Jean-Louis Clement,[1] *Raoul Hagege,*[2] *Albert Le Pareux,*[1]
Jeanine Connet,[2] *and Gisèle Gastaldi*[2]

New Concepts About Hair Identification Revealed by Electron Microscope Studies

REFERENCE: Clement, J. L., Hagege, R., Le Pareux, A., Connet, J., and Gastaldi, G., **"New Concepts About Hair Identification Revealed by Electron Microscope Studies,"** *Journal of Forensic Sciences*, JFSCA, Vol. 26, No. 3, July 1981, pp. 447-458.

ABSTRACT: Current methods of hair identification are based on differential characteristics of the medulla observed with the light microscope. In this study, we propose a new method based on electron microscope observation of the medullary ultrastructure. Our studies revealed numerous new anatomical characteristics of the medulla, and a modern classification for mammalian hair identification was established.

KEYWORDS: pathology and biology, human identification, hair

The identification of hair belonging to man or animal has played an important role in numerous criminal cases; therefore, hair identification must be based on specific and well-defined characteristics. In the forensic sciences, all hair comparisons can be categorized into the following three groups:

(1) human hair to human hair,
(2) human hair to mammalian hair, and
(3) mammalian hair to mammalian hair.

Since the beginning of the century, forensic scientists have referred to the microscopic observations made by earlier authors—such as Waldeyer and Grimm [*1*] in 1884 or Lambert and Balthazard [*2*] in 1910—who based their classification of hair on the anatomical characteristics of the medulla (namely the features of its structure and the cortico/medullary ratio). These earlier classifications are usually taken into account in modern examinations although different authors have proposed various methods for hair identification. Among them, those based on the characteristics of the cross section (such as Glaister [*3*] for all mammalian hairs or Stoves [*4*] for hairs of rodents and other carnivores) must be noted. Several atlases have been published, and the latest is that of Brunner and Coman [*5*] on the hairs of Australian mammals.

In all these light microscope studies, the differential characteristics of the medulla are rather badly defined because of two main obstacles to a good observation:

1. The entire hair is illuminated by transmitted light so the inner layer (the medulla) is observed through the thickness of the cuticle and the cortex.

Received for publication 10 July 1980; revised manuscript received 28 Oct. 1980; accepted for publication 29 Oct. 1980.
[1]Biologists, Laboratoire Identité Judiciaire, Paris, France.
[2]Doctors, Morphology Department, Institut Textile de France, Paris, France.

2. The resolution and the depth of focus of the light microscope are not sufficient to observe the fine structure of the hair medulla.

Studies of the hair medulla with a light microscope show that this layer is composed of stacked and juxtaposed cells and of air chambers (the aerial vesicles), but the real form of these cells and vesicles and their relationships cannot be observed.

More recently, scientists have tried to modernize hair examination by observing the hair cuticle first with the light microscope and then with the scanning electron microscope. These studies seem promising mainly for the identification of mammalian hairs. Kassenbeck [*6*] has classified cuticle scales isolated by means of an appropriate solvent according to their form and length, and some workers are now looking for an individual human identification based on the morphologic characteristics of these cuticle scales.[3]

The results of these studies are very interesting and have answered some identification questions but until now have been, on the whole, limited because of the weak relative diversity of the cuticle. They have not provided a coherent system for hair examination that could take the place of those based on the medullary characteristics. For this reason we studied the general morphology of the medulla on longitudinal sections of hairs with the scanning electron microscope and the structural characteristics of the medullary cells on cross sections with the conventional transmission electronic microscope.

Material and Method

From a light microscope study of hairs from more than a hundred species of animals, we sorted the different kinds of medulla into some 20 different patterns. These types of medulla, already optically distinct by their shape and content, were further studied with the electron microscope. Mammalian guard hairs (awns and bristles) freshly plucked from living animals were cut at both ends (root and distal), the fat was taken off, and the hairs were treated with a 5% glutaraldehyde solution (to harden the proteins) and then washed with alcohol. Two specimens were made: one for the scanning electron microscope (SEM) and one for the transmission electron microscope (TEM).

For the SEM the hairs were stretched across the empty part of a bristol paper, stuck onto a sample holder previously covered with double-faced adhesive, and securely fixed by means of two drops of glue at the ends. The bristol paper was removed and the longitudinal splitting was made, by hand, under the binocular with a razor blade. The "gold-metalized" specimens (by cathodic pulverization) were examined with a Cambridge Mark II SEM.

For the TEM the hairs were placed in Teflon® tubes with Araldite and transversely cut with a Fibrotom®. The sections were then put into a bath of Epon 812 under weak air pressure (to fill the medulla) and recut with an ultramicrotome. The ultrathin sections were stained with uranyl acetate and lead citrate. The stained sections were carbon-coated just before they were observed with a Philips EM 400 TEM.

Background

Before presenting the results of this work it seems necessary to review the process of medulla morphogenesis. The hair bulb is composed by mass of undifferentiated cells (matrix cells) that divide themselves by successive mitosis and push the oldest ones upward; these latter form the different layers of the hair (medulla, cortex, cuticle, and inner root sheath).

