IDENTIFICATION OF WOLF PREY USING HAIR AND FEATHER REMAINS WITH SPECIAL REFERENCE TO WESTERN CANADIAN NATIONAL PARKS

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CANADIAN WILDLIFE SERVICE

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1981

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MEMORANDUM **NOTE DE SERVICE**

TELFER/DOE/CWS/435-7320/ek

TO / À
Director General
Canadian Wildlife Service
Ottawa, Ontario

FROM / DE
Regional Director
Canadian Wildlife Service
Western & Northern Region
Edmonton, Alberta

SECURITY - CLASSIFICATION - DE SÉCURITÉ
OUR FILE/NOTRE RÉFÉRENCE
YOUR FILE/VOTRE RÉFÉRENCE
DATE: February 23, 1981

SUBJECT / OBJET: Report entitled: "Identification of wolf prey using hair and feather remains with special reference to Western Canadian National Parks" by A.J. Kennedy and L.N. Carbyn.

I attach a copy of this report for your information. The document sets out useful techniques for analysis of predator food habits. The authors adapted the techniques to regional conditions as part of studies conducted for Parks Canada by our Region. Many requests from colleagues for information on their techniques prompted the authors to prepare this internal report.

M. R. Robertson
Regional Director

Encl.

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ACKNOWLEDGEMENTS

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Messrs. C. Gates, R. Hudson, University of Alberta; S. Oosenbrug, D. Patriquin and T. Trottier, CWS; B. Treichel, Alberta Fish and Wildlife Division; and Parks Canada Wardens collected hair samples for our hair reference collection from which type hairs for the present key were obtained.

The following individuals volunteered their time to test the effectiveness of the present key: T. Burkholz, D. Downie, H. Kiliaan, G. Munro, S. Myer, J. Nolan, P. Pryor and A. Smith. P. Kroeger, CWS, provided laboratory assistance throughout the project.

S. Popowich, CWS, drafted the figures. W.D. Bowen and E. Telfer reviewed an earlier draft of this manuscript.

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ABSTRACT

A system for accurate identification of hair and feather remains recovered from wolf (*Canis lupus*) feces (scats) or at kill sites is presented. A pictorial hair key, using hair medulla and cuticle scale microstructure, supplemented with macroscopic information, has proven effective in separating the hair of 32 reported mammalian wolf prey species. Microstructure of feather barbules is successful in separating the only two common taxonomic groups of avian prey.

A literature review, outline of methodology and discussion of the advantages, limitations and future of the hair identification process are also included.

INTRODUCTION

1. PURPOSE OF STUDY

For centuries the wolf (Canis lupus) has remained a controversial species, due in part to its predation on livestock and ungulates. Thus, food habits have been a primary concern in wolf research since the early studies of Murie (1944) and Cowan (1947). One method of determining wolf diets has been, and is at present, the analysis of fecal droppings (scats).

The Canadian Wildlife Service (CWS) has for some time been involved in wolf food habits studies within the National Parks of Northwestern Canada. Previous wolf research in Jasper, (Carbyn 1975), Wood Buffalo (Oosenbrug et al. 1979) and Riding Mountain, (Carbyn et al. 1979) national parks demonstrated the need for an accurate method to determine wolf prey items from the hair, feather and bone remains recovered in scats. The variety of potential prey species, and the fact that existing identification aids were not specific to the appropriate geographic areas, prompted this analysis of hair characteristics. The intention was to establish a set of definite, readily recognizable criteria to separate all bone, hair and feather remains recovered in wolf scats collected in national parks within the western and northern region of Canada.

We used photomicrographs, electron microscopy, and line drawings of various hair forms to establish a pictorial key to all reported wolf prey species in northwestern Canadian national parks. The key has proven useful for the prey species eaten by wolves in Northwestern Canada. However, users in other regions may find it necessary to use additional criteria to separate different conbinations of prey.

2. LITERATURE REVIEW

In Europe, hair analysis began with Saxinger (1925), who first attempted to obtain information on cuticle scale structure through impressions of hair. He pressed hair into a mold containing a balsam medium and from these subsequently formed impressions to be used for identification. Herzog (1927) refined this technique of hair examination and obtained clearer hair impressions. Manuals were later prepared that included photographs of the microstructure of hair from a limited number of mammal species (Brummond 1937). Recent work is aimed at the compilation of hair keys on a wide variety of mammalian species (Dziurdzik 1973) using a set of more specific hair criteria.

Although hair identification research in North America began during the late 1800's (McMurtie 1886), it was first reported in a series of papers by L.A. Hausman between 1920 and 1932. Inititially, Hausman (1920) reported on the general microanatomy of hair and suggested that medulla form and cuticle scale pattern were quite different among animal groups. Using these structures he devised a classification scheme to separate the hairs of 166 different mammalian species. Hausman (1924) later refined his techniques and added many new terms to his hair classification scheme. He also began stressing the taxonomic value of hair to separate many closely related animal groups. Hausman (1930) subsequently expanded this work to emphasize the relationship between the advancement of hair form, size and complexity and the evolution of general body form within many taxonomically diverse mammalian orders. Hausman (1932) believed that pigment granules, or cortical fusi, could be a valuable

microscopic criteria for the separation of hairs. He reported that distribution and shape of the cortical fusi were different among some mammalian groups and that these differences were consistent and therefore important.

Hausman's baseline work is valuable in that it provided a definite set of nomenclature on hair morphology and prompted much research aimed to improve the hair analysis technique. A weakness of his work was a simplistic approach to the documentation of hair microstructure (Short 1978, Homan and Genoways 1978). Data from those studies indicated that his classification scheme was inadequate to separate the hairs of many mammalian species.

Later, improved techniques to view hair microstructure (Hardy 1940) led to the compilation of sets of hair characteristics presented in key form (Mathiak 1933, Williams 1938). These keys represented good initial efforts for the systematic identification of hair, but, relied heavily on very general hair criteria for hair identification. Nomenclature in those early keys was confusing and hair characteristics overlapped considerably among the species presented. Development of more precise hair descriptions and improved diagrammatic representations of hair allowed the formation of more accurate hair keys that included hairs of mammals with extensive ranges (Williamson 1951, Mayers 1952, Stains 1957, Day 1966). These keys were much improved from the previous but still lacked sufficiently detailed descriptions of individual hairs.

Further advancement of hair preparation methods (Carter and Dilworth 1971, Wienhart 1973) and the advent of modern photomicrography and scanning electron microscopy improved the modern hair keys by enabling an exacting

representation of hair structure (Spiers 1973). Most recent published work on hair identification have stressed the inclusion of hair photographs to show clearly and exactly the structure of each hair (Adorjan and Kolenosky 1969, Brunner and Coman 1974, Moore *et al.* 1974).

The appearance of hair when cross sectioned has been considered a valuable aid in hair identification (Mathiak 1938, Dearborn 1939). The most useful scheme for separation of hairs from a diverse mammalian fauna using cross sections was developed by Appleyard (1960), Coman and Brunner (1971) and Brunner and Coman (1974). These studies showed differences between hair cross sections of some distantly related animal groups.

Cross sections were not useful for the present key because: a) cervid hair composed a major portion of our samples and this hair type was found to be thick and keratenous and therefore, it could not be cross sectioned properly; b) cross sections of hair of closely related species such as those considered here, have similar medulla form, and appear very similar under the microscope. For these reasons we did not include cross sections in the present key.

A separate hair key for use in wolf predation studies was needed for several reasons. First, some characteristics used in general keys are not applicable to wolf food habits work. For example, details such as hair color and length are often not recognizable in the fragmented hair recovered from wolf scats. Second, the great number of descriptions of various mammal hair types presented in general keys makes them difficult to follow. The great amount of detail is also unnecessary because wolves usually feed on only a few species in a given area. The greatest drawback with the majority of available hair keys is the nature of the diagnostic

characteristics presented. For these reasons we have prepared a concise pictorial key using a variety of hair characteristics.

METHODS

1. GENERAL TECHNIQUES

1.2. Scat collections

Wolf scats found on trails, at dens or rendezvous sites should be collected individually in durable plastic bags and immediately labelled with location, date, estimated age and any other pertinent data. Scats should be frozen as soon as possible to retard decomposition.

Care should be taken to include only scats that are greater than 27 mm in diameter to avoid confusion with other canid scats. From our studies in Riding Mountain Park, and previous work by Thompson (1952), Peterson (1977) and Weaver and Fritts (1979), the approximate maximum diameter ranges for canid scats are: coyote (*Canis latrans*) 15-24 mm, wolf pup 17-20 mm and wolf adult 25-40 mm. Juvenile wolves may fall within both the wolf pup and adult categories. Domestic dog (*Canis familiaris*) scats present a problem because they also can fall into any of the categories. However, by knowing and heeding field signs common to wolves domestic and wild samples are not often confused.

Transmission of the granular tapeworm (*Echinococcus* spp.) to man through wolf feces has been documented by Rausch (1967). Field personnel should be aware of this fact and take the appropriate hygenic measures when collecting wolf scats. Direct contact with scats should be avoided. In the field this is accomplished by collecting material using a plastic

bag as a glove. The scat should then be double bagged in plastic. Wolf scats should never be close to food or drink. When collecting dry crumbling scats care must be taken not to inhale the "dust" that rises, because it may contain parasite ova.

1.3 Preparation and examination of hair remains

In the laboratory the scat samples should first be autoclaved at temperatures of 130 C or more and then washed to remove extraneous detrital matter. Autoclaving will destroy the eggs of the granular tapeworm. The washed hair, feather, bone and flesh can now be placed in heat resistant plastic containers, covered and oven dried at 70 C.

The dry sample should next be spread out in an observation pan to gain a general impression of the biomass of various components of the scat. Guard hairs, with tip and base intact, should be selected for examination. Other potentially identifiable material, such as teeth and bone, can be collected at this time.

Following collection of all identifiable material from the scat, the hair sample should be examined as follows. The first step is to clean the hair. Running water is usually not sufficient, therefore the hairs may be further cleaned with organic solvents. In this study methyl salicylate has proven effective as a cleaning agent. The most common of the larger primary guard hairs in the sample should be chosen for examination. This is done for two reasons. First, the common larger hairs will probably give an accurate picture of the major contents of the stomach and secondly, the widest hairs generally have the greatest diagnostic value. It is best to

prepare a slide using between two and four hairs, allowing good representation without interference between hairs.

