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ACID NUCLEASES IN HUMAN SKIN*

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There is abundant histochemical evidence for the presence of acid hydrolyses in human and other mammalian epidermis (1, 2, 3, 4). Many of these enzymes are of the same type that are present in lysosomes, and it has been suggested that those occurring in the epidermis are also of lysosomal origin (3, 5, 6). It was therefore thought worthwhile to search for other hydrolyses which could be considered to be of lysosomal origin. Deoxyribonuclease (DNase) and ribonuclease (RNase) both belong to this category (7) and the work reported here is the investigation of these two enzymes in human epidermis.

The earlier methods for the demonstration of deoxyribonuclease and ribonuclease as described by Daoust (8, 9) depended upon the hydrolysis of DNA or RNA contained in a thin gelatine film applied to a histological section of the tissue under investigation. The sites of activity were revealed by an absence of staining for nuclear protein by the subsequent application of toluidine blue O. There were several disadvantages to this ingenious method. First, the technique depends upon the diffusion of the enzyme into the gelatine film before it can digest the contained nuclear-protein, and this diffusion results in poor localization. Secondly, one cannot help wondering whether non-specific proteolytic enzymes might partly digest the gelatine film allowing the dissolved nuclear-protein to escape and thus giving a false positive reaction. Cathepsins are present in lysosomes and these might readily give such a false reaction. Doaust and Cantero (10) reported epidermal RNase, but stated there was little activity in the dermis.

The work of Aronson and his co-workers (11) and that of Vorbrodt (12) brought a new approach to the problem. Their method consisted in the use of an acidic aqueous solution of deoxyribonucleic acid as substrate in the presence of acid phosphatase and lead. The rationale of the technique is that the DNase of the tissue hydrolyses the DNA in the substrate with the release of dinucleotides, trinucleotides, and polynucleotides: during this part of the reaction free phosphate is not released. The acid phosphatase in the substrate then acts upon the nucleotides with the liberation of free phosphate which is then captured by the lead in the substrate and precipitated as lead phosphate at the site of reaction. The lead phosphate is subsequently visualized by its conversion to lead sulphide by ammonium sulphide. This method gives a very accurate localization and does not appear to be affected by the presence of proteolytic enzymes. In the present work this technique has been extended to include the demonstration of RNase, and this is essentially the same except that RNA is substituted for DNA in the substrate.

MATERIALS AND METHODS

In the present study the lead method for the demonstration of deoxyribonuclease was employed, as described by Aronson and his co-workers (11) and Verbrodt (12). This method is also described in detail by Thompson and Hunt (13). For convenience the constituents of the incubation medium are given below:

Deoxyribonucleic acid*	1 or 2 mg
Acid phosphatase†	5 mg.
0.2 M acetate buffer pH 5.9	12.5 ml.
0.4 M lead nitrate	0.25 ml.
Distilled water	50 ml.

* Supplied by Sigma Chemical Co., St. Louis, Missouri

† Supplied by Worthington Biochemical Corp., Freehold, N.J.

The pH of the incubating medium is critical and a variation of as little as 0.2 units is sufficient to alter the results (see below). Sections of normal human skin from the abdomen were incubated in the above medium at pH 5.4, 5.7, and 5.9. An identical incubation medium, except that ribonucleic acid† was substituted for deoxyribonucleic acid, was also used at these three different pH values.

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† Supplied by Sigma Chemical Co., St. Louis, Missouri.

