THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 51, No. 1
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Printed in U.S.A.

ARYL SULFATASE IN THE SEBACEOUS GLANDS OF MOUSE SKIN

A COMBINED ELECTRON MICROSCOPE AND CYTOCHEMICAL STUDY*

G. ROWDEN, M.Sc.

The contribution of hydrolytic enzymes to the events occurring in the secretion and discharge of sebaceous glands has received a considerable amount of attention in recent years. The acid hydrolases contained within lysosomes are known to play important parts in the physiological autolysis that occurs in holocrine secretion (1). Acid phosphatase and esterase have been demonstrated in sebaceous glands at the light microscope level (2). The ultrastructural investigations of Brandes and his associates have proved particularly illuminating in the implication of acid phosphatase and non-specific esterase in the sequence of events occurring in holocrine secretion of sebaceous glands (3). Examination of sebaceous glands from adult rats by means of electron microscopy and combined cytochemistry produced convincing information implicating the smooth surfaced endoplasmic reticulum and the Golgi complex with the formation of lysosomes.

Lysosomes have been demonstrated at the ultrastructural level in keratinizing epithelia in a number of different species (4, 5, see review in 6). The autolysis occurring in the upper layers of keratinized epithelia seems to be part of a system of "programmed cellular disruption" (7). A programmed partial or total cell disruption also seems to be involved in holocrine secretion in sebaceous glands.

The importance of complete characterizations of a number of different lysosomal enzymes, both at the optical and electron microscopic levels, has been stressed by a number of workers (7, 8, 9). Adequate methods are now available for ultrastructural localization of other lysosomal hydrolases besides acid phosphatase. Aryl sulfatase (10, 11) and E.600-resistant esterase (see 12) are lysosomal enzymes which are currently receiving considerable attention.

The heterogeneous nature of lysosomes is well known and it is to be expected that differential distributions of the various hydrolases may be detected by application of a number of methods for ultrastructural cytochemistry. The present report stems from an investigation of aryl sulfatase in murine keratinized epithelia (13). Some preliminary observations concerning aryl sulfatase activity in sebaceous glands have become apparent.

METHODS

Specimens of skin were obtained from the pinnae of male Swiss albino mice. Six mice were used in the cytochemical study and a further 6 as controls. Primary aldehyde fixation, subsequent washing and preparation of 50 micron frozen sections for cytochemistry were as previously reported (13). Aryl sulfatase activity was demonstrated using the incubation medium as proposed by Hugon and Borgers (12). Subsequent to incubation for periods of up to 60 minutes at 37°C, the sections were processed for electron microscopy as previously reported (13, 14).

Control unincubated blocks were used for electron microscopic determination of the normal ultrastructure of the mouse sebaceous glands. A correlated light microscopy study was performed using 20 micron frozen sections incubated in the same medium as for the ultrastructural study. In both light and electron microscopic studies control histochemical incubations were performed (13). Thin sections cut from the Araldite embedded sections were examined in an A.E.I. EM6B microscope, initially without density enhancement. After identification of sites of aryl sulfatase activity the sections were stained by the standard uranyl acetate and lead citrate methods (see 15).

Sections for light microscopy were examined under phase contrast conditions.

RESULTS

Normal Ultrastructure

The ultrastructure of the sebaceous glands of the mouse was essentially similar to the previous morphological reports of other in-

Supported by the provision of an A.E.I. EM6B electron microscope by the United Kingdom Science Research Council.

Accepted for publication March 4, 1968.

* From the Department of Biology, Royal Free Hospital School of Medicine, 8, Hunter Street, London, W.C.1.