The hairs are next laid on an acetate strip (thermoplastic film) which is in turn laid on a microscope slide. A second slide covers the acetate and hairs forming a "sandwich", which is held together by clamps (large paper clips). The sandwich is then numbered and heated to approximately 120°C for 15-20 minutes. Colored (green) acetate is recommended for these preparations since it is least irritating to the eyes during microscopic examination. After heating, the unmarked slide is removed and the hairs are displaced a millimeter or so from their impressions and a cover slip fastened. From this single slide the observer can view the general microscopic appearance of the hair, the scale impression in the acetate, and the whole-mount view of the medulla. Medulla examination sometimes requires the observer to increase the light intensity to see transmission through the hair itself and to focus on several levels throughout the hair shaft. A list of materials and a summary of the hair analysis procedure is presented in Appendices B and C.

Spray lacquer (Carter and Dilworth 1971), nail polish remover (Weinhart 1974) and gelatin (Korschgen 1963) have been used as mounting media for hair preparation. Although these mounting substances provide faster hair impressions we have found that the heat-plastic method gives a superior specimen.

Washed scat samples can be saved for future reference by storage in air tight glass jar to prevent decomposition. Hair slides may be stored for extended periods simply by applying a permanent mounting medium (permount) to the microscope slides.

2. METHODS FOR HAIR KEY CONSTRUCTION

Primary dorsal guard hairs were collected from live, captive animals and museum study skins. These specimens were used to complete a hair reference collection of all reported wolf prey species within Northwestern Canadian National Parks (Appendix A). Typical hairs were selected for each prey species and a list of microscopic and macroscopic criteria compiled for each. Emphasis was placed on the more concrete, quantitative features of the hair rather than subjective aspects such as general form and gross morphological characteristics. Each criteria was then judged and the type hair placed in a suitable location in the key. The accuracy of the key was then checked by examiners working through the key with known hair samples. Photomicrographs were then taken of the criteria found to be most valuable. The photographs were taken using a Lietz Ortholux II microscope fitted with a Lietz camera and using Kodak Panatomic X film. Scanning Electron micrographs were obtained using facilities at the Department of Entomology, University of Alberta, Edmonton, Alberta. Finally, the key was retested using a series of different, known hair samples also from the hair reference collection.

RESULTS

BACKGROUND

To use the hair key effectively an analyst must have a good comprehension of basic hair anatomy. Knowledge of both gross anatomy and an understanding of microscopic hair structure are essential prerequisites to using the present key. The following discussion is designed to prepare the analyst in these areas.

1. HAIR MACROSTRUCTURE

1.2. Hair type

For the purpose of this report there are two recognisable types of mammalian hair: guard hair and underfur or body hair.

Guard hairs can be classified as either primary or secondary. The former has been described as "the elastic, horny, large, shiny outer coat fibres that give animals their characteristic appearance" (Moore *et al.* 1974:8). These hairs consistently show species specific features. Secondary guard hairs, although they closely resemble primary hair, are shorter, thinner and most often fill in the spaces between primary hairs in the animal's coat. The differentiation between guard hairs is made here because secondary hairs generally do not show the hair characteristics as consistently as primary hairs. A typical primary guard hair is shown in Figure 1. Other types of guard hairs such as spines, barbs, bristles and awns are also recognisable (Deblase and Martin 1975) but are not included here because they are not readily identifiable.

Underfur, (or wool, velli, vellum) because it functions mostly as insulation, is a fine, short, soft fibre usually lying under the guard hair (except in domestic sheep). These hairs have no value in identification because the microstructure is difficult to examine and is not species representative.

1.3. Hair color

Hair color by itself is generally only a marginally useful criterion because of the discoloring that occurs during the digestive processes.

However, a brief description is given here because hair found on trails or at kill sites may retain useful color properties. Hair color is determined by the manner in which light passes through the hair shaft. Therefore, the thickness, texture and viscosity of the hair are important to color. These properties of the hair are controlled largely through the type, amount and distribution of pigment granules.

Individual hairs through placement of pigments may be bicolor, multicolor or occasionally banded. Frequently color banding of hair (Figure 1) is species specific; although overlap between species has been observed. Despite this, some keys have used color banding or general color patterns as primary criteria to separate hair. This was not done in this key, but we did occasionally include these features as a secondary criterion.

1.4. Hair classes and regions

There are two classes of primary guard hairs; the shield type and the standard type. Shield hair has the upper shaft region flattened to form a disk like upper region. This hair type is common to rodents. Standard primary guard hair does not have a flattened middle region (Figure 2).

Mammal hair has two general regions: the lower region or area from the middle of the shaft to the end of the root (lower shaft); and the upper shaft or upper region from the middle of the hair to the distal tip of the shaft (upper shaft). Specific regions of the hair shaft were selected for the key to serve as reference points for the examiner, and were defined as follows. The Base (Basal) or bottom of the shaft

containing the entire root and continuing distally to about one third of the distance upward on the shaft; the mid-shaft (medial) portion of the shaft or the area within the middle one third of the hair shaft; and the tip portion or the final distal one third of the hair shaft, often this region is constricted to a point. The regions of a hair are shown in Figure 2.

2.2. Hair Microstructure

Mammalian hair is recognized as having four distinct structural microcomponents. These are the cuticle or outside covering; the cortex or inner shealth; the medulla or central core; and pigment granules which can be found scattered throughout the shaft in the cortex region (Hausman 1920). For the purpose of this hair key only an understanding of medulla form and cuticle pattern is required. Mammalian hair microstructure is shown in Figure 3.

The medulla (or core) of the hair shaft takes on various shapes that are specific to certain species and therefore useful to identification. Unfortunately, the literature on medulla structure is confusing because details of the microanatomy are inaccurately reported, not included, or inconsistently described. To overcome this problem we have formed a three part naming system for the different medulla types in the hair key. The three parts of the nomenclature system in order are: 1) medulla length (and continuity) along the hair shaft; 2) medulla thickness across the hair shaft; and 3) general medulla type. The medulla classification scheme used in the hair key is presented in Table 1, and are shown in Figure 4.

Table 1.

Medulla terminology used in hair key. Figure 4 presents graphic representation of medulla types.

Length	Thickness	Type	Example
Continuous	Complete	Lattice	cervidae
Continuous	Incomplete/complete	Flattened lattice	porcupine
Discontinuous	Incomplete	Intrusive bar	sciuridae
Discontinuous	Incomplete	Vaculated intrusive bar	sciuridae
Continuous	Incomplete	Granular	marmot
Continuous	Incomplete	Unicellular ladder	microtine
Continuous	Incomplete	Dicellular ladder	microtine
Continuous	Incomplete	Multiserial (spiral) ladder	leporidae
Discontinuous	Incomplete/complete	Granular	muskrat
Discontinuous	Incomplete	Fragmental	cow
Discontinuous	Incomplete/complete	Homogeneous column	wolf, bison
Discontinuous	Incomplete	Irregular column	bear
Discontinuous	Incomplete	Absent	beaver

We have also formulated a nomenclature scheme for reference to the hair cuticle. The hair cuticle is broken into units known as scales (Hausman 1920), and the various patterning of these units are important for identification. Our classification is based on features described by Hausman (1920) and is as follows: 1) distance between scale margins; 2) scale margin description; and 3) scale type. A summary of scale pattern classification format is given in Table 2 and shown in Figure 5.

3. FEATHER MICROSTRUCTURE

The present feather identification procedure is modified from that presented by Day (1966). A typical feather rachis (vein) has many small side branches known as barbs. These barbs, in turn, also have smaller appendages referred to as barbules (Pettingill 1961). The microstructure of these barbules from the downy region of the feather are unique in appearance. For example, the nodes along the pennulum of the barbule have certain distinctive forms. Using the structural differences one can accurately separate to taxonomic order the reported avian prey of wolves. The microstructure of a typical feather is shown in Figure 6.

4. HAIR KEY

1A. Medulla a continuous, complete, lattice along the entire hair shaft. -----Cervidae (deer family) and some Bovidae (sheep family; except bison and cow).----------(13) Figure 7

1B. Medulla other than above.----------(2)

Table 2.

Cuticle scale nomenclature used in hair key. Figure 5 shows cuticle scale nomenclature.

Scale Margin Distance	Scale Margin Form	Scale Pattern Type				
		Mosaic	Wavy	Dentate	Cevron	Coronal
Close	Smooth	Regular	Regular	Elongate	Single	Single
Intermediate	Rough	Regular	Irregular	Diamond	Double	
Distant	Crenate					

2A. (1) Medulla a continuous, complete or incomplete, flattened lattice along entire shaft.

Cuticle scales at base a distant, smooth, regular mosaic pattern; medially the scale becomes intermediate, rough, wavy; at tip of shaft the scales are close, rough, and irregular to wavy in form. Figure 8

---------- Erethrizontidae (porcupine)

Note: Superficial microscopic examination by the examiner will cause confussion between the hairs of some cervidae and porcupine, however, careful examination of the incomplete medulla and the details of the scale pattern will eliminate any doubt. The recovery of quills in the sample is also often possible. Macroscopically, porcupine hair is long (up to 100 mm) and wide (up to 250 µm dia.) and often black banded or grey and white bicolor. Figure 9

2B. Medulla other than above.----------(3)

3A. (2) Medulla may be continuous, incomplete granular or one of discontinuous, complete intrusive barred or discontinuous incomplete fragmental. Hair always with a shield region. Figure 10

----------Sciuridae (4)

Note: Members of the squirrel family have similar hair characteristics and are difficult to separate accurately. A listing of the squirrel species known to the study area is beneficial in that it may limit the number of choices involved. This key pertains only to documented wolf prey species, others may be involved.

3B. Medulla not as above.----------(5)

4A. (3) Medulla discontinuous, incomplete, and intrusive barred in form; cuticle scales at base intermediate, smooth to crenate and an elongated dentate pattern; becoming close, smooth to rough and a regular wavy pattern in shield region.

Figure 11

----------Red squirrel

Note: Red squirrel hair is most easily differentiated by its dentate scale pattern at the base of the shaft. Macroscopically the hair is short (15-25 mm) and color banded red, brown and grey or occasionally black and white.

4B. Medulla similar to 4A, however, bars often broken and vacuoles present; cuticle scales not as dentate at base but usually distant, crenate, regular mosaic pattern; and close, rough, irregular wavy pattern in the shield region.