Electron microscope studies on the medulla morphogenesis of laboratory animal hairs (mouse, rat, guinea pig) have shown that the cells of this layer undergo a sort of progressive degeneration: the nucleus disappears and the cytoplasm fills up with granules and small air chambers (the vacuoles) while the cell organelles (such as mitochondria) are destroyed [*7,8*].

[3]C. Orfanos, private communication, 1979.

The vacuoles gather and their number decreases while their size increases [*8*]; the granules also coalesce to form bigger masses.

Only in the mature cell do the very large aerial vesicles and the cytoplasmic remnants remain. We can thus infer that the aerial vesicles result from the coalescence of small intracytoplasmic vacuoles. The cytoplasmic remnants of the medullary cells are not made of true keratin because of their lack of sulfur (they contain only traces of cystin but they are rich in citrulline and glutamic acid [*8-11*]).

The observations on medulla morphogenesis tend to compare this layer with the inner root sheath by their chemical nature and to contrast them by the nature of their cytoplasmic remnants (granular and amorphous in the medulla, fibrous in the inner root sheath). This distinction is debatable since it is based on the study of hairs of rodents whose characteristics, as we shall see, are peculiar and cannot be generalized to all mammalian hairs.

Results

From all our observations, we can classify the different kinds of medullary cells into three main groups.

First Group

The first group comprises the medullary cells of hairs of lesser mammals that have dense cytoplasmic remnants, more or less uniform in size, which constitute a kind of "nucleus" we call "cytoplasmic nuclei." The TEM observations on the hairs of marsupials, rodents, and lagomorphs show that these nuclei apparently contain an amorphous substance (therefore confirming the previous discussion on the morphogenesis of the hairs of rodents [*7,8*]).

The cytoplasmic nuclei are alternated with aerial vesicles and the vacuolization process of the cellular cytoplasm is, in a way, polarized because the vacuoles gather in a part of the cell and leave a large part of cytoplasm undamaged.

This first group seems to correspond to a primitive stage in the mammalian hierarchy: it encompasses hairs of marsupials (Fig. 1), rodents (Fig. 2), and lagomorphs (Fig. 3). This is probably a pattern descending directly from ancestral form.

In the hairs from the insectivores (Fig. 4) and from the lesser apes (Fig. 5), it seems that the cytoplasmic nuclei begin to have an apparently organized ultrastructure: two phases are often perfectly obvious.

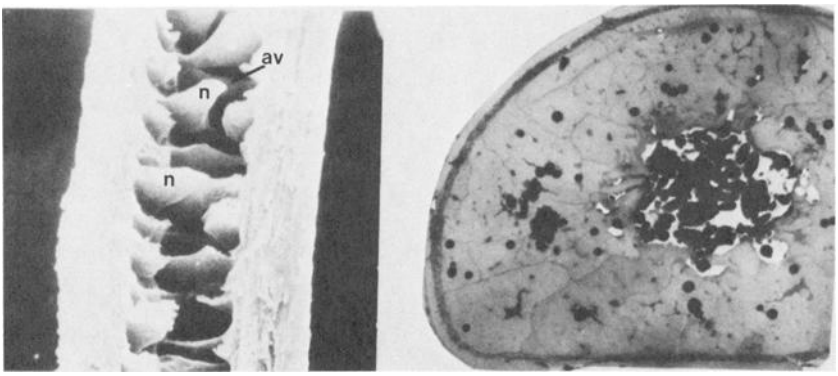

FIG. 1—*Hair from an opossum* (Didelphis marsupialis). *Note the "nuclei"* (n) *alternating with aerial vesicles* (av); (left) *SEM and* (right) *TEM.*

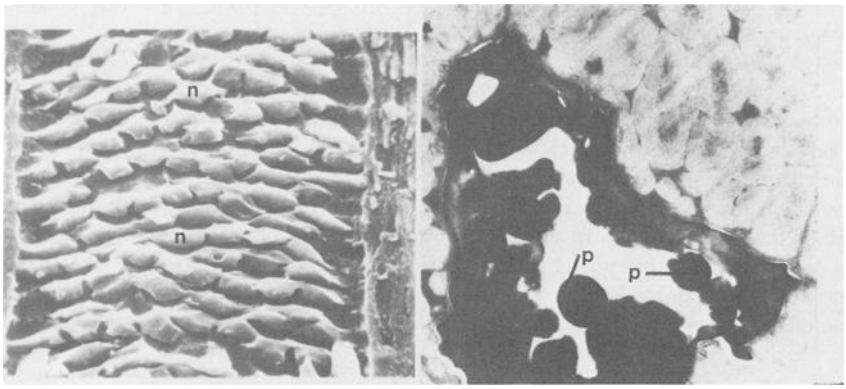

FIG. 2—*Hair from a coypu rat* (Myocastor coypus). *Note* (left) *the regularly shaped and displayed "nuclei"* (n) (SEM). *Also note* (right) *the pigment* (p) *not included in the cytoplasm of the medullary cells but lying on the surface of the aerial vesicle walls* (TEM).