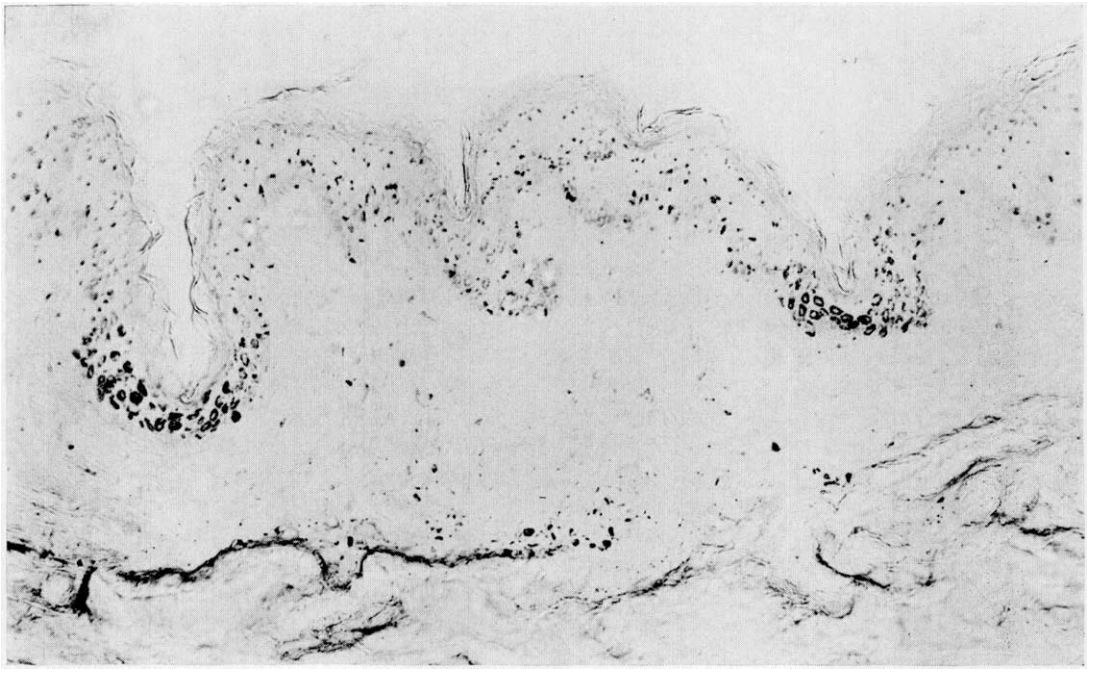

FIG. 1. *DNase at pH 5.4.* (low power). There is a marked perinuclear reaction in the cells associated with epidermal depressions. In other regions there is a punctate activity which does not appear to be related to nuclei. Note that the dermal cells do not give a positive reaction at this pH. × 100

Unfixed cryostat sections, cut at about 8 μ and mounted on cover slips, were allowed to air dry before being transferred to the incubating medium. Incubation times varied from one to twenty hours. After incubation the sections were washed in distilled water and then post-fixed for three minutes in calcium formaldehyde (Pearse) at room temperature. After this they were transferred to 1% acetic acid for one minute, and then into a weak ammonium sulphide solution for 3 minutes. The sections were finally well washed in distilled water and mounted in a water-mounting medium.

RESULTS

Deoxyribonuclease

Positive results were obtained within the pH range of 5.4 to 5.9.

pH 5.4

When sections were incubated at this pH punctate activity was detected in the epidermis. Nuclear reactions were more marked in certain sites, particularly in the epidermal depressions related to hair follicles (Fig. 1). The nuclei of dermal cells failed to give a positive reaction at this pH.

pH 5.7

At this pH the nuclei of the epidermal cells showed a markedly positive reaction in all regions of the epidermis. It is worthy of note that the nuclei of the basal cells sometimes tended to show a dense diffuse reaction whilst the reaction in higher epidermal cells was predominantly perinuclear (see RNase at pH 5.7 and Fig. 4). The nuclei of the dermal cells also showed a positive reaction and like some of the nuclei of the basal cells this was often a diffuse reaction. The granular layer failed to show any activity.

pH 5.9

Incubation at this pH gave a similar reaction as that at pH 5.7, except that all the nuclei of basal cells and the dermal cells now showed a punctate rather than a diffuse reaction. The granular layer showed only a very slight response and was virtually negative (Fig. 2).

Ribonuclease

Positive results were obtained in the pH range 5.4 to 5.9. The results were, however,