----------Arctic ground squirrel

Figure 12

----------Franklins ground squirrel

----------Richardsons ground squirrel

Note: Although these species appear identical in microscopic appearance, they are quite different in gross appearance. Golden-mantled ground squirrel hair has a distinctive, yellowish band <1 mm from the tip. Arctic ground squirrel hair is fine, with a small whitish band and a distinctive black tip. Richardsons ground squirrel hair is completely yellow or with a very slight black band. Franklins is much similar to Richardsons but slightly more orange in color. Knowledge of the North American distribution of these species improves differentiation of these species (see Banfield 1974 pps. 119-219).

4C. Medulla changes in pattern from fragmented, to intrusive bar, to granular from base to shield to tip respectively. Cuticle scales are distant, smooth wavy to mosaic at the base becoming close, crenate, regular wavy in shield region. Figure 13

----------Columbian ground squirrel

Note: Columbian ground squirrel hair is very distinctive. The only possible confusion could be with Golden-mantled squirrel, and medulla form is quite different between these two species (compare Figure 12 to Figure 13).

4D. Medulla a continuous, incomplete, granular form at base, near shield region medulla may appear slightly barred; cuticle scales are distant, smooth, and irregular mosaic at base becoming close, rough to crenate and irregular wavy at shield.

----------Marmot

Note: Marmot hair is similar to the hair of both Columbian and Golden-mantled ground squirrels in gross appearance, microscopic examination of medulla form along the shaft is therefore essential to identification. Figure 14

5A. (3) Medulla a continuous, incomplete, unicellular ladder at base; medulla may be dicellular pattern near the shield region but never a multicellular ladder. Hair very short (<100 µm) generally with a shield region. Figure 15

----------Cricetidae (6)

Note: Cricetidae hair is often conspicuous due to its macroscopic appearance; the hairs are very short and limp with diameters of <20 µm. Skeletal material is almost always present in scat samples containing mice or voles.

5B. Medulla not as above. At base thin and granular, may be absent in shield region; when present in shield the medulla is discontinuous, incomplete and granular form.----------Beaver; muskrat (7)

Figure 16

6A. (5) Medulla at base a discontinuous, incomplete unicellular ladder becoming dicellular with prominent vacuoles in shield region. Cuticle scales at base distant, crenate and simple coronal in form; becoming close, smooth and regular wavy in shield region.

Figure 17 ----------Cricetinae (mice)

6B. Medulla at base a discontinuous, incomplete unicellular ladder becoming dicellular and intrusive barred in shield area. Scales at base distant, crenate and simple coronal becoming intermediate, smooth,

Figure 18 regular wavy in shield region.----------Microtinae (voles)

Note: Although adequate separation is possible at the sub-family level; species separation by hair structure is impossible without skeletal material. For skeletal and dental classification see Banfield (1974).

7A. (5) Medulla at base a discontinuous, incomplete, granular form; becoming a thick granular form in the preshield region, then absent in shield. Cuticle scales at base intermediate, crenate and irregular wavy to irregular mosaic in form, becoming close, rough, irregular

Figure 19

wavy in shield and at tip.----------Beaver

Figure 20

7B. Medulla discontinuous, incomplete granular form throughout entire hair shaft; may be fragmental at tip. Cuticle scales at base a distant, crenate single chevron form becoming intermediate, rough and irregular wavy in shield and tip regions.----------Muskrat Figure 21

Note: A fast, effective method of separating beaver and muskrat is accomplished by a comparison of basal and shield medulla combined with examination of basal scale form of each species. Figure 22

7C. Medulla not as above.----------(8)

8A. (7) Medulla a continuous, complete, multiserial (spiral) ladder. Figure 23
----------Leporidae (9)

8B. Medulla a discontinuous, incomplete, homogeneous colum.----------(10)

9A. (8) Medulla a multiserial ladder, most often in a spiral; cuticle scales at base distant, smooth, and elongated dentate becoming close, rough, and regular wavy in shield region.----------Snowshoe hare Figure 24

9B. Medulla fragmental in form at base, becoming a homogeneous column in midshaft.----------(11) Figure 25

10A. (8) Medulla never fragmental, cuticle scales distant, smooth, and diamond dentate at base becoming intermediate, rough, double chevron in midshaft, and finally close, rough, and irregular wavy at tip.
----------Canidae

10B. Cuticle scales distant, smooth, and diamond dentate both at base; becoming intermediate, rough, and irregular wavy near the tip. Both primary and secondary guard hairs show definite waviness; hair length ranges from 50-122 mm, color white (or grey) with black tip or black and white banded.----------Coyote Figure 26

10C. Cuticle scales as above but only secondary guard hairs wavy; hair length always over 122 mm. Color as above but may also be black.
----------Wolf

Figure 27 *Note:* Hairs of wolves and coyotes are almost identical in microscopic appearance, accurate separation using microstructure is therefore questionable. The macroscopic characteristics presented often are the only means of separating hairs of these species.

11A. (9) Medulla at base of hair shaft an incomplete, discontinuous, homogeneous column form; medulla becomes an incomplete, incontinuous fragmental form medially, and is absent at the tip. Cuticle scales at base are distant, crenate, and a regular wavy pattern, becoming intermediate, rough, and irregular wavy at tip.----------Bison

Figure 28 11B. Medulla and scale pattern as above (11A), but hair on gross inspection appears very limp and curly; distinctly and entirely red colored; cuticle scales may be more distant, crenate and irregular wavy than in adult hair samples.----------Bison calf

Note: Bison calf hair is undistinguishable from that of an adult after the first summer of the calf's life.

11C. Medulla a complete discontinuous homogeneous columm very similar to bison (11) but always complete when homogeneous. Often fragmental in form at the base as well the midshaft and tip. Cuticle scales at base with edges much more close and rough (often crenate) than bison. Scales are close in midshaft and tip regions and scale edges become

Figure 29 smooth.----------Domestic cow

Note: The present distribution of bison and domestic cow in North Western Canadian National Parks is such that the simultaneous recovery of hair of both species is highly unlikely.

11D. Medulla a discontinuous, homogeneous column at base, becoming fragmental in the medial region of the hair shaft; cuticle scales at base intermediate, smooth and regular mosaic becoming a close, rough, irregular mosaic near the medial portion of the hair shaft.

Figure 30

----------Domestic horse

11E. Medulla a discontinuous, incomplete irregular column form often becoming fragmental in midshaft area.----------(12)

12A. (11) Medulla as above (11E); cuticle scale intermediate, rough, regular wavy at base; becoming close, rough, irregular wavy at mid-shaft and tip.----------Black bear

Figure 31

12B. Hair microstructure as above (12A), but gross appearance of hair slightly different. Hair longer and light brown in color (never black).----------Grizzly bear

13A. (1) Hair diameter at base ranging from 50 µm to 100 µm, at middle shaft ranging from 20 µm to 90 µm. The number of cuticle scales across the base of hair shaft never more than 4. Cuticle scales a intermediate, smooth, irregular mosaic pattern throughout entire hair shaft.----------Cervidae young (14)

13B. Hair diameter ranging from 180 µm to 520 µm at base and from 170 µm to 460 µm medially. The number of cuticle scales at base ranging from 5-12. Cuticle scales at base are distant, smooth, and regular mosaic becoming regular wavy in form at the tip.

Figure 32

----------Cervidae and some Bovidae adults (15)

Figure 33

14A. (13) Cuticle scales intermediate, smooth, regular wavy until mid-shaft then becoming close, rough, irregular wavy. Hair often appears red and is limp and thin. Cuticle scales at base close, average 10 ± 2.2 to 100 µm of hair shaft.----------Elk calf

Figure 34

14B. Cuticle scales as above however, much more robust and distant, average 5 ± 2.5 in 100 µm of hair shaft. Color similar to elk calf.

Figure 35

----------Deer fawn

14C. Cuticle scales as above (14A, 14B) but much more regular mosaic in appearance, especially at base. Hair diameter much greater than above (up to 100 µm); hair never reddish, limp and curly; hair black basally, red-brown medially, and black distally.----------Moose calf

Figure 36

Note: Young cervidae hairs are very difficult to separate within themselves because of overlapping structure of the cuticle scales. Special attention must therefore be given to this group. Macroscopic form and microscopic structure must be combined in order to identify the members of this group accurately. Young cervids begin to develop adult hair characteristics after about 4 months of life; therefore, hair of young cervids in scat samples from September or later are essentially indistinguishable from adults.

15A. (13) Cuticle scales appear elongated; number of scales across base less than 8.----------Cervidae adults group A (16)

15B. Cuticle scales appear hexagonal; number of scales across base greater than 8.----------Cervidae adults group B (17)

Figure 37
Figure 38

16A. (14) Cuticle scales at base distant, smooth, regular mosaic, becoming close, smooth, regular wavy at midshaft and tip. Hair dark with gold or reddish brown band. The base of the hair may be gold in color. Hair thick, generally >250 µm.----------Elk

Figure 39

16B. Cuticle scales similar to elk at base but more elongated and with rough edges and very elongated medially. Hair is characteristically thick, keratinized and very kinky.----------Sheep

Hair light brown basally becoming white medially.

----------Rocky Mountain Bighorn sheep Figure 40

Hair entirely white, basal scales rough and wavy.

----------Dall sheep Figure 41

Note: Sheep hair is generally quite recognizable, however, separation of dall and bighorn samples is difficult. The distribution of these species is such that there is little opportunity for recovery of both in one wolf scat sample (see Banfield 1974, page 416).

16C. Cuticle scale pattern at base similar to sheep, however, individual scales tend to have prominent leading edges. Structure of scales medially similar to sheep but more elongated. Hair thick, brown to grey at base becoming white at tip; never kinky.----------Caribou Figure 42

16D. Cuticle scales at base similar to caribou but individual scale edges more rough; scales also slightly more elongate. Hair entirely white thin, limp, less keratenous than others in Group A.

----------Mountain goat Figure 43

Note: Proper separation of members of Group A initially appears difficult, however, distribution of these species are quite different which allows the examiner to eliminate the most confusing pairs.