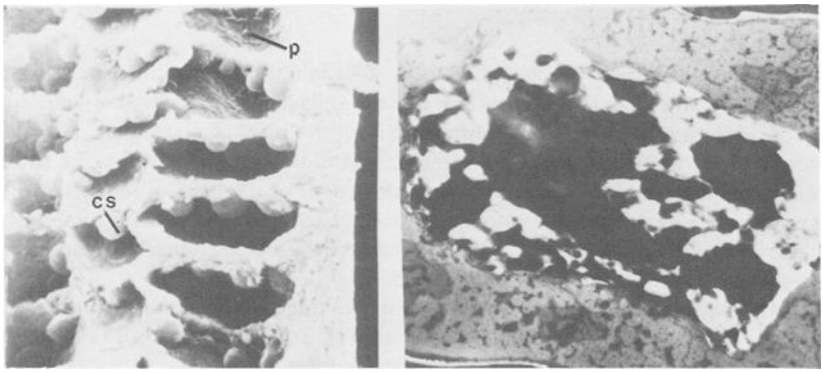

FIG. 3—*Hair from a rabbit* (Oryctolagus cuniculus). *Note* (left) *the aerial vesicle walls with small cytoplasmic spherules* (cs) *on the inferior surface (towards the bulb) whereas the other face (towards the tip) is coated with pigment* (p); (left) *SEM and* (right) *TEM.*

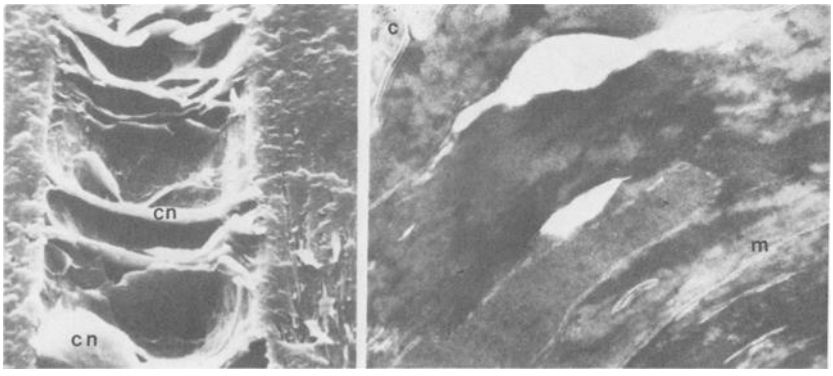

FIG. 4—*Hair from a hedgehog* (Erinaceus europaeus). *Note* (left) *the flattened "cytoplasmic nuclei* (cn) *with a flaky structure (SEM) and* (right) *another view of the flaky structure* (TEM).

Second Group

In the second group, the medullary vacuoles do not gather preferentially in a part of the cytoplasm but tend to fill it up entirely. The vacuoles make the cytoplasmic nuclei disappear (totally or partly) and their place is taken by aerial vesicles that are more or less large, have various forms, and are separated by thin cytoplasmic walls. We can subdivide this group into two subgroups.

Subgroup I—Hairs in Subgroup I are characterized by the presence of vacuoles in the wall thickness of the aerial vesicles; these all belong to carnivores. We can distinguish these:

(1) the hairs of Felidae, whose aerial vesicles are generally large and empty (Fig. 6),

(2) the hairs of Mustelidae, whose aerial vesicles are small and often septated by smaller vesicles (Fig. 7), and

(3) the hairs of Canidae, whose aerial vesicles are small and very often septated by smaller vesicles (Fig. 8).

The distinction between hairs of these three families is not easy and transitional patterns can be found.

Subgroup II—The hairs belonging to the second subgroup are characterized by medullary cells whose aerial vesicles have full walls (all the vacuoles fall into the aerial vesicles and the

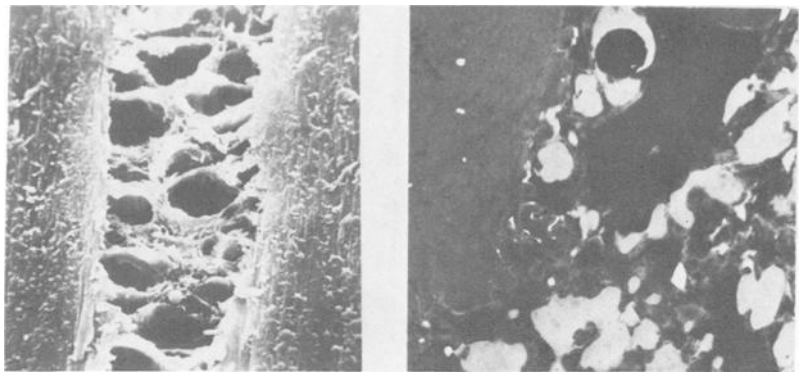

FIG. 5—*Hair from a lesser ape* (Cercopithecus aethiops). *Note the often dense medullary matter that separates the aerial vesicles;* (left) *SEM and* (right) *TEM.*