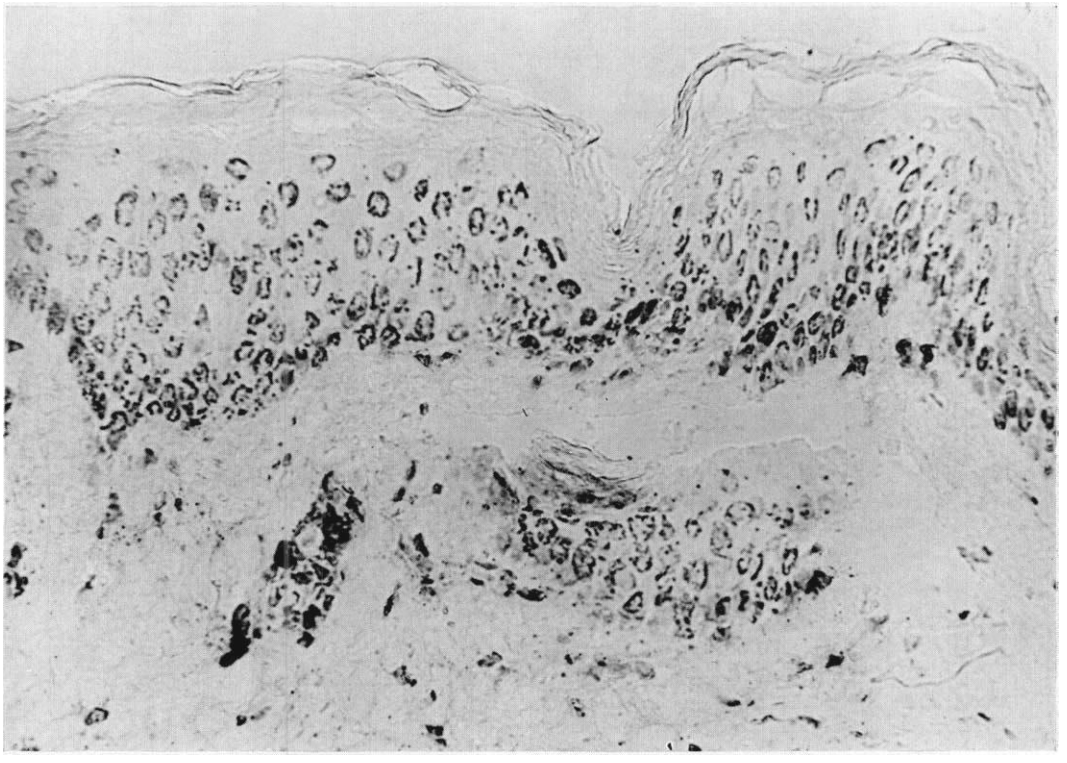

FIG. 2. *DNase at pH 5.9.* All the nuclei of the epidermal cells show a positive perinuclear reaction. Those of the dermal cells are positive and some of these show a more intense and diffuse nuclear activity. × 220

more strikingly altered by changes in pH than the previous series.

pH 5.4

At this pH only the nuclei of the basal cells and the dermal cells gave positive results. The reaction tended to be diffuse, and like the DNase reaction at this pH, there was some punctate activity in the cells of the epidermis (Fig. 3).

pH 5.7

A shift of 0.3 units of pH resulted in the nuclei of all the epidermal cells becoming positive. Those of the basal cells showed a dense diffuse reaction whilst the upper epidermal cell nuclei showed a delicate perinuclear punctate reaction (Fig. 4). The granular layer also gave a positive reaction and this was most marked in the regions around hair follicles (Fig. 4).

pH 5.9

A further shift of 0.2 units of pH resulted in a complete absence of activity in the nuclei of the cells of the basal layer and of those of the dermal cells. However, a definite positive perinuclear punctate reaction remained in the other epidermal cells (Fig. 5).

DISCUSSION

The results reported here indicate that both DNase and RNase are present in human epidermal cells. Although there is some evidence that activity may be scattered throughout the cells, the main reaction site appears to be related to the cell nucleus.

De Duve has classified both acid RNase and acid DNase as lysosomal in origin, and this is therefore additional circumstantial evidence for the existence of these bodies in human epidermis. The function of these enzymes would be to remove the nucleus and also perhaps the cellular ribosomes during the normal