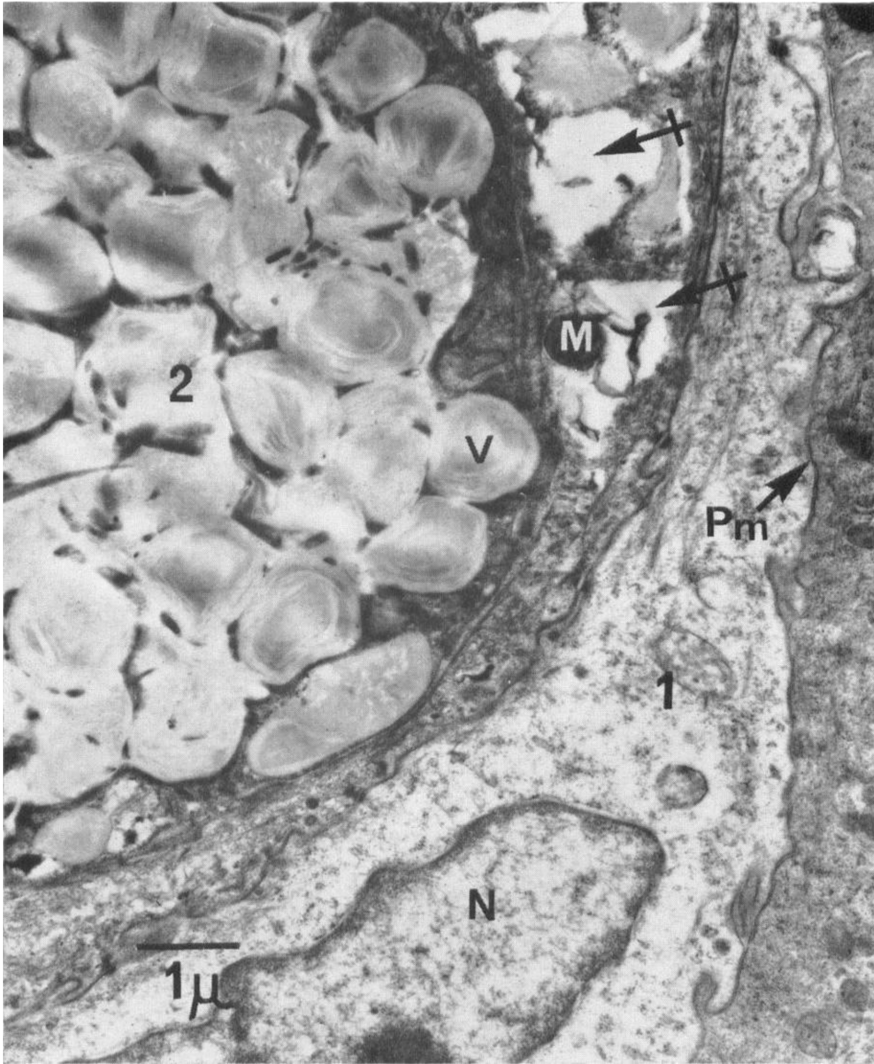

FIG. 1. Mouse sebaceous gland. Control unincubated material. Part of a sebaceous acinus. Cell 1 is a peripheral cell showing no vacuolation. Cell 2 is a fully mature cell with vacuoles (V) filling the cytoplasm. ↔ indicate the lysis and loss of structural detail occurring in a portion of a disintegrating cell. Mitochondrion (M), nucleus (N), plasma membrane (P.m.), × 13,800.

vestigations of rat (3) and human (16) material. The classification of the stages in the life history of cells in an acinus into a number of arbitrary groups was as follows: 1. peripheral undifferentiated cells lying on a basement membrane 2. differentiating cells with increasing degrees of vacuolation towards the center of the acinus 3. disintegrating cells with disrupted cell contents and pyknotic nuclei. These stages in holocrine secretion in the sebaceous gland are similar to those reported elsewhere for rat and human sebaceous glands.

Figure 1 illustrates examples of both the peripheral cell type and the fully mature disintegrating type. As lipogenesis proceeds in the cells, the number of vacuoles present in the cytoplasm increases. A marked increase in the total area of cytoplasm occupied by the agranular reticulum and the Golgi complex is correlated to the production of lipid

vacuoles (Fig. 2). Very few organelles having the morphological appearance of lysosomes were observed in the peripheral cells, but they were more numerous in the actively differentiating cells (Fig. 3).

Light Microscope Histochemistry

Figure 4 illustrates the observed pattern of reaction product after incubation for 45 minutes for aryl sulfatase activity. The bulk of the activity in the center of the glands appears to be of a diffuse nature. Punctate staining is present in all preparations and is more easily identifiable in regions of the glands that are nearer the periphery. The hair follicle shows only weak diffuse activity.

Electron Microscope Histochemistry

The reaction product (barium sulphate) of the aryl sulfatase incubation technique was finely granular and strongly electron-dense. The precipitate was evenly located over the