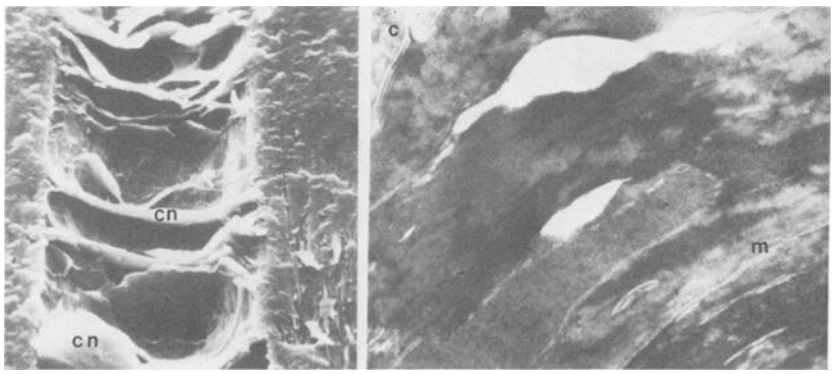

FIG. 6—*Hair from a cat* (Felis domesticus). *Note the large aerial vesicles with perforated walls;* (left) *SEM and* (right) TEM.

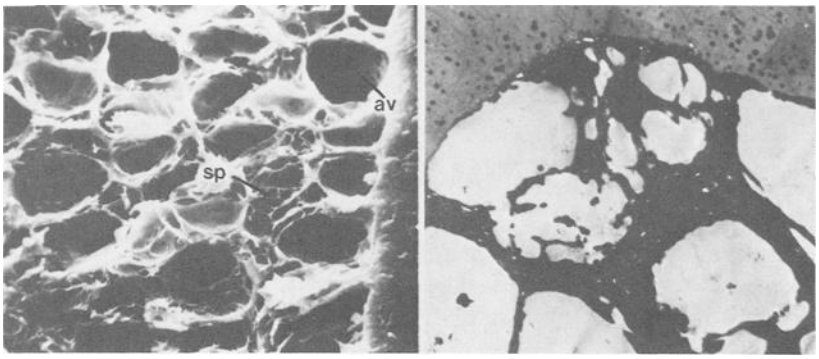

FIG. 7—*Hair from a fisher* (Martes pennanti). *Note the often septated* (sp) *aerial vesicles* (av); (left) *SEM and* (right) *TEM.*

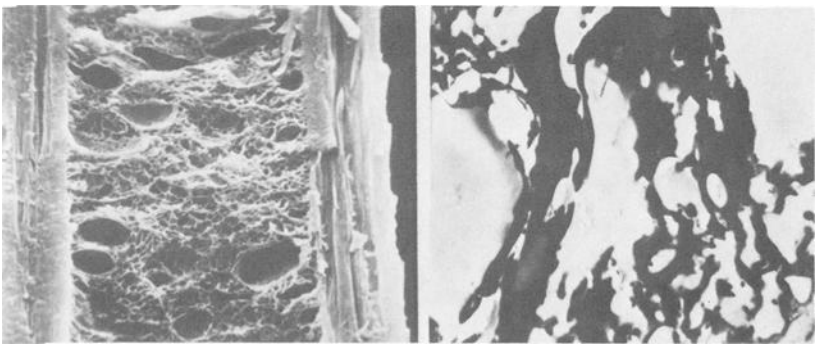

FIG. 8—*Hair from a fox* (Vulpes vulpes). *Note the very often septated aerial vesicles;* (left) *SEM and* (right) *TEM.*

walls that separate the latter are composed of cytoplasmic remnants almost without vacuoles). This characteristic can be found only in the hairs of the hoofed mammals (Ungulata).

Hairs belonging to the hoofed mammal suborder can be separated by the accessory morphological characteristics of the medulla:

1. In the hairs of perissodactyls (Fig. 9) the surface of the aerial vesicle walls is rough because of the presence of fused vacuole marks. The walls are thick but not of equal thickness (their section has no parallel edges).
2. In the hairs of most of the true-ruminant artiodactyls (Figs. 10 and 11) the surface of the walls of the aerial vesicles are smooth, of fairly regular thickness (their section has almost parallel edges), and thin. The aerial vesicles are distributed according to a regular pattern (honeycomb) in the hairs of Cervidae (Fig. 10) and a less regular pattern in the great majority of cavicorns (Fig. 11).

