is hydrolysed with difficulty.) A negative result was observed on most occasions, but after alkaline hydrolysis a slight positive result was obtained twice. Tiny fragments of wool, hair and hoof, after alkaline hydrolysis, readily gave a well-marked positive result with the sodium nitroprusside test. When boiled with 25 % KOH for 30 min., enamel protein showed no blackening with lead acetate, while wool, hair and hoof all readily showed a marked blackening. The findings of Rosebury [1930] with regard to the difficulty of hydrolysis of enamel protein are thus confirmed, but not the "slight darkening" which he observed with lead acetate.

These observations on enamel protein have now been extended in order to get more definite evidence as to the nature of the protein.

For the tests to be described the enamel protein was collected as before, by decalcifying the enamel with acid and washing the protein; 2 % HCl was used in place of the acetic acid used before. The teeth had not been in formalin.

The material collected in this way contained N, 12.1 % (Weiler), S, 1.2 %, ash, 8.0 % (Schoeller). There was about 2 % Ca and a small amount of fatty material. The protein gave a marked reaction for tyrosine.

Now keratins are usually regarded as proteins very rich in sulphur and particularly in cystine. Human hair contains 16.5 % cystine, sheep wool contains 10.0 % cystine, while silk fibroin which has hitherto been classed with the keratins, contains no cystine [Block & Vickery, 1931].

Block [1937] and Block & Bolling [1939] have subdivided the keratins into the eukeratins (cattle horn is the typical eukeratin), which contain histidine : lysine : arginine in the molecular ratio 1 : 4 : 12, and pseudokeratins (human skin is a typical pseudokeratin) which do not give these ratios and which are less resistant to enzymic hydrolysis than the former.

While it would be desirable to ascertain how enamel protein compares with keratins in respect to its amino-acids, such examination appears at present impracticable because of the small quantities of material available.

A more profitable line of investigation, suggested by Dr M. M. Murray and Dr Neuberger, appeared to be that followed by Goddard & Michaelis [1934]. Two tests were applied: (1) to compare the solubility of enamel protein with that of known keratins, e.g. hair, wool, horn and hoof, in solutions of Na₂S, KCN and

Na thiolacetate; (2) to test the action of trypsin and pepsin on such of these proteins as remained undissolved after they had been treated with the solutions mentioned above.

Hair and wool dissolved in 0.75 *M* Na₂S, 4 *M* KCN or 1.6 *M* Na thiolacetate in 2 days; horn and hoof took rather longer, while enamel protein was more resistant still. If all these proteins were exposed to the solutions for 24 hr., enamel protein resisted subsequent exposure to trypsin longer than did the other substances. It was previously reported that KCN attacked enamel protein; this observation is now thought to be incorrect. Limitation in the supply of enamel protein necessitates collection over a considerable time, so that such moulds as penicillia may contaminate the tiny collections of protein. It has been found that moulds, which may have attacked enamel protein, are themselves readily attacked in turn by KCN, and in this way the error probably arose. Most of the work has been carried out on pieces of material less than 3 mm. square and very thin; supply of suitable human material is limited.

It has been stated above that wool and hair dissolved in Na₂S or in Na thiolacetate in a couple of days; if horn, hoof and enamel proteins were treated with Na₂S or Na thiolacetate for 4 days, only enamel protein resisted subsequent exposure to trypsin for 24 hr.; enamel protein is eventually but slowly attacked by trypsin. Experiments with pepsin gave similar results.

Enamel protein thus appears to differ from characteristic keratins. It has a low sulphur content; it does not give a positive test for cystine. The protein does not dissolve in such reagents as Na₂S, KCN and Na thiolacetate; after exposure to these reagents it is attacked by trypsin and pepsin at a much slower rate than the typical keratins. Enamel protein is therefore unlike hair, wool, horn and hoof and yet it cannot be classed as one of Block & Vickery's pseudo-keratins, since it is more and not less resistant to enzymic digestion than the true keratins; it appears to be much more resistant than human skin. The results of the tests are set out in Table I, which also provides the summary of results.

SUMMARY OF RESULTS

Table I. *Results of tests on keratins and enamel protein*

(A) Lead acetate test			
Wool, hair, horn, hoof	All +ve	Enamel protein -ve	
(B) Sodium nitroprusside test			
Wool, hair, horn, hoof	All +ve	Enamel protein -ve	
(C) Solubility and enzymic hydrolysis			
(a) KCN	KCN	KCN	
(b) Na ₂ S	Na ₂ S	Na ₂ S	
(c) Na thiolacetate	Na thiolacetate	Na thiolacetate	
		Contact with one of the above for 4 days followed by trypsin for 24 hr.	
Contact with one of the above for 2 days	Contact with one of the above for 4 days		
Wool	Dissolves	—	—
Hair	Dissolves	—	—
Horn	Resists	Resists	Dissolves
Hoof	Resists	Resists	Dissolves
Enamel protein	Resists	Resists	Resists

This last series of tests (C) was carried out at 37°.

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