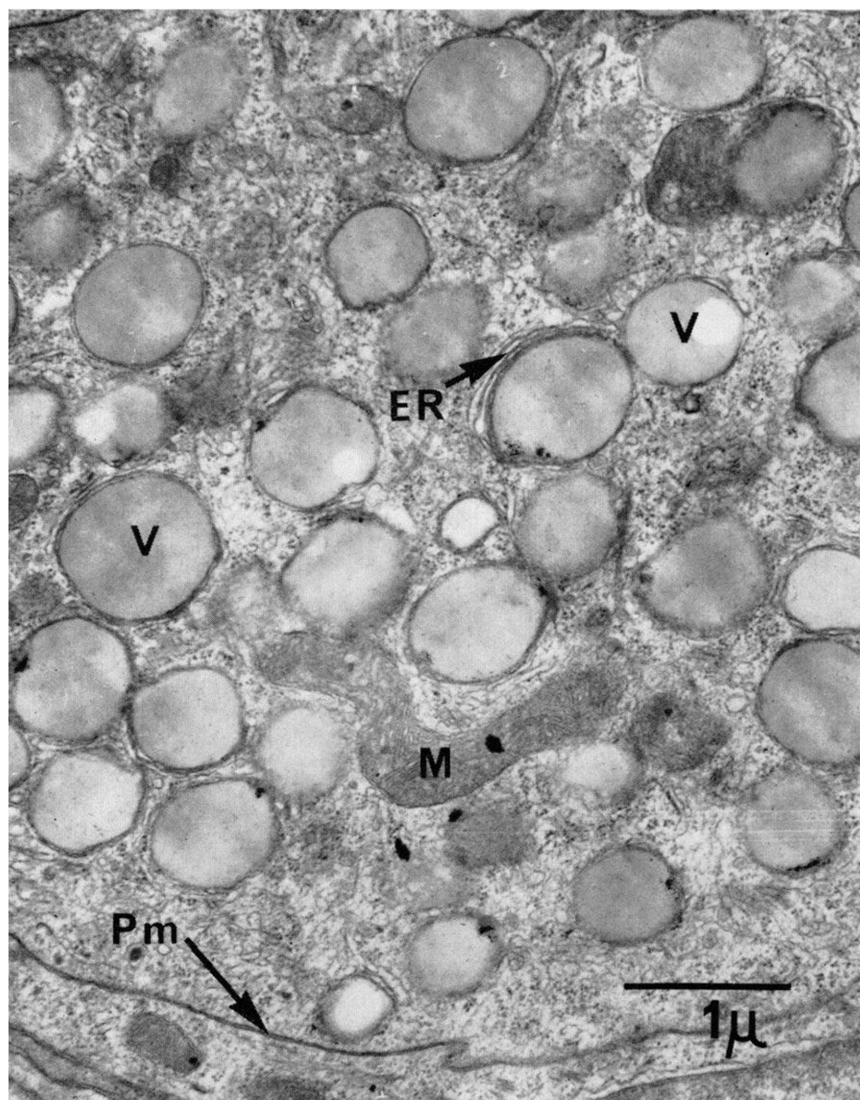

FIG. 2. Mouse sebaceous gland. Control unincubated material. Part of a differentiating cell of a sebaceous acinus. Large numbers of vacuoles (V) are present and the association of the smooth endoplasmic reticulum (E.R.) with these vacuoles is indicated. Mitochondrion (M), plasma membrane (P.m.), × 22,000.