17A. (15) Cuticle scale distant, smooth and regular mosaic in form at base becoming close, rough and regular wavy at tip. Hair diameter wide at base (ranging from 330 µm to 520 µm). Number of scales across base averaging 7.5. Medially hair diameter ranges from 190 - 450 µm,

number of scales averages 4.9. Macroscopically hair appears very thick and kertenous. Hair is generally white below and brown-black above.----------Moose adult

Figure 44

17B. Cuticle scale pattern similar to moose, however, leading edges of scales very pronounced forming a "looping" effect. Hair diameter at base from 180 µm to 380 µm basally; 170 - 380 µm medially, number of scales across base averaging 5.8. Hair usually grey brown to white with a prominent orange band on upper 1/3 of hair shaft.

----------Deer adult

Figure 45

Note: With present techniques it is impossible to separate white-tailed deer and mule deer with a high degree of confidence. We have therefore lumped these species.

5. KEY TO AVIAN PREY

1A. Barbule nodes restricted to tip (upper 1/3 of barbule) of pennulum and distinctly triangular shaped.

----------Anseriformes (waterfowl)

Figure 46

1B. Barbule nodes along entire pennulum and appear rounded.

----------Galliformes (game birds)

Figure 47

DISCUSSION

1. LIMITATIONS OF PRESENT KEY

Confining the key to only the prey items previously reported in the literature allows a possibility of overlooking prey species that occur only occasionally. In the present key we have taken the approach that specific, detailed descriptions of the major prey species is less confusing and hence more accurate than using general descriptions of many possible prey

species. Further, the key describes the hair of most prey encountered in wolf scats from northwestern Canada. It is unlikely that the examiner will encounter any sample that will not fit this key to at least the family level. If it is not possible to assign a specimen to species, the examiner may find it useful to refer to the more general publications to obtain these identifications. Moore *et al.* (1974) and Adorjan and Kolenosky (1969) have presented examples of hair structure for a large number of mammal species.

The chief disadvantage to the use of keys is that it is time consuming. Many hours of preliminary work with known samples are required before the analyst will feel confident with his/her identifications. However, at present we see no other viable means to identify hair.

2. PROBLEM AREAS IN SCAT/HAIR ANALYSIS

Two major concerns exist in wolf predation analyses. The first involves the correctness of the relationship of frequency occurrence data obtained from scat analysis and the actual wolf predation rate on various prey species. The second is involved with the accuracy of the identification of prey items.

Researchers agree that the number of occurrences recorded from scat samples accurately reflect the number and biomass of individual prey items eaten (Scott 1941, Lockie 1959). Floyd *et al.* (1978) hypothesized that smaller prey animals such as snowhoe hare are over represented in terms of biomass and under represented with regard to numbers. Further, they postulated that with an understanding of the rate of prey consumption it is possible to calculate a correction factor for prey biomass estimates

based wolf scat analyses. Based on data from a controlled feeding experiment a linear equation representing the relationship between the weight of prey per scat and total prey weight was calculated.

This equation has predictive value in calculating prey weights of a known number of prey items, however, some qualifications are necessary before this technique is applied to other wolf food habits data. First, Floyd *et al.* (1978) base their regression calculation on only collectable scats, not non-collectable fecal material (liquid scats). Non-collectable scat must be omitted from biomass considerations when using the regression equation. A second consideration required concerns prey weight assumptions. When using the equation one must correct the weights given by Floyd *et al.* (1978) for prey animals actually taken in other study areas. For example, there is considerable difference between the indicated weight of white-tailed deer from areas of northern Canada. Also, more biomass from larger prey species is left uneaten than in smaller species. This factor introduces problems when calculating the biomass of these large prey. The amount of prey left in these cases (ie. moose or elk) is undoubtedly much higher than that of deer. Further wolf feeding trials are needed to evaluate these prey weight calculations. Finally, frequency occurrence data, because it includes scats that contain more than one item, cannot be directly compared to the regression data. The error is small if only a few scats contain more than one item but increases linearly as the number of scats with multiple food items increases.

The second problem encountered in wolf scat analysis concerns the identities of the remains recovered. Carbyn (1973) has shown that the possibility of errors in identification of prey items in wolf diets is high.

This problem appears related to the type of hair being examined. For example, the hairs of certain animal groups (i.e. cervidae) require closer examination than others. The present work is intended to reduce errors in scat analysis.

3. FURTHER RESEARCH REQUIREMENTS

Hair identification by anatomical components is effective but suffers from inherent subjectivity. Quantitative physiological and biochemical hair tests could be more accurate methods of hair identification. Preliminary work carried out mainly for forensic studies, have shown that biochemical and physiological differences are detectable in hair of different species.

Studies documenting hair trace elements (Franzman *et al.* 1976) and the activation these trace elements (Kennington and Ching 1971) have shown that there are definite significant differences between hairs of different mammal species. Problems occur in the interpretation of such data, however because there are also signficant differences in hair trace elements within the same species inhabiting separate habitats. Such differences are also being used in birds to determine where they were raised. At present it is impossible to be sure that range condition does not effect those data found in trace element experiments.

Electrophoresis has been used to separate specific protein chains from hairs to subsequently determine sex (Degraf and Larson 1972) or to separate animal groups (Swart and Joubert 1968). Although initial results are promising, to date no significant species specific amino acids have been found.

Biochemical identification of hair from fecal passages may not be possible for it is not known what biochemical changes occurred in the hair as it passes through the digestive system of an animal. A further deterrent to biochemical studies is the cost. Therefore, although these studies may prove to be accurate at present, there are severe limitations to their use in predation studies. The need for further studies to determine practicabilities of biochemical methods is clear.

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APPENDICES

APPENDIX A. LISTING OF REPORTED PREY SPECIES OF WOLVES FROM NORTHWESTERN CANADIAN NATIONAL PARKS.

CERVIDAE (deer family)

Elk - *Cervus canadensis*
Moose - *Alces alces*
Deer - *Odocoileus* sp.
Caribou - *Rangifer* sp.
Elk calf - *Cervus canadensis*
Moose calf - *Alces alces*
Deer fawn - *Odocoileus* sp.

BOVIDAE (sheep and allies)

Dall sheep - *Ovis dalli*
Rocky Mountain Bighorn sheep - *O. canadensis*
Rocky Mountain goat - *Oreamnos americanus*
Domestic cow - *Bos* spp.
Bison - *Bison bison*
Bison calf - *B. Bison*

EQUIDAE (horse family)

Horse - *Equis caballus*

CANIDAE (dog family)

Timber wolf - *Canis lupus*
Coyote - *C. latrans*

URSIDAE (bear family)

Grizzly bear - *Ursus arctos*
Black bear - *U. americanus*

LEPORIDAE (rabbits and allies)

Snowshoe hare - *Lepus americanus*

CASTORIDAE (beaver)

Beaver - *Castor canadensis*

ERETHRIZONTIDAE (porcupine)

Porcupine - *Erethrizon dorsatum*

SCIURIDAE (squirrel family)

Columbian ground squirrel - *Spermophilus columbianus*
Golden-mantled ground squirrel - *S. lateralis*
Franklins ground squirrel - *S. franklinii*
Richardsons ground squirrel - *S. richardsonii*
Arctic ground squirrel - *S. parryii*
Red squirrel - *Tamiasciurus hudsonicus*
Marmot - *Marmota caligata*

CRICETIDAE (mice, voles and allies)

Deer mouse - *Peromyscus maniculatus*
Meadow vole - *Microtis pennsylvanicus*
Red-backed vole - *Clethrionomys gapperi*

RODENTIA (rodents)

Muskrat - *Ondatra zibethicus*

AVIAN (birds)

Anseriformes - (waterfowl)
Galliformes - (grouse)

APPENDIX B. LISTING OF MATERIALS REQUIRED FOR HAIR EXAMINATION.

1. Microscope slides (2.6x7.6 mm, 1.1 to 1.3 mm thickness).
2. Cover slips (22x22 mm, 22x40 mm, 22x50 mm, No. 1 thickness).
3. Single-sticky slides clear tape.
4. Slide labels.
5. Methyl salicylate (synthetic) laboratory grade.
6. Standard rule (in mm).
7. Dissecting microscope.
8. Compound microscope with 10X and 25X objectives.
9. Ocular micrometer with calibrated μm scale.
10. Clear polyvinyl chloride plastic, 0.03 mm, 0.09 mm or 0.13 mm thickness.
11. Xylene.
12. Microforceps.
13. Mounting medium (permount).
14. Oven, temperature range 100-130°C with thermostatic control.
15. Scale cast press made from two pieces of smooth 3/4 inch hardwood, cut three inches wide and five inches long. A hole is drilled in both ends of each block and fitted with small bolts, each with a wingnut. Tightening the nuts serves to compress items held between the blocks. Large bulldog style paper clips will suffice if a press is not available.

APPENDIX C. STEPWISE SUMMARY OF LABORATORY TECHNIQUES USED IN SCAT ANALYSIS.

1. Wash sample and dry in covered heat resistant containers at 150°C.
2. Collect distinctive primary guard hairs, and any other identifiable material from sample.
3. Clear hairs with an organic solvent; carbon tetrachloride, methyl salicylate, xylene or acetone have been used with varying degrees of success.
4. Place 1-4 clean primary guard hairs on a plastic base and sandwich these between two microscope slides. Large paper clips can be used to hold the hairs in place.
5. Heat preparation to 120°C for 10-15 minutes.
6. Displace hair with scalpel and cover with cover slip (20 mm x 60 mm).
7. After examination hair samples can be stored in slide boxes for future reference.