Third Group

The hairs in the third group have a medulla with a granular or dense aspect when observed with the light microscope. The aerial vesicles are small and the volume of the

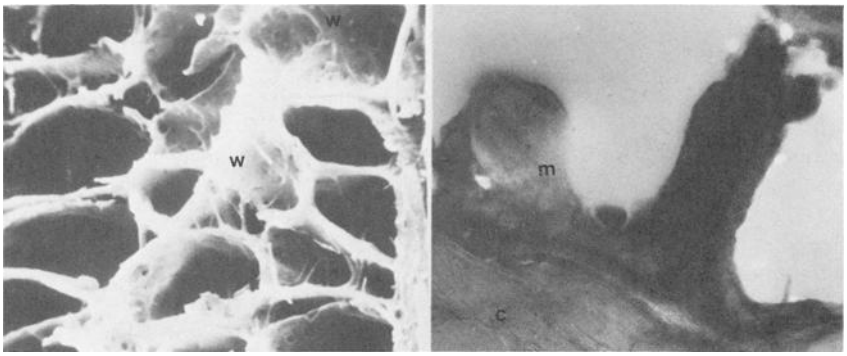

FIG. 9—*Hair from a horse* (Equus caballus). *Note the aerial vesicle walls* (w) *with a rough surface;* m = *medulla and* c = *cortex;* (left) *SEM and* (right) *TEM.*

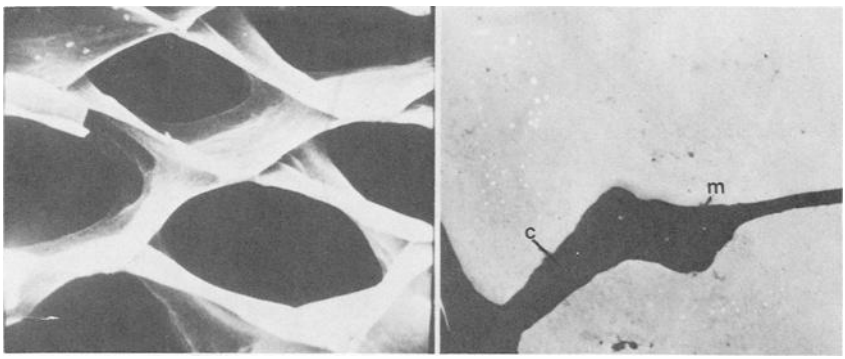

FIG. 10—*Hair from a reindeer* (Rangifer tarandus). *Note* (left) *the curious shape of the vesicle walls* (SEM) *and* (right) *the cortex* (c) *penetrating into the medulla* (m) (TEM).

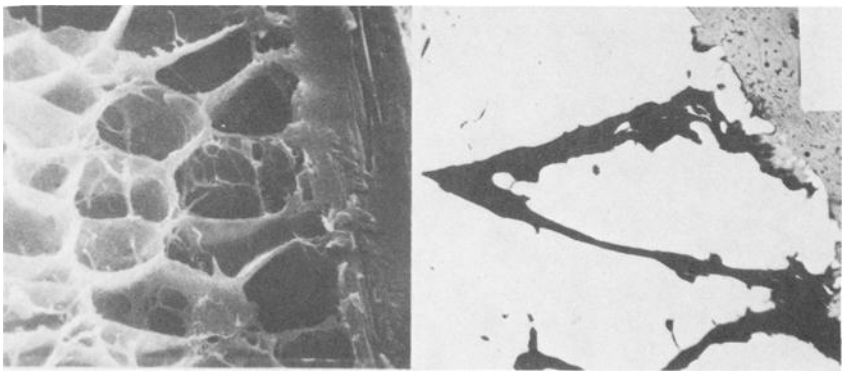

FIG. 11—*Hair from a springbok* (Antidorcas marsupialis). *The aerial vesicles are less regular than those shown in Fig. 10;* (left) *SEM and* (right) *TEM.*

cytoplasmic remnants is very important. There is a certain stricture of the medulla, which probably produces a "squeezing" of the medullary matter. We can divide this group into two subgroups:

Subgroup I—In the first subgroup, the medullary cells of the hairs have a biphasic ultrastructure and are rich in small vesicles. The medulla has a more or less filamentous structure when observed with the scanning microscope but medullary cells do not have an ultrastructure organized into microfibrils and macrofibrils (TEM). This sort of hair belongs to Camelidae (Fig. 12), to some true-ruminant artiodactyls (sheep), and to nonruminant artiodactyls, or pig-like artiodactyls (Fig. 13). The hairs of Anthropoidea (man-like apes) (Fig. 14) also have a similar medulla and must be classified in this subgroup.

Subgroup II—In the second subgroup, the medullary cells of the hairs have a microfibrillar and macrofibrillar structure like that of the cortex cells (Fig. 15), and this ultrastructure is characteristic of all human hairs without consideration of sex, race, or age. There are two main differences between the macrofibrillar ultrastructure of the medulla and that of the cortex: the medullary cells have great affinity with dyes like uranyl acetate (which means that these medullary cells are still sulfur-poor, unlike those of the cortex) and the orientation of the medullary fibrillar matter is not strictly parallel to the hair axis, unlike that of the cortex.