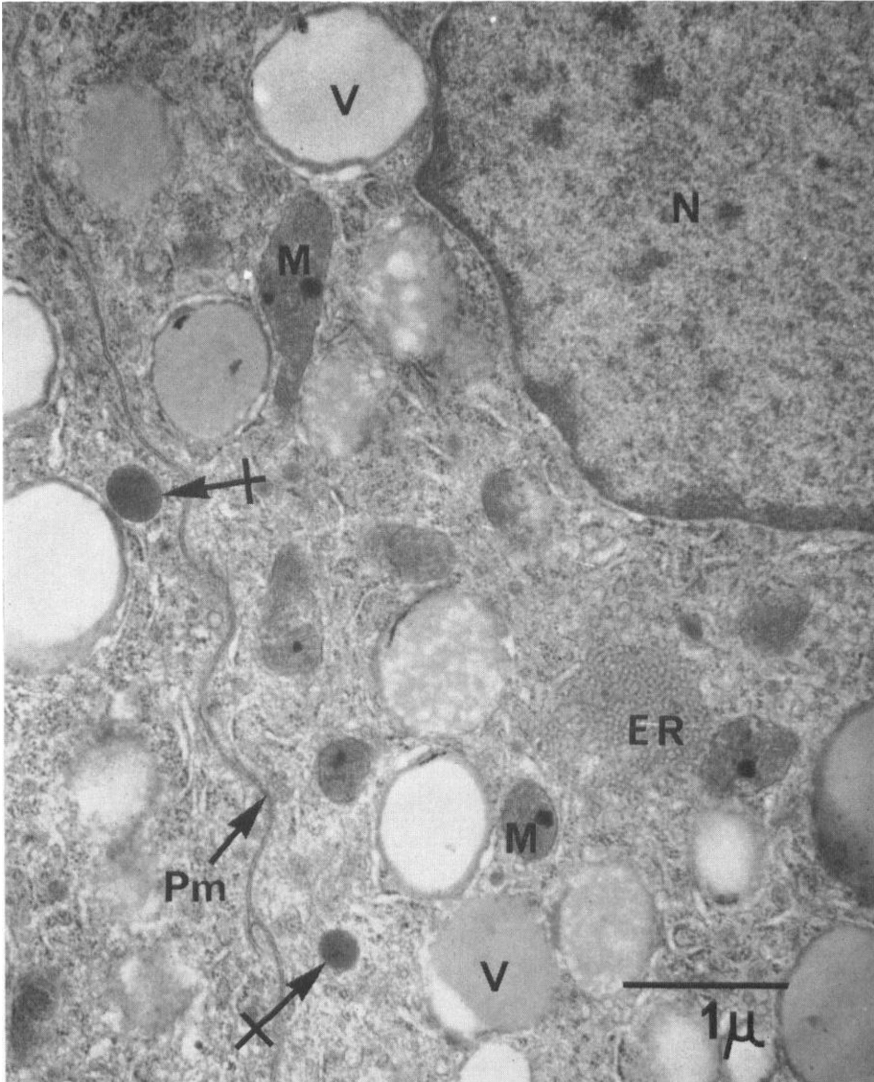

FIG. 3. Mouse sebaceous gland. Control unincubated material. Part of a differentiating cell of a sebaceous acinus showing crowded elements of the agranular reticulum (E.R.) and vacuoles (V) of various sizes. ↮ indicate organelles with the morphological appearance of lysosomes. Mitochondrion (M), nucelus (N), plasma membrane (P.m.), × 22,000.

lysosomes observed in all cases. This is in agreement with previous reports (11, 14).

Very few positively stained granules were observed in the peripheral cells. In the differentiating cells the numbers of positively stained lysosomes increased markedly. Examples are shown in Figures 5, 6, and 7. Incubation periods of 15–20 minutes produced minimal quantities of enzymatic reaction product associated with lysosomes. Thirty to forty minutes incubation produced dense deposits of the barium sulfate. Over-incubation for periods of 60 minutes produced artefactual redistribution of the reaction product, giving the impression of a diffuse component in the vicinity of the lysosomes. The mature disintegrating cells in the centre of the acinai show diffuse staining reaction after incubation periods of as little as 20 minutes. Often, the pyknotic nuclei of such cells have marked

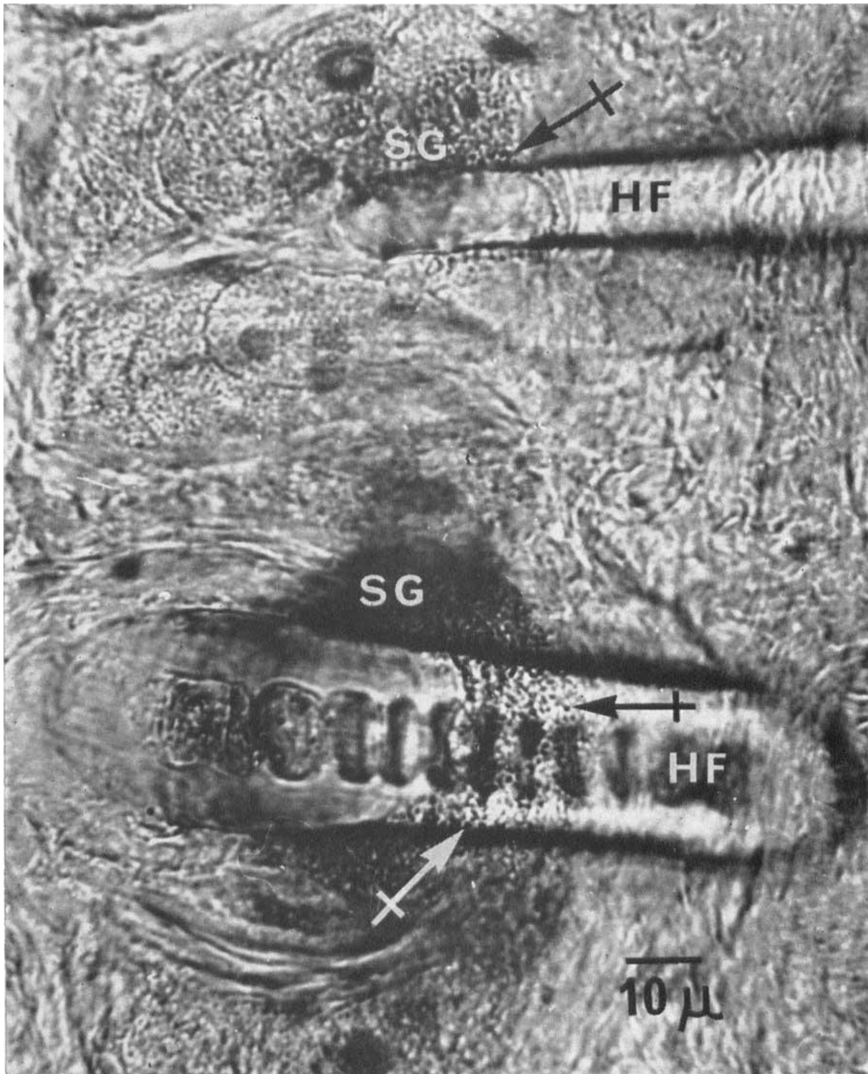

FIG. 4. Mouse skin. 10–20 micron light microscopy section incubated for aryl sulfatase activity for 45 minutes. Reaction product localization is both diffuse and granular. ↔ indicate punctate staining reaction over the sebaceous gland (S.G.) Hair follicle (H.F.) × 986.