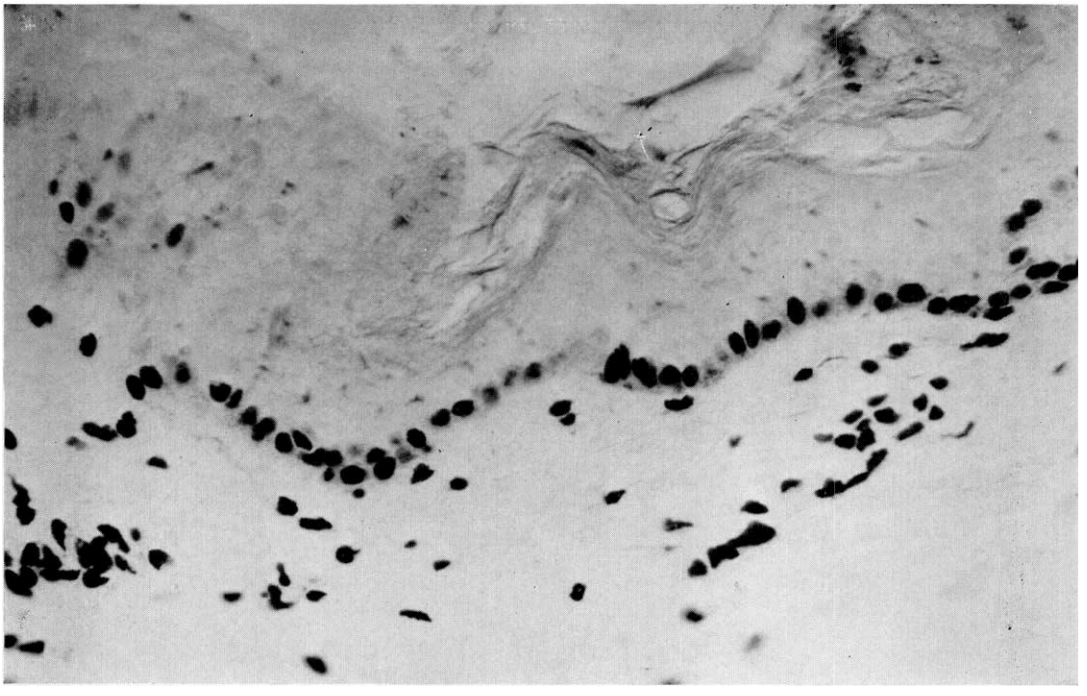

FIG. 3. *RNase at pH 5.4.* The basal cells of the epidermis all show a strong diffuse perinuclear reaction. There is a punctate reaction in the upper epidermis and it is therefore similar to the DNase reaction at pH 5.4 at sites removed from hair follicles. The dermal cells also show an identical diffuse nuclear activity. × 220

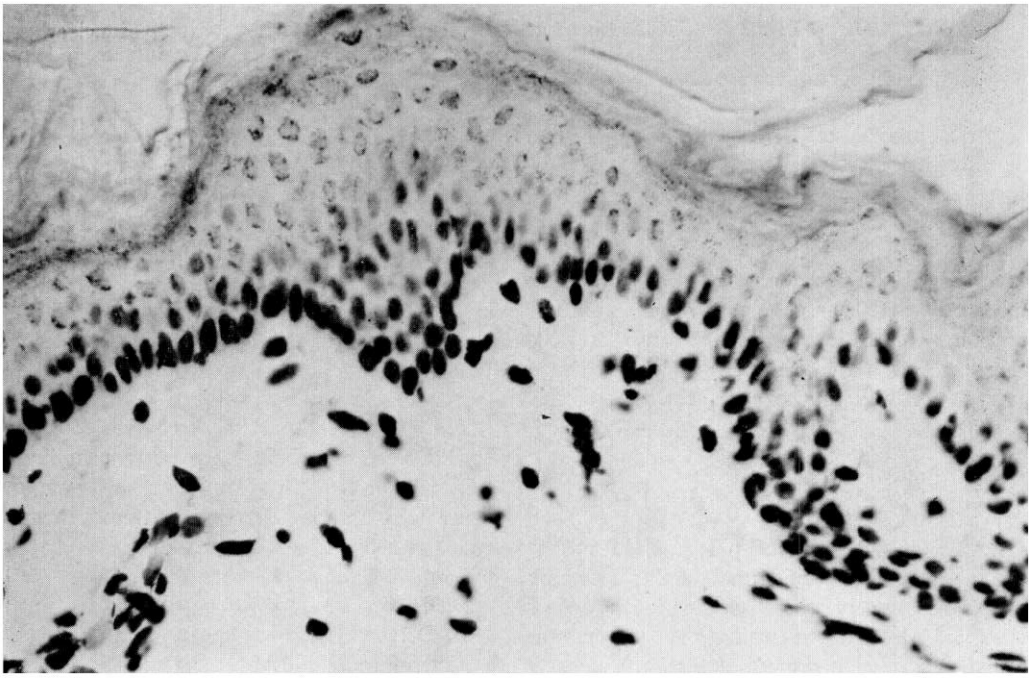

FIG. 4. *RNase at pH 5.7.* At this pH the nuclei of the cells in the upper epidermis become positive. They show a punctate perinuclear reaction which is clearly different from the diffuse activity nuclei of the basal cells and of the dermal cells. The granular layer gives a definite positive reaction. × 220