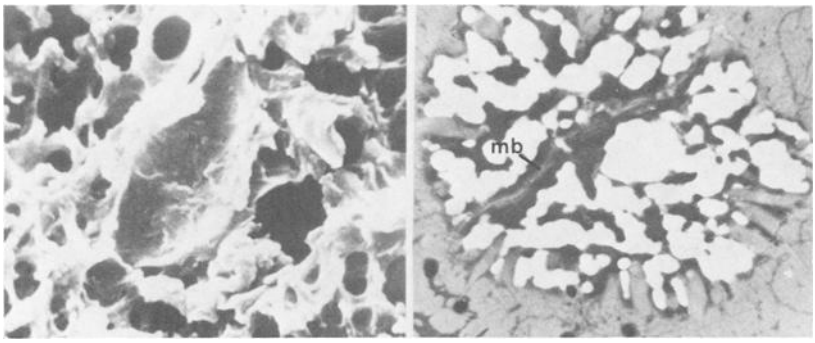

FIG. 12—*Hairs from a llama* (Lama glama huanachus). *Note* (left) *the very small aerial vesicles* (SEM) *and* (right) *the medullary cell membrane* (mb) *included in the cytoplasm* (TEM).

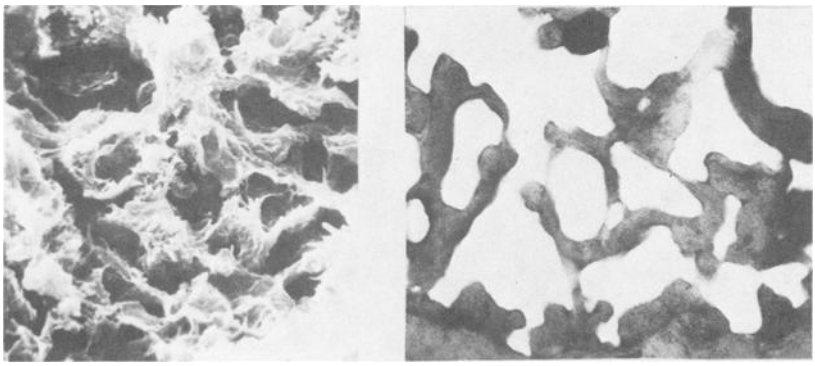

FIG. 13—*Hair from a wild boar* (Sus scrofa). *Note the dense structure of the medulla;* (left) *SEM and* (right) *TEM.*

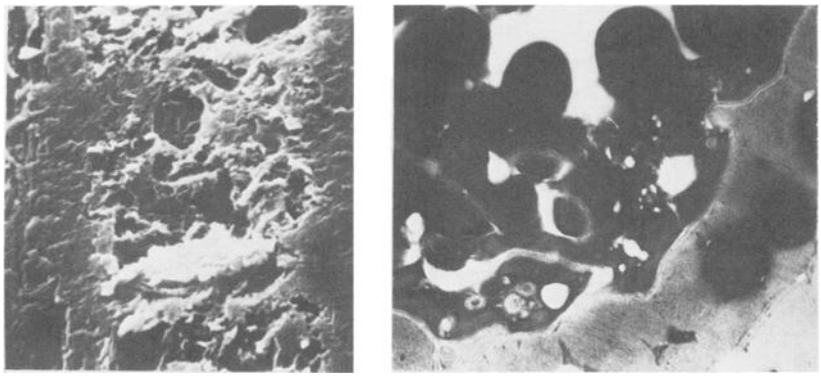

FIG. 14—*Hair from an anthropoid ape* (Pongo pygmaeus)*:* (left) *SEM and* (right) *TEM.*

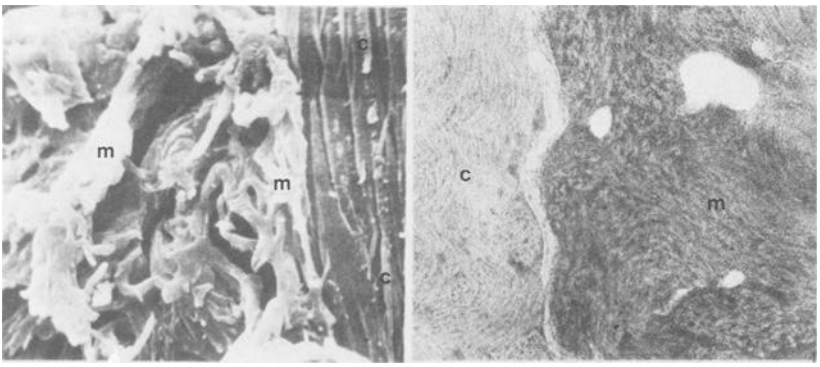

FIG. 15—*Hair from a human. Note the macrofibrillar and microfibrillar structure of the medulla* (m)*, which looks like the cortex* (c)*:* (left) *SEM and* (right) *TEM.*

Discussion

These results must be compared with the classification established by Hausman in 1930 [*12*], which distinguished five types of hair according to the medulla shape (Fig. 16):

(1) hairs whose diameter varies from 0 to 10 μm, with no medulla;
(2) hairs 10 to 45 μm thick with a discontinuous medulla;
(3) hairs 45 to 75 μm thick with an intermediate medulla;
(4) hairs 75 to 90 μm thick with a continuous medulla; and
(5) hairs 100 μm or more in diameter with a fragmental medulla.