concentrations of diffuse reaction product in their vicinity (Fig. 8). Occasionally, heavily stained lysosomes are seen in the cytoplasm of such cells after short periods of incubation. This suggests that there is either an increase in permeability of the membrane or an actual increase in enzyme content in the lysosomes. In no case was aryl sulfatase activity demonstrable in the Golgi complex or in the small vesicular component of the agranular reticulum. All control histochemical sections were negative.

DISCUSSION

The observations reported in this investigation support the contention that the acid hydrolases contained in lysosomes play an important part in holocrine secretion in sebaceous glands. Identification of aryl sulfatase

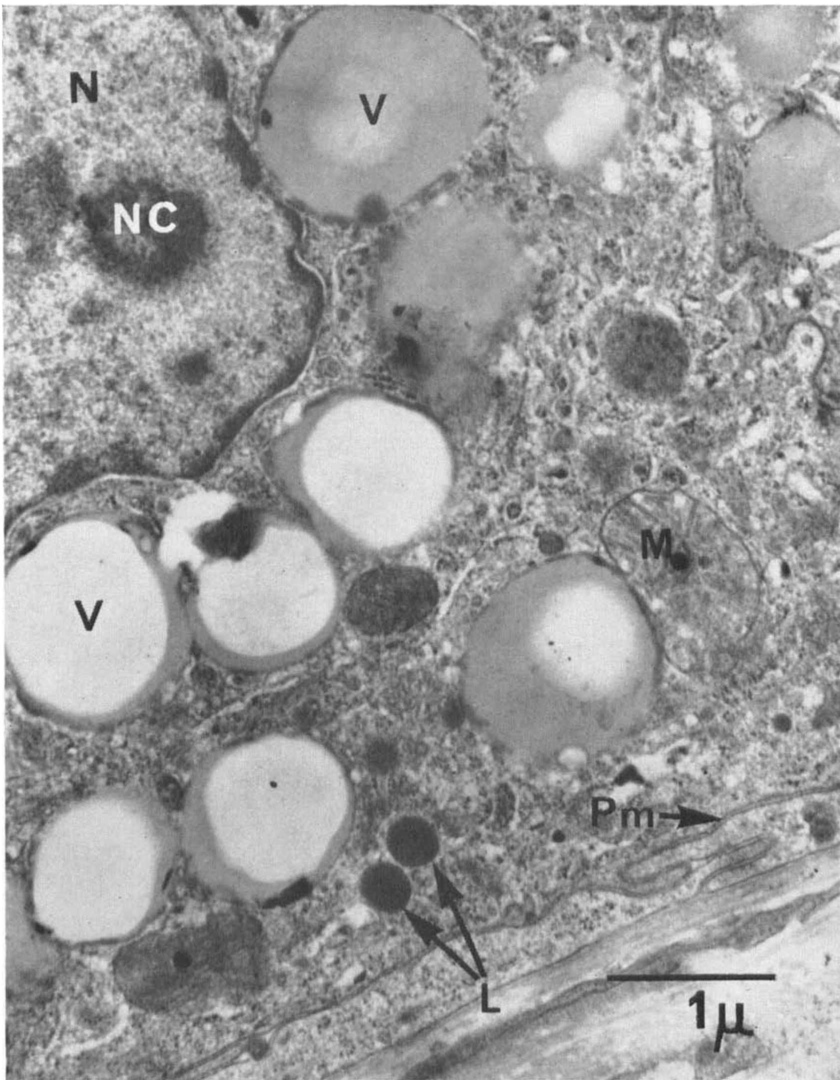

FIG. 5. Mouse sebaceous gland. Incubated for aryl sulfatase activity for 40 minutes. Part of a differentiating cell of a sebaceous acinus. Lysosomes (L) with associated electron-dense enzymatic reaction product are present. Mitochondrion (M), nucleus (N), nucleous (N.C.), plasma membrane (P.m.), vacuoles (V), × 22,000.

in organelles conforming to the accepted morphological definition of lysosomes lends weight to the previous reports concerning acid phosphatase (3). The distribution and relative abundance of aryl sulfatase-positive organelles throughout cells of the acini is essentially similar to the previous reports for acid phosphatase. There is, of course, no evidence from this report that both hydrolases are contained within the same population of lysosomes. The well known heterogeneity of components of the vacuolar system make it unlikely that all the hydrolases will be present in every lysosome at any particular time. Biochemical analysis of cell fractions will provide information concerning the differential distribution of the various hydrolases. The type of sulfatase identified by the procedure utilized in the present study is likely to be aryl sulfatase B, since the incubation medium was buffered at pH 5.2 as in the previous reports (13, 14).