APPENDIX D

FIGURE CAPTIONS

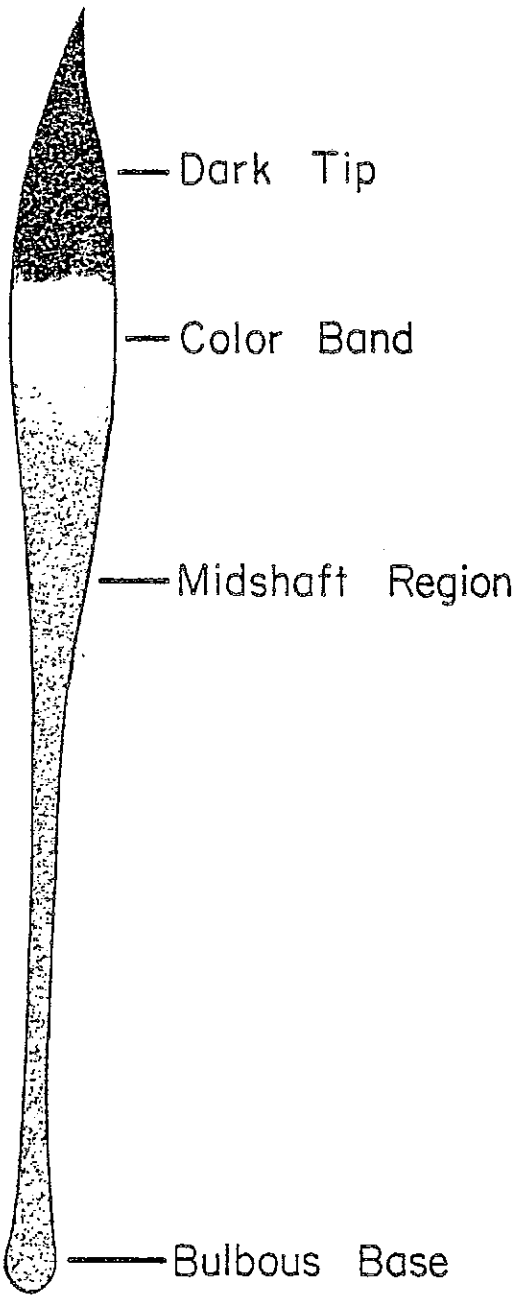


Figure 1.

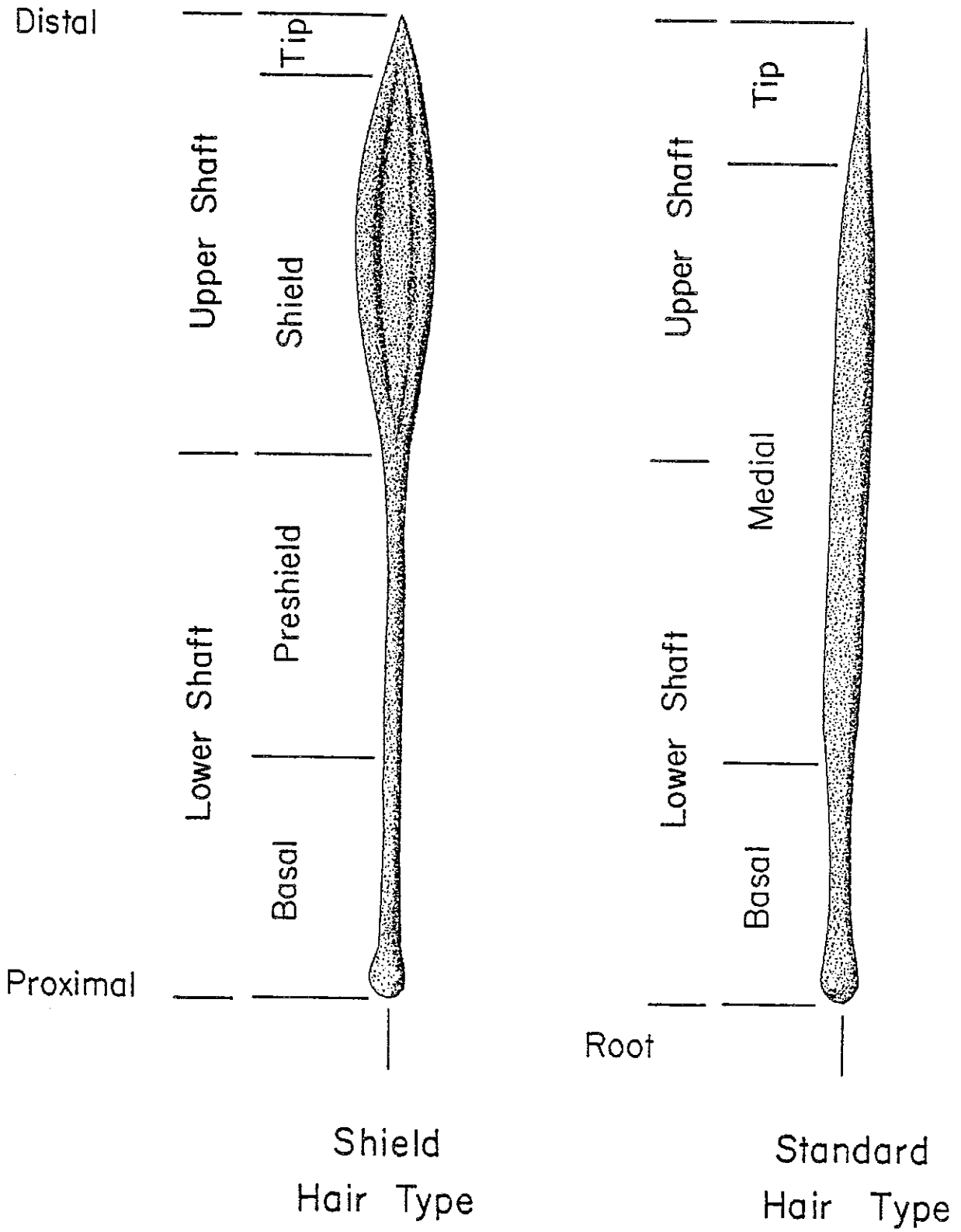

Distal
Tip
Upper Shaft
Shield
Lower Shaft
Preshield
Basal
Proximal
Shield
Hair Type
Tip
Upper Shaft
Medial
Lower Shaft
Basal
Root
Standard
Hair Type

Figure 2.

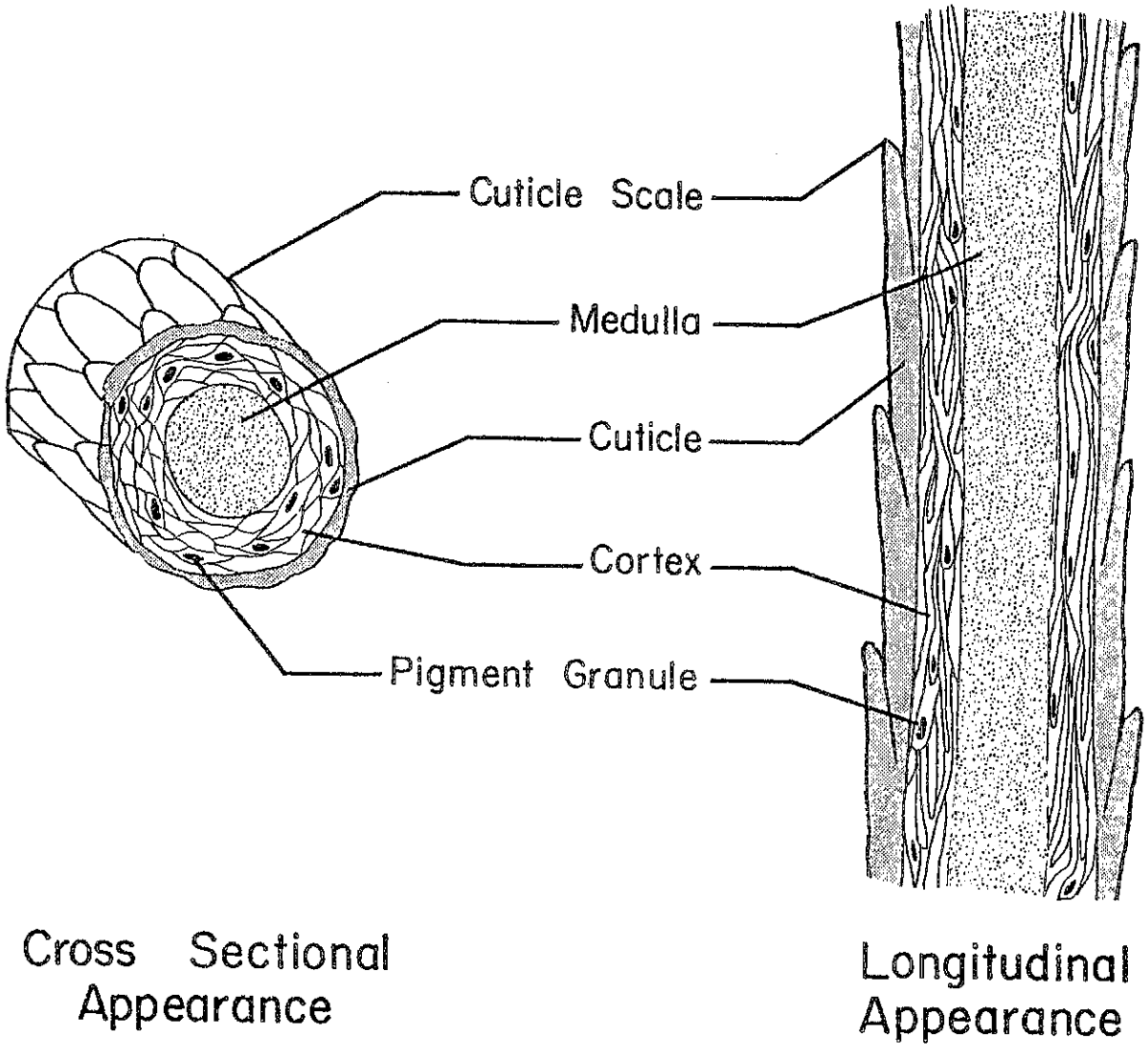
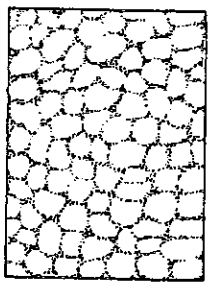
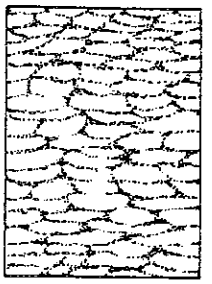

Cuticle Scale
Medulla
Cuticle
Cortex
Pigment Granule
Cross Sectional Appearance
Longitudinal Appearance

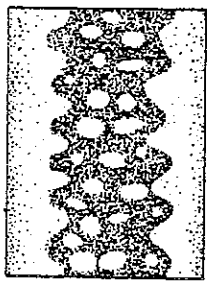
Figure 3.