This correlation between hair diameter and medulla shape must not be interpreted as a rigorous rule but rather as a wide mean (central area of a Gaussian curve).

By inserting our observations on the medullary cells into this classification of the medulla shape, we can establish the system given in Table 1. This table, the outcome of our work, outlines the fundamentals of a modern hair identification scheme based on the general structure (light microscopy) and on the fine structure (SEM) of the hair medulla; for a more detailed determination, one must use the cortico/medullary ratio.

All these data arrange themselves in a coherent and well-ordered system and allow us to bring the genera and the families together according to their relationships and to the general scheme of mammalian evolution. This latter point is one on which many observers have

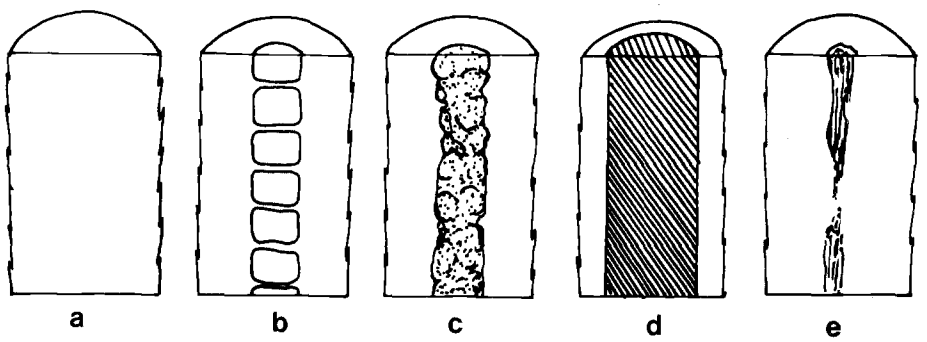

FIG. 16—*The different types of medulla according to Hausman* [12]: (a) *hair without medulla;* (b) *discontinuous medulla;* (c) *intermediate medulla;* (d) *continuous medulla; and* (e) *fragmental medulla.*

TABLE 1—*Identification of mammalian hair.*

I. Discontinuous medulla (Fig. 16b)	
Medulla with "cytoplasmic nuclei"	
(A) "Nuclei" composed of amorphous matter	
Somewhat regular in size and architecture	marsupials (Fig. 1)
Regular in size and structure	rodents (Fig. 2)
Small aerial vesicles arranged in juxtaposed columns	lagomorphs (Fig. 3)
(B) "Nuclei" composed of biphasic matter	
Wide or cubic aerial vesicles	insectivores and lemurs (Fig. 4)
Flattened aerial vesicles	lesser apes (Fig. 5)
II. Continuous medulla (Fig. 16d)	
Wide medulla composed of cytoplasmic remnants with a biphasic ultrastructure	
(A) Walls of the aerial vesicles perforated with vacuoles	carnivores
Large and well-individualized aerial vesicles rather rarely septated	Felidae (Fig. 6)
Small aerial vesicles, often septated	Mustelidae (Fig. 7)
Small aerial vesicles, very often septated	Canidae (Fig. 8)
(B) Aerial vesicles have solid walls	hoofed mammals
Walls of variable thickness, with a rough surface	perissodactyls (Fig. 9)
Walls of equal thickness, with a smooth surface; wide aerial vesicles with a rectilinear outline	most of the true-ruminant artiodactyls
Very regularly displayed aerial vesicles (honeycomb)	Cervidae (Fig. 10)
More or less regularly displayed aerial vesicles	cavicorns (Fig. 11)
III. Intermediate and fragmental medulla (Figs. 16c and e)	
Thin medulla with a dense aspect	
(A) Cytoplasmic remnants with a biphasic ultrastructure	
Very small aerial vesicles, sinuously outlined, non-filamentous cytoplasmic remnants	Camelidae (Fig. 12)
Small or middle-sized aerial vesicles; cytoplasmic remnants more or less filamentous	nonruminant artiodactyls (Fig. 13) and some true-ruminant artiodactyls (sheep) anthropoid apes (Fig. 14)
(B) Cytoplasmic remnants with a microfibrillar and macrofibrillar ultrastructure	man (Fig. 15)

failed and have concluded that medullary morphology is related not to the taxonomic group of the animal possessing the hair but to the hair diameter [*13,14*]. The reasons for this failure were tied to the methods and the equipment, which could give only limited results.

Two essential remarks must still be made: (1) the hairs of animals classified in one of three main groups look like each other and are totally unlike those classified in one of the other groups and (2) in the medullary cells of human hairs we have found, for the first time, a very clear microfibrillar and macrofibrillar ultrastructure, a characteristic that absolutely differentiates human hairs from all other animal hairs and that can be used every time it is necessary to determine the human or animal origin of one hair.

However, this characteristic should not be used to determine the age, the sex, or the race of a person.

Our study completes Rosen's [*15*] because it shows that human hairs are quite different from those of most of the other primates and slightly different from those of gorilla and chimpanzee [*16*].