It has been suggested that the release of

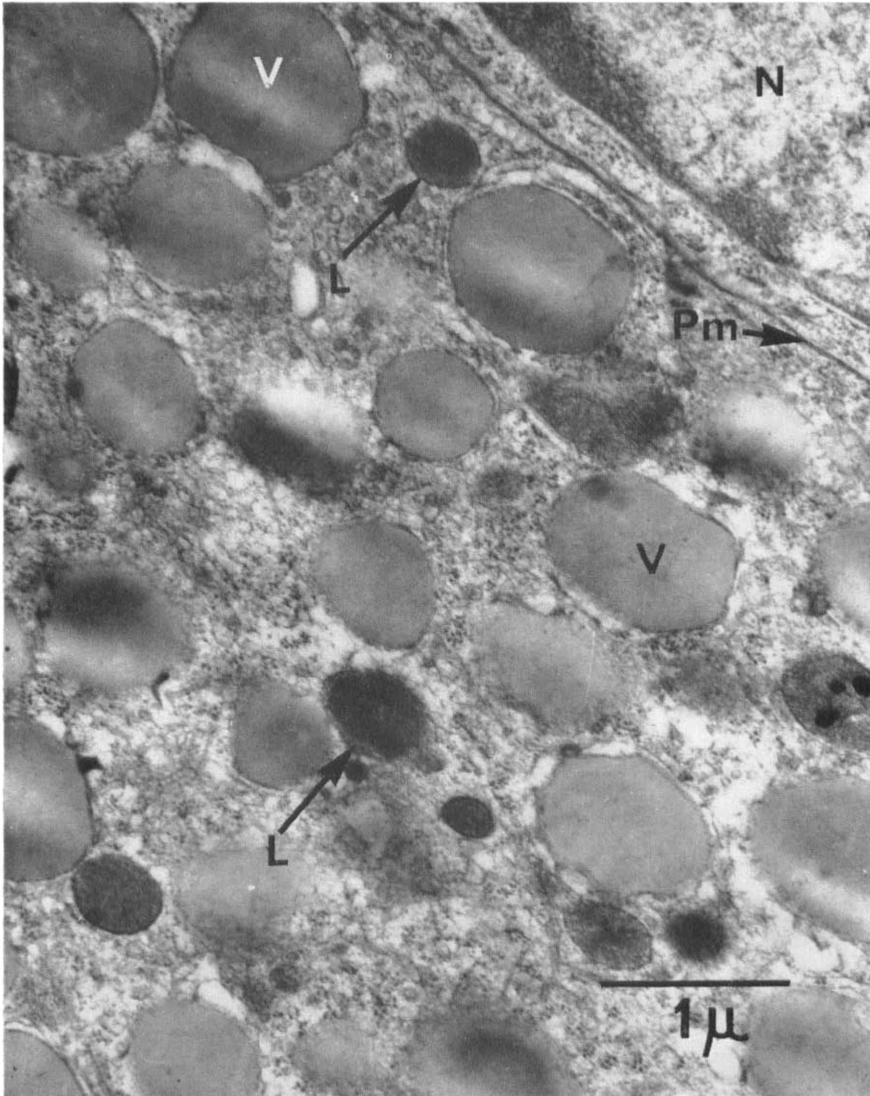

FIG. 6. Mouse sebaceous gland incubated for aryl sulfatase activity for 35 minutes. Lysosomes (L) with heavy deposits of enzymatic reaction product present in the cytoplasm of a differentiating cell which has numerous developing vacuoles (V). Nucleus (N), plasma membrane (P.m.), × 25,000.

hydrolases from lysosomes is a normal occurrence in certain physiological and pathological phenomena involving cell degradation and autolysis (17). This indeed seems to be the case in holocrine secretion of the sebaceous gland. Probably the major function of the hydrolases that are synthesized as the cells differentiate is reserved for the final autolytic stages of the life cycle. The increase in diffuse reaction product observed in degenerating cells in the present investigation indicates a marked alteration in the normal membrane delimitation of the aryl sulphatase.