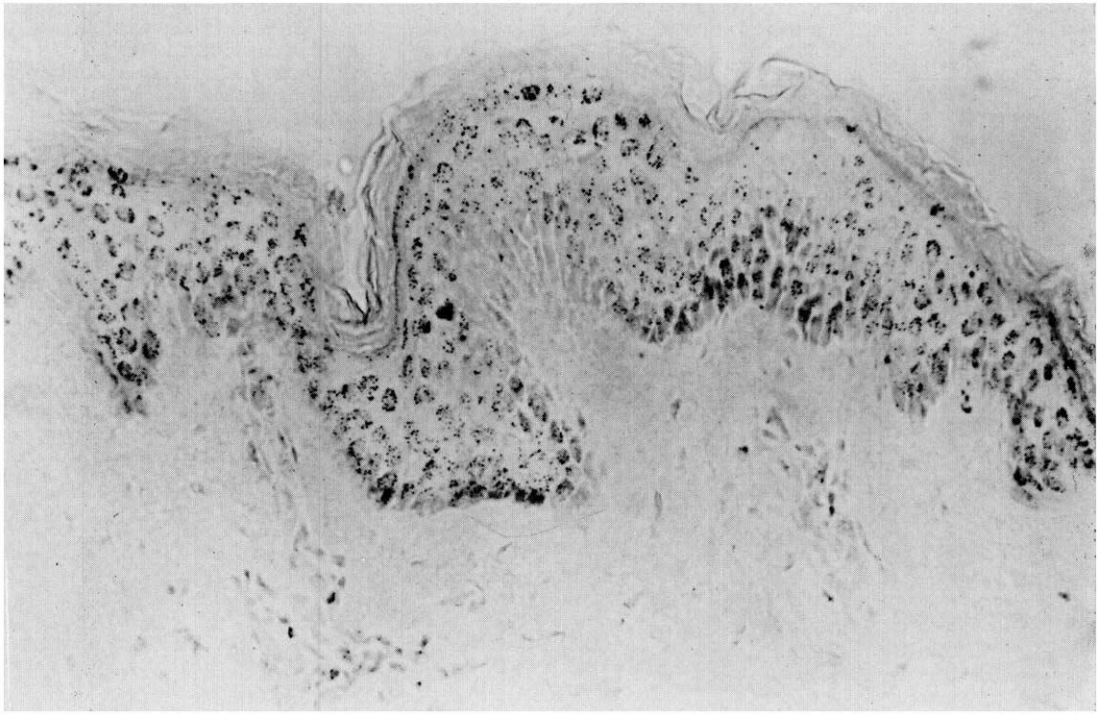

FIG. 5. *RNase pH 5.9.* The basal nuclei and those of the dermal cells fail to give a positive reaction. The higher epidermal cell nuclei, however, still show a marked perinuclear reaction. × 220

process of keratinization. In this respect it is interesting that it has been observed that there is always a high acid phosphatase reaction in the epidermal cells at the time they are losing their nuclei during the process of normal keratinization. It is, therefore, possible that the normal mechanism of nuclear removal consists of two stages. First, the nuclear acids are hydrolysed to di-, tri-, and polynucleotides by the action of RNase and DNase, and secondly these nucleotides are then further degraded by acid phosphatase. In other words, the usual manner in which the nuclei are removed from epidermal cells prior to keratinization might be analogous to the sequence of events employed in the techniques described here for the detection of deoxyribonuclease and ribonuclease.

The effects of hydrogen ion concentration. From the results reported here it is clear that these enzymes are sensitive to small changes in pH. These effects are most interesting, and indicate differences between the basal cells and those at higher levels in the epidermis. Thus, at pH 5.7 the DNase activity of the basal cells showed a diffuse nuclear reaction, whereas the higher epidermal cell nuclei had a perinuclear reaction pattern. At pH 5.9 both groups of cells gave perinuclear reactions.

In the case of RNase at pH 5.4 only the basal cells and the dermal cells showed any activity. At pH 5.7 the higher epidermal cells showed perinuclear activity, but the basal and dermal cell nuclei still showed the diffuse type of reaction. At pH 5.9 both the basal cells and the dermal cells lost their diffuse nuclear activity, whereas a perinuclear reaction was still present around the higher epidermal cell nuclei.

It will be seen, therefore, that there appears to be a similarity between the basal cells and the dermal cells in their nuclear reaction pattern of DNase and RNase. The significance of this finding is not understood but it is worthy of note, and may well warrant further investigation.

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