The fact that we have found in human hair medullary cells a peculiar ultrastructure is a little disturbing as we cannot adopt a teleological (and anthropocentric) point of view that would consider this medullary ultrastructure superior to all other animal hairs. The only possible explanation is bound to the medulla itself, whose index (diameter of the medulla divided by the diameter of the hair) in human hairs is among the few to be less than 0.30 (this is one of the essential characteristics that differentiates human from animal hairs). It is not excluded that this compression of the medulla has led the medullary cells of human hairs to evolve towards a new form.

Summary

These coordinated studies using SEM and TEM on the fine structure and the ultrastructure of hair medulla have brought to light a number of new anatomical characteristics and have modernized hair identification by renewing the fundamentals of its conception.

Former studies of the medulla anatomy by light microscopy through the cortex led only to rough differentiation whereas electron microscopy has permitted, for the first time, the real morphology of this layer to be seen and new structures that can be used in identification to be distinguished. From these new characteristics, a general hair identification scheme in accordance with the taxonomy of mammals has been established. Three patterns of medulla can be distinguished:

(1) discontinuous medulla with "cytoplasmic nuclei" in the hairs of marsupials, rodents, insectivores, lemurs, and nonanthropoid apes;

(2) continuous medulla with small or large aerial vesicles whose walls are perforated with vacuoles in hairs from carnivores and not perforated in hairs from hoofed mammals; and

(3) intermediate and fragmental medulla with a dense aspect in Camelidae, nonruminant artiodactyls, and human hairs.

Human hairs differ from all other animal hairs because of the clear microfibrillar and macrofibrillar ultrastructure of their medullary cells.

References

[*1*] Waldeyer, W. and Grimm, J., *Atlas der menschlichen und Tierischen Haare, sowie der Ahnlichen Fasergebilde,* Druck und verlag Von Moritz Schauenburg, Lahr, 1884.

[*2*] Lambert, M. and Balthazard, V., *Le poil de l'Homme et des Animaux,* Steinheil Edit., Paris, 1910.

[*3*] Glaister, J., *A Study of Hairs and Wools,* Misr Press, Cairo, 1931.

[*4*] Stoves, J. L., "The Appearance in the Cross Sections of the Hairs of Some Carnivores and Rodents," *Proceedings of the Royal Society, Series B. Biological Sciences,* Vol. 62, 1944, pp. 99-104.

[5] Brunner, H. and Coman, B., *The Identification of Mammalian Hairs*, Inkata Press, Melbourne, Australia, 1974.
[6] Kassenbeck, P., "Morphologie und Feinstruktur der cortex und cuticulazellen von Humanhaaren," in *First International Congress of Hair Research Compendium*, German Society of Dermatology, Hamburg, W. Germany, 1979, p. 4.
[7] Parakkal, P. F. and Matolsty, A. G., "A Study of the Differentiation Products of the Hair Follicle Cells with the Electron Microscope," *Journal of Investigative Dermatology*, Vol. 43, No. 1, July 1964, pp. 23-34.
[8] Roth, S. I. and Helwig, E. B., "The Cytology of the Dermal Papillae, the Bulb, and the Root Sheaths of the Mouse Hair," *Journal of Ultrastructure Research*, Vol. 11, No. 1-2, Aug. 1964, pp. 52-67.
[9] Rogers, G. E. and Simmonds, D. H., "Contents of Citrulline and Other Amino Acids in a Protein of Hair Follicles," *Nature* (London), Vol. 182, No. 4629, July 1958, pp. 186-188.
[10] Rogers, G. E., "Occurrence of Citrulline in Proteins," *Nature* (London), Vol. 194, No. 4834, June 1962, pp. 1149-1151.
[11] Rogers, G. E., "The Localization and Significance of Arginine and Citrulline in Proteins of Hair Follicles," *Journal of Histochemistry and Cytochemistry*, Vol. 11, No. 6, Nov. 1963, pp. 700-705.
[12] Hausman, W., "Recent Studies of Hair Structure Relationships," *Scientific Monthly*, Vol. 30, 1930, pp. 258-277.
[13] Noback, C. R., "Morphology and Phylogeny of Hair," *Annals of the New York Academy of Science*, Vol. 53, March 1951, pp. 476-492.
[14] Smith, H. H., "The Relationships of the Medulla and Cuticular Scales of the Hair Shaft of the Soricidae," *Journal of Morphology*, Vol. 55, 1933, pp. 137-149.
[15] Rosen, S. I., "Identification of Primate Hair," *Journal of Forensic Sciences*, Vol. 19, No. 1, Jan. 1974, pp. 109-112.
[16] Clement, J. L., Sotton, M., Gastaldi, G., Le Pareux, A., Hagege, R., and Connet, J., "Etude comparative des poils des singes anthropoides et de l'Homme," *L'Anthropologie*, Vol. 84, No. 2, 1980, pp. 243-253.

Address requests for reprints or additional information to
J. L. Clement
Laboratoire de l'Identité Judiciaire
3, quai de l'Horloge
75001 Paris, France