In addition to the proposed involvement with autolysis, it has been suggested that the lysosomes may have an additional function to perform in the early differentiating cells of sebaceous glands. Participation in the hydrolysis of proteins to provide the basic metabolites for utilization in the synthesis of fats has

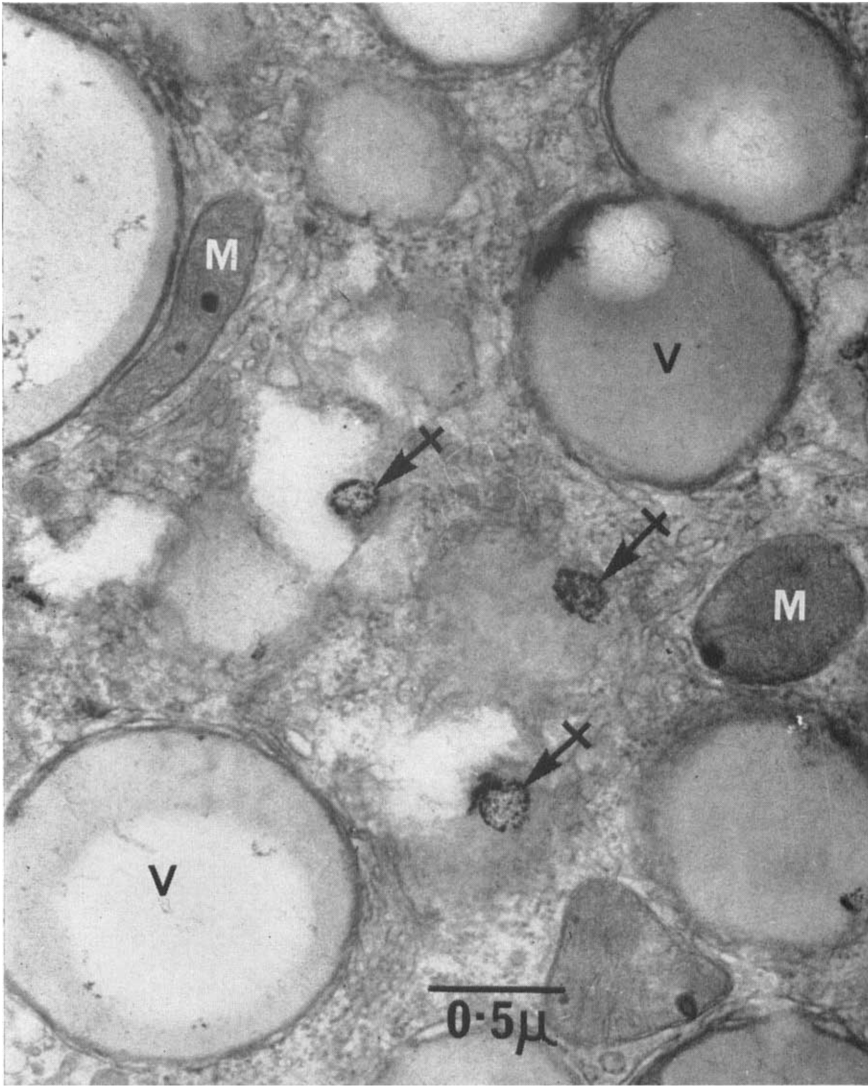

FIG. 7. Mouse sebaceous gland incubated for aryl sulfatase activity for 30 minutes. ↔ indicate lysosomes with enzymatic reaction product accumulating. The structure of the vacuolar boundaries is shown with the characteristic "husk" appearance. Mitochondrion (M), vacuole (V), × 36,000.

been postulated (3). There were, however, very few observations of large or small autophagic vacuolar structures in the present study in the early differentiating cell stage. This may suggest that autophagy of cellular components plays a minor role in the provisions of basic metabolites for lipogenesis. An alternative hypothesis suggests that lysosomes may have a function in sebaceous glands in connection with detoxification during sebum lipid synthesis (18). This involves inactivation of potentially toxic esters of endogenous and exogenous origin.

The functional involvement of lysosomes with the early stages of lipid secretion requires further investigation if the significance of this section of the life cycle of the components of the "vacuolar apparatus" is to be made clear. In addition, the present study provides no new information regarding the site of synthesis of the hydrolases present in lysosomes. The lack of demonstrable aryl