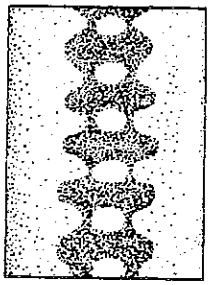
A. Continuous, complete lattice form



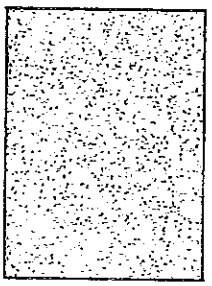
B. Continuous, complete/incomplete flattened lattice form



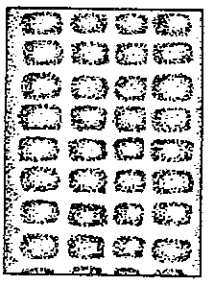
C. Discontinuous, incomplete intrusive bar



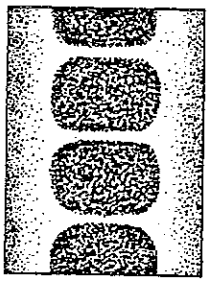
D. Continuous, incomplete vacuolated intrusive bar



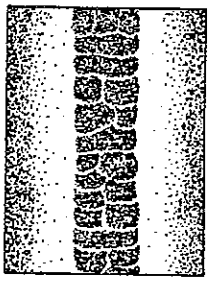
E. Continuous, complete/incomplete granular



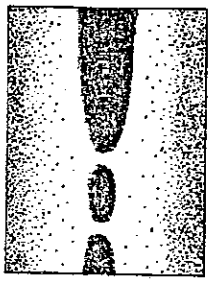
F. Continuous, incomplete Multiserial (spiral) ladder



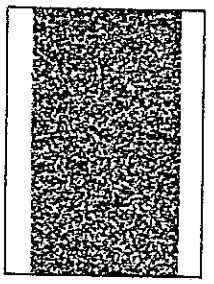
G Continuous, incomplete unicellular ladder

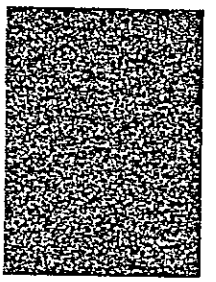


H. Continuous, incomplete dicellular ladder

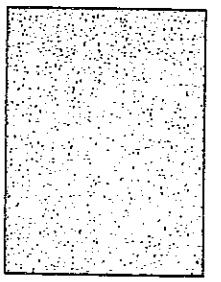


I. Discontinuous, incomplete fragmental

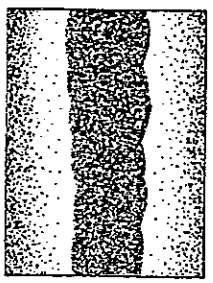




J. Discontinuous, incomplete/complete homogeneous tube



K. Discontinuous, incomplete absent



L. Discontinuous, incomplete amorphous tube

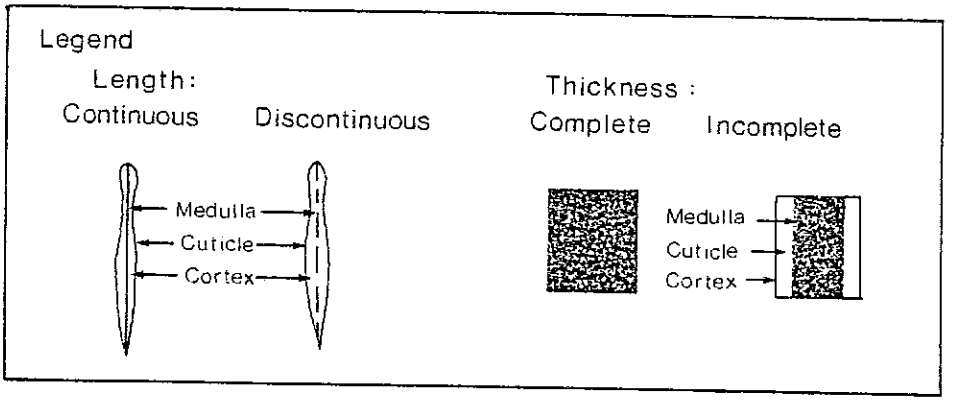
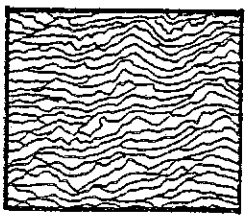


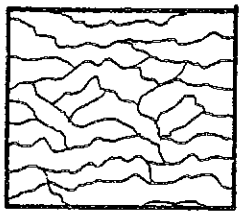
Figure 4.

SCALE DISTANCE



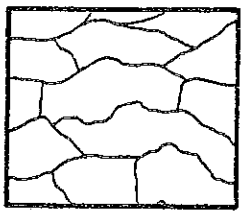
Close

5 µm
between margins



Intermediate

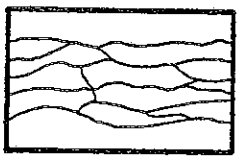
5 - 10 µm
between margins



Distant

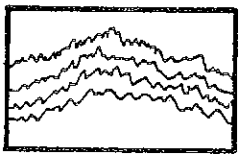
10 µm
between margins

SCALE MARGIN



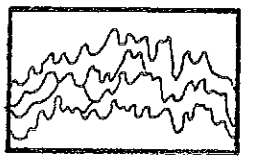
Smooth

no interruptions
irregularities or
indentations



Rough

saw-toothed
appearance

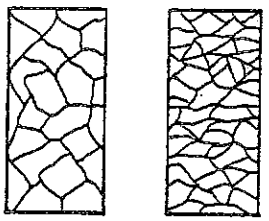


Crenate

similar to rough
but with scallop
like indentations

SCALE PATTERNS (form)

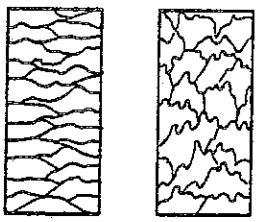
Mosaic



regular
mosaic

irregular
mosaic

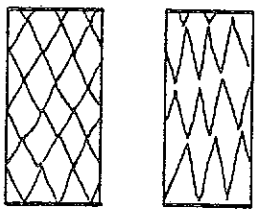
Wavy



regular
wavy

irregular
wavy

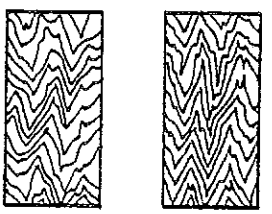
Dentate



diamond
dentate

elongated
dentate

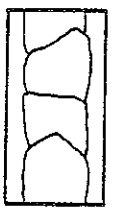
Chevron



single
chevron

double
chevron

Coronal



simple
coronal

Figure 5.

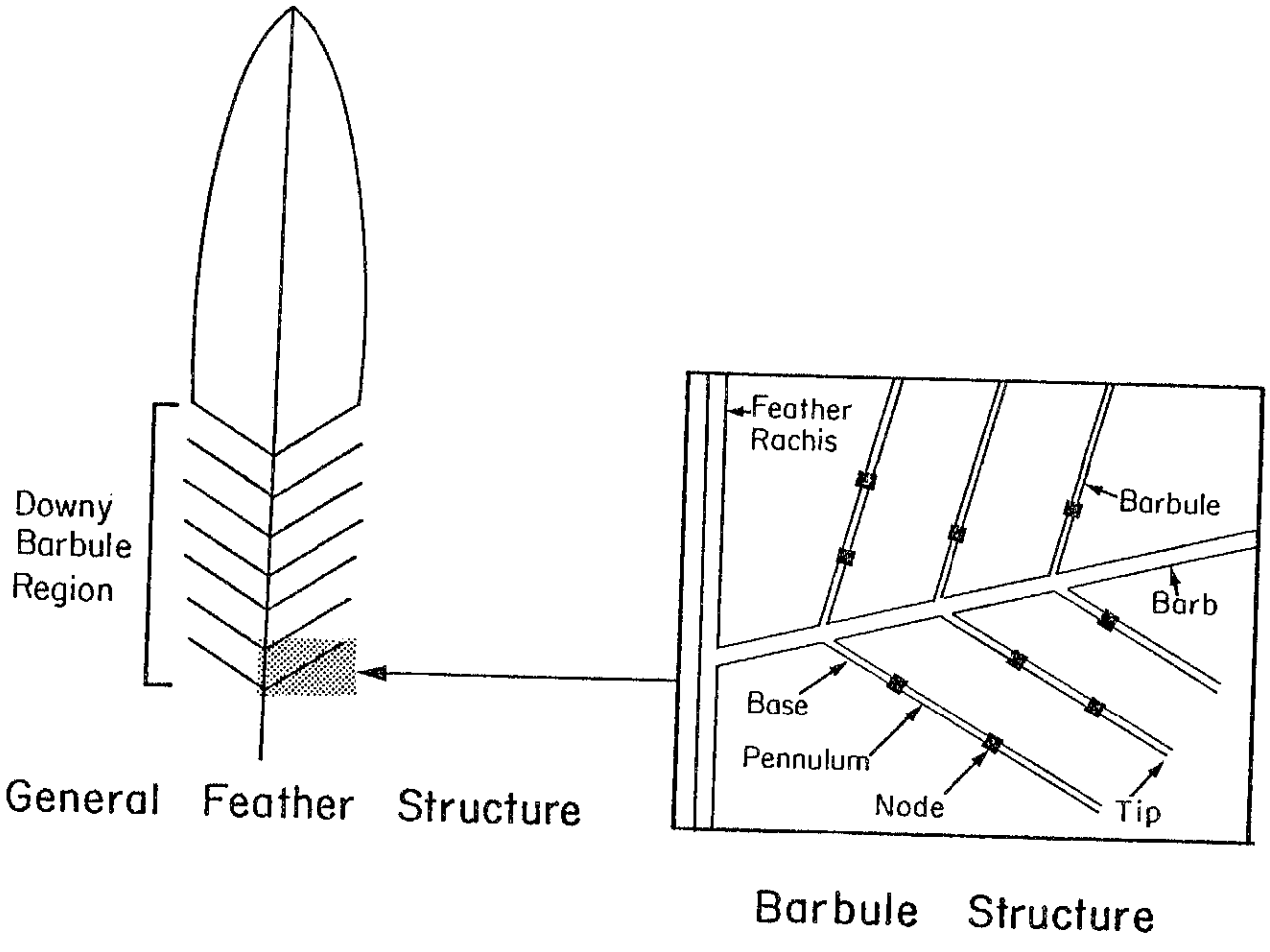
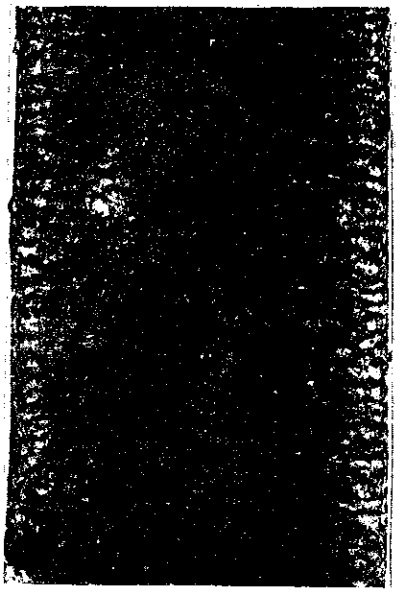

Downy
Barbule
Region
General Feather Structure
Feather
Rachis
Barbule
Barb
Base
Pennulum
Node
Tip
Barbule Structure
Node Structure of:
Galliformes and Anseriformes

Figure 6.