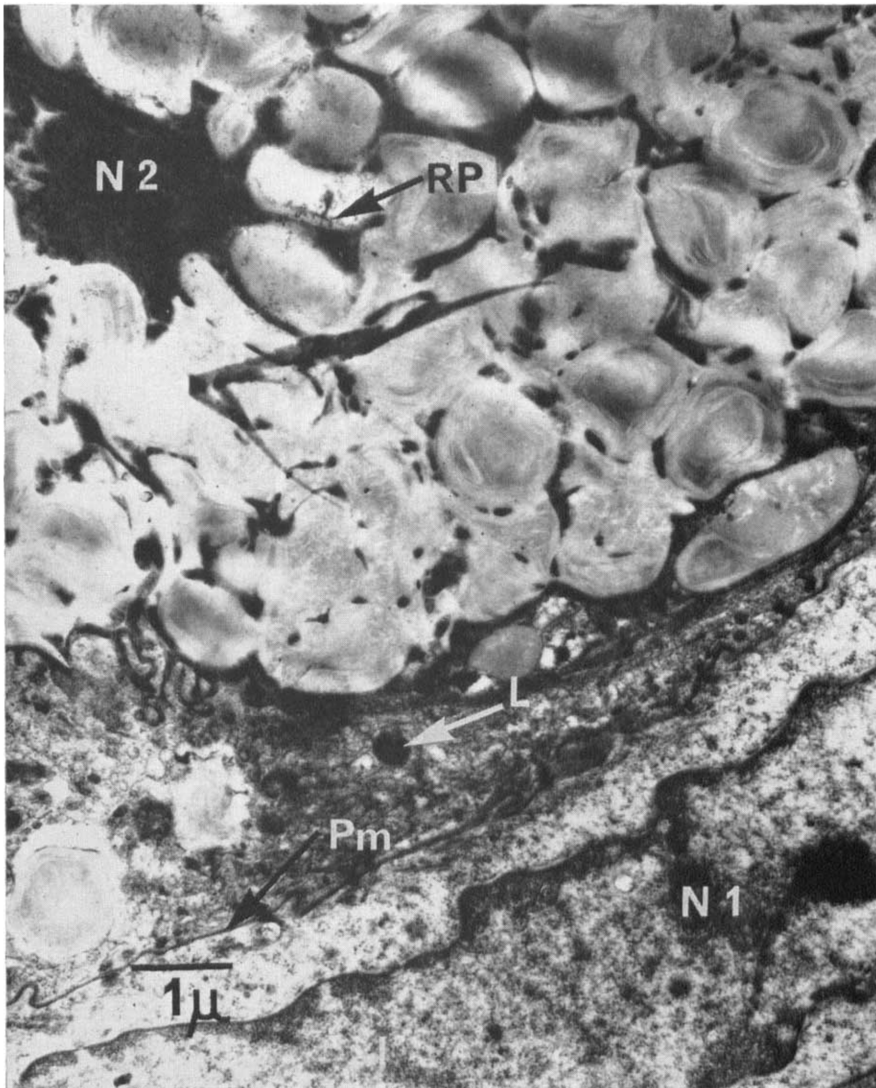

FIG. 8. Mouse sebaceous gland incubated for aryl sulfatase activity for 20 minutes. Part of a disintegrating cell of a sebaceous acinus is shown. Enzymatic reaction product (R.P.) is of a diffuse distribution in the moribund cell, and heavy deposits are present associated with the pyknotic nucleus (N 2). A lysosome (L) in an adjacent cell shows a strong positive reaction. Nucleus of a peripheral cell (N 1), plasma membrane (P.m.), × 13,800.

sulphatase activity in the Golgi complex is unexpected when compared to the results of previous workers with acid phosphatase (3). It may be that the present method for demonstration of aryl sulfatase is insufficiently accurate to detect small quantities of enzyme, or alternatively, that the fixation procedure inhibited the enzyme if it was present in the Golgi complex. The number of reports implicating the Golgi complex with synthesis of acid hydrolases and in particular acid phosphatase is increasing rapidly (1, 8, 9, 19) and it is hoped that further investigation of the present material may provide an explanation for the lack of demonstrable aryl sulfatase activity.

SUMMARY

1. The sites of aryl sulfatase activity were examined in the sebaceous glands of mouse skin

using combined cytochemistry and electron microscopy.

2. Examination of control material revealed the various stages in holocrine secretion. Three arbitrary divisions were proposed to characterize the significant cell types. These were as follows:

a) peripheral undifferentiated cells lying on a basement membrane

b) differentiating cells with increasing degrees of vacuolation towards the center of the acinus

c) disintegrating cells with disrupted cell contents and pyknotic nuclei

3. Aryl sulphatase activity was demonstrated in membrane-limited organelles corresponding to the morphological appearance of lysosomes.

4. The lysosomes showed a gradient in their abundance from the periphery towards the centre of the acini.

5. An increase in the numbers of lysosomes, concomitant with the differentiation of cell contents involved in lipogenesis was observed.

6. Moribund or disintegrating cells had very few lysosomes and aryl sulfatase activity appeared to be largely diffuse in such cells.

7. The implications of these observations as regards the previous speculations on the contribution of hydrolytic enzymes to the physiological autolysis occurring in holocrine secretion in sebaceous glands is discussed.

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