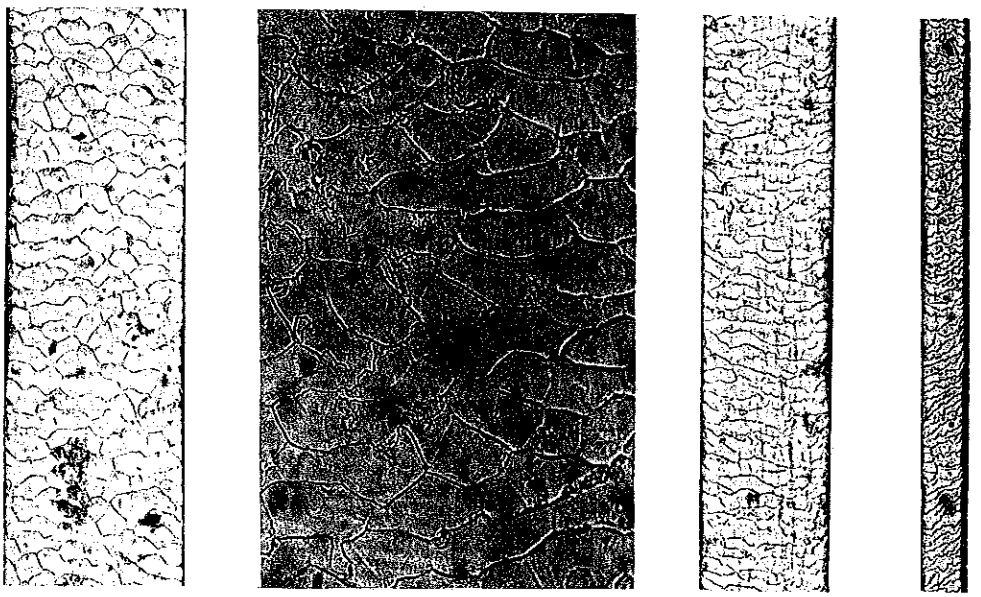
A

Fig. 7



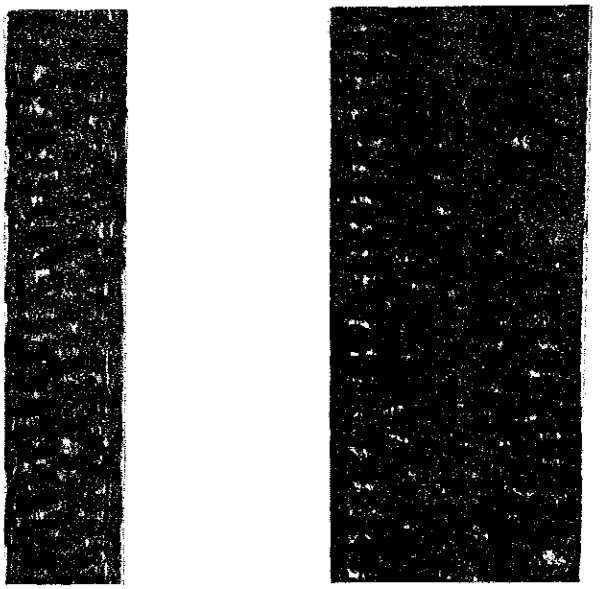
A

Fig. 8



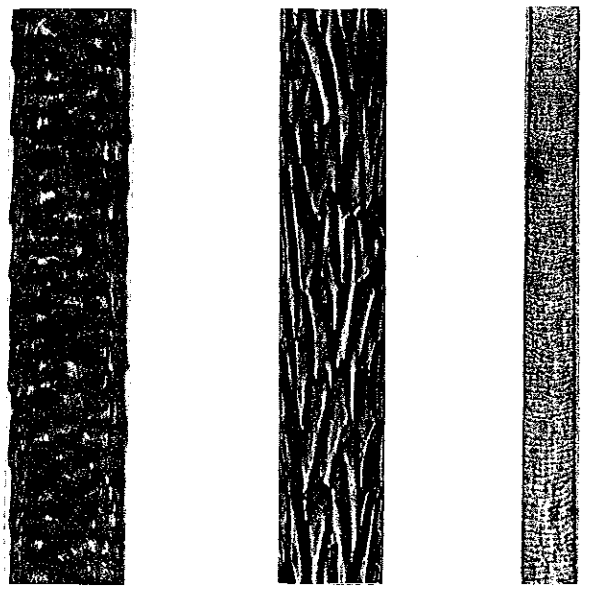
A B C D

Fig. 9



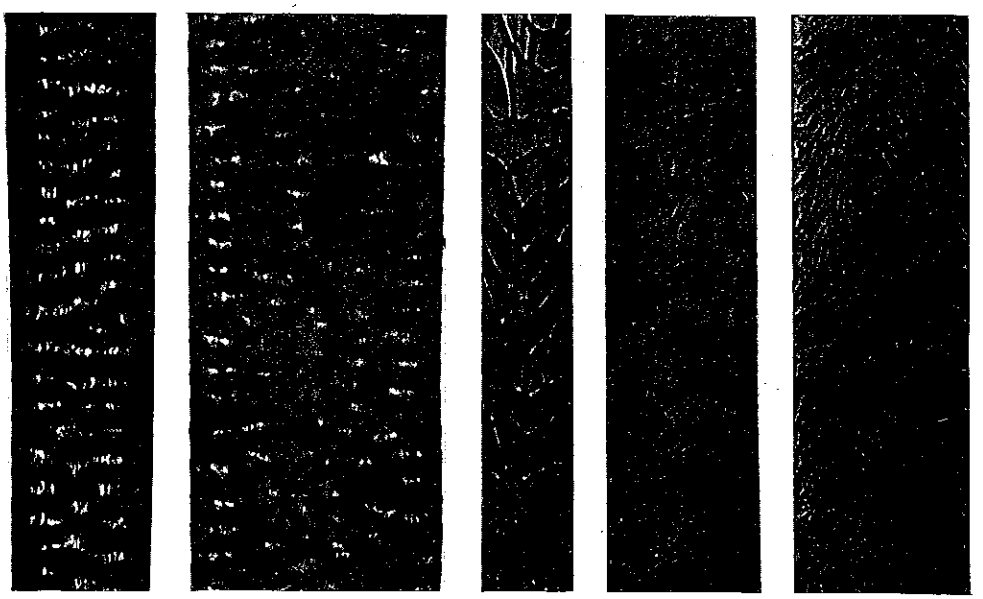
A B

Fig. 10



A B C

Fig. 11



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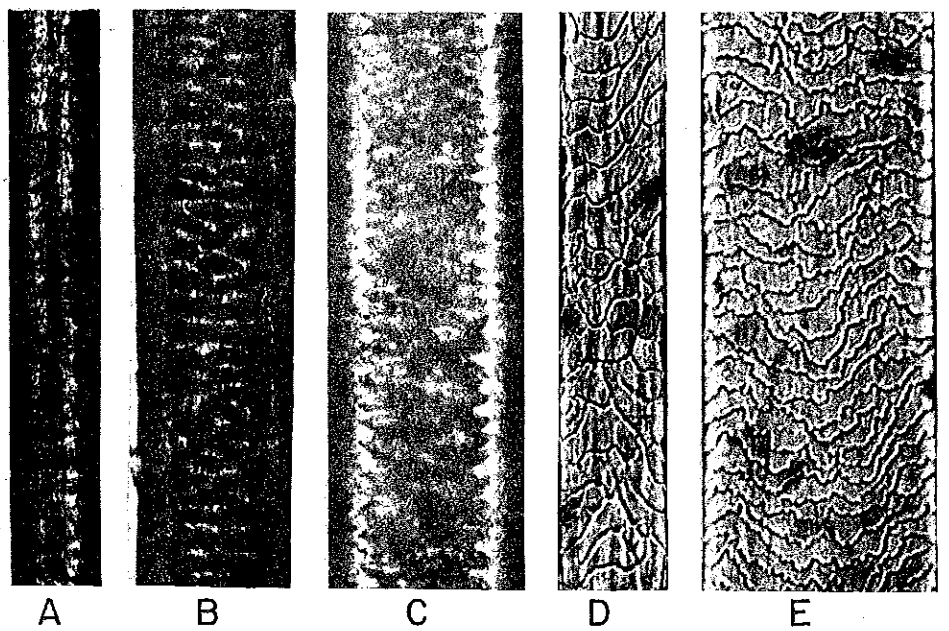


Fig. 13

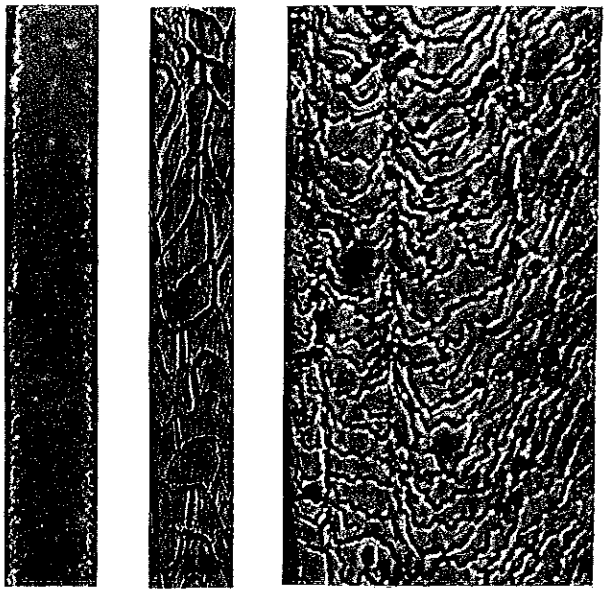


Fig. 14

Fig. 15

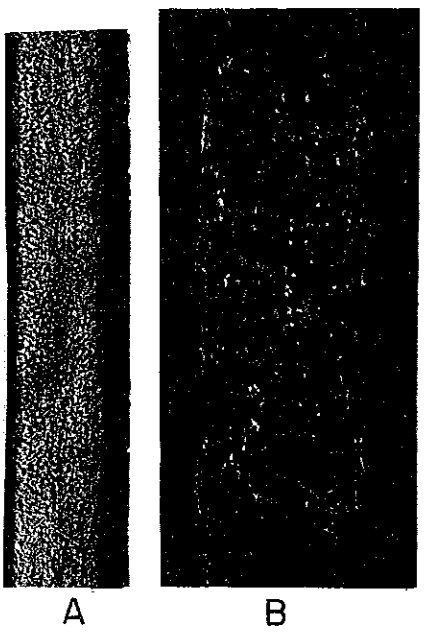


Fig. 16

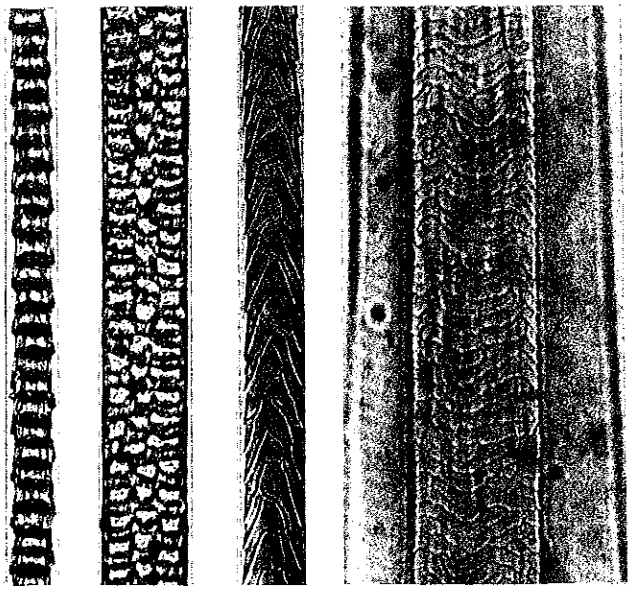


Fig. 17

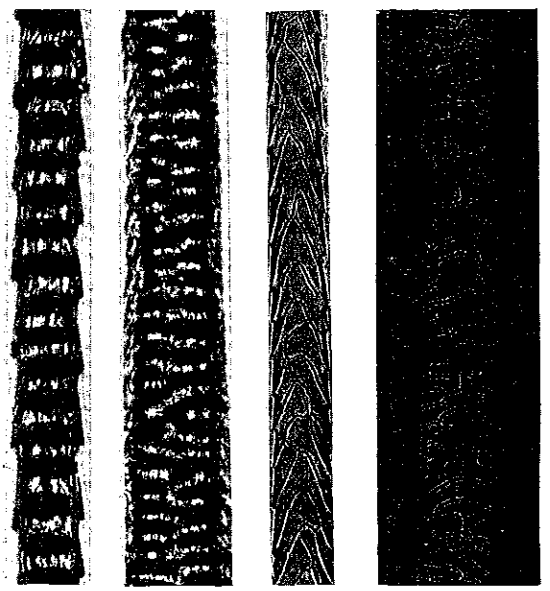


Fig. 18

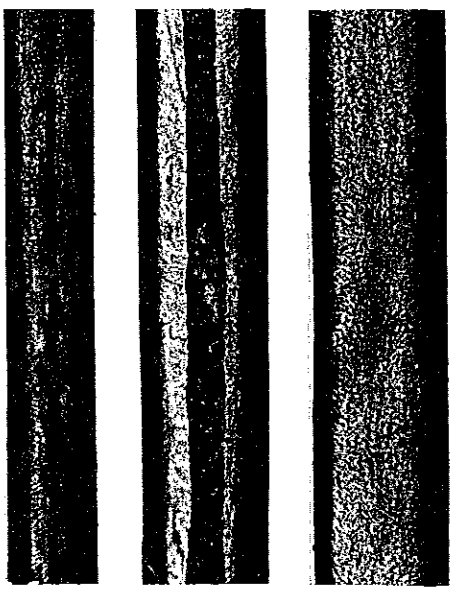


Fig. 19

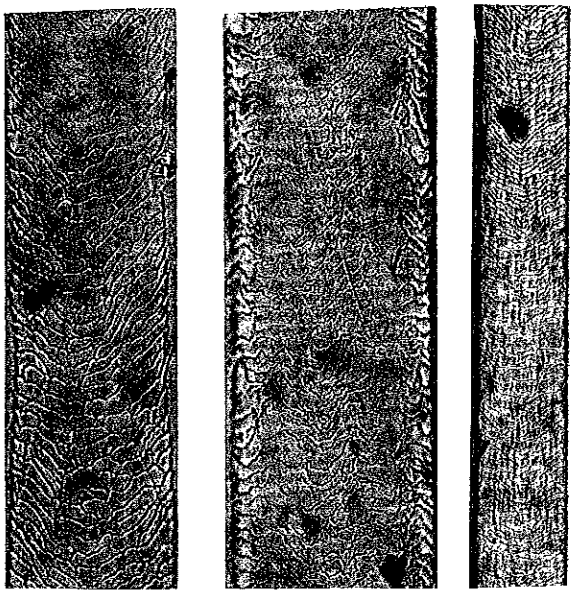


Fig. 20

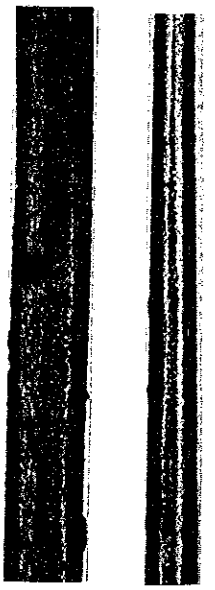


Fig. 21

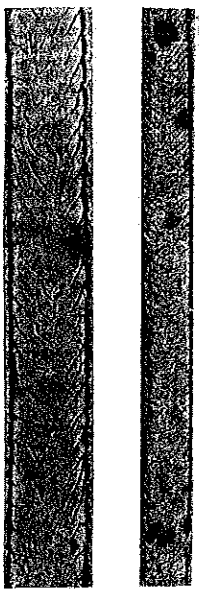


Fig. 22

Fig. 23

Fig. 24

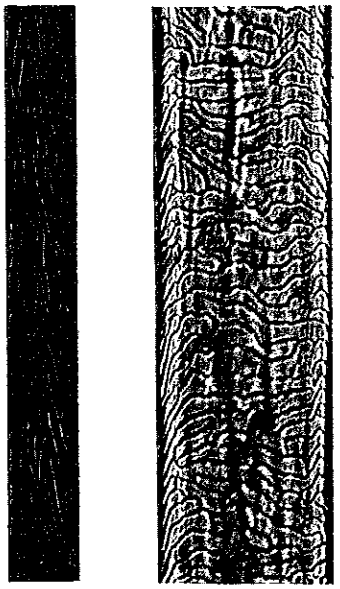


Fig. 25

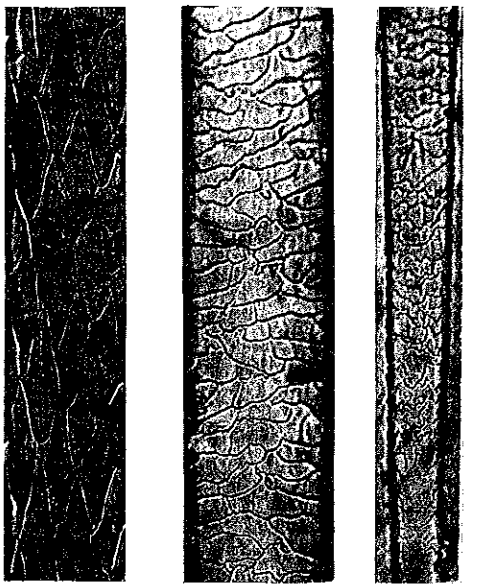


Fig. 26

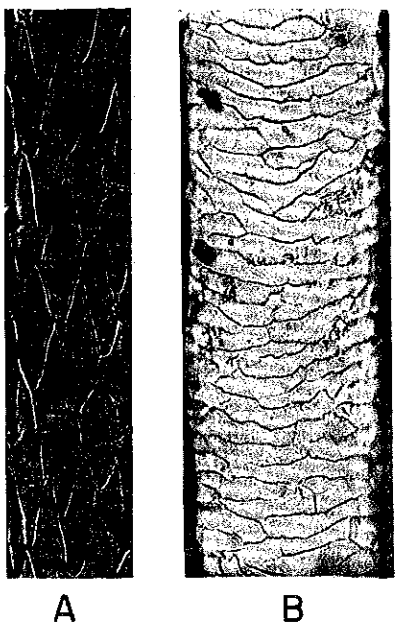


Fig. 27

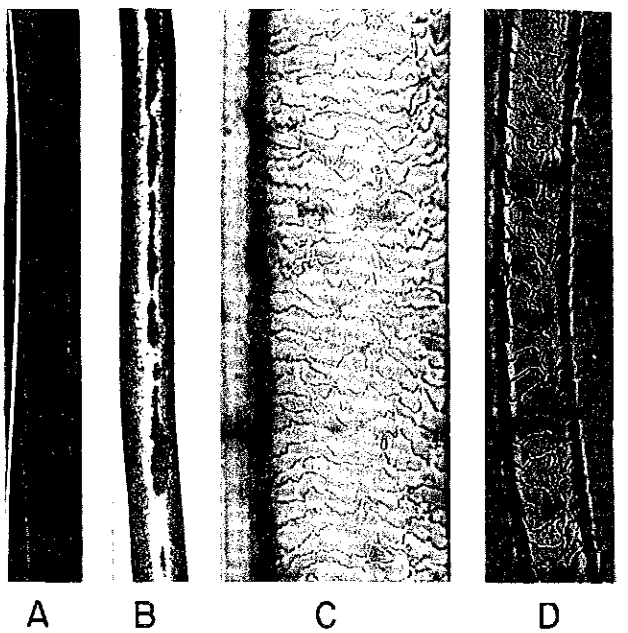


Fig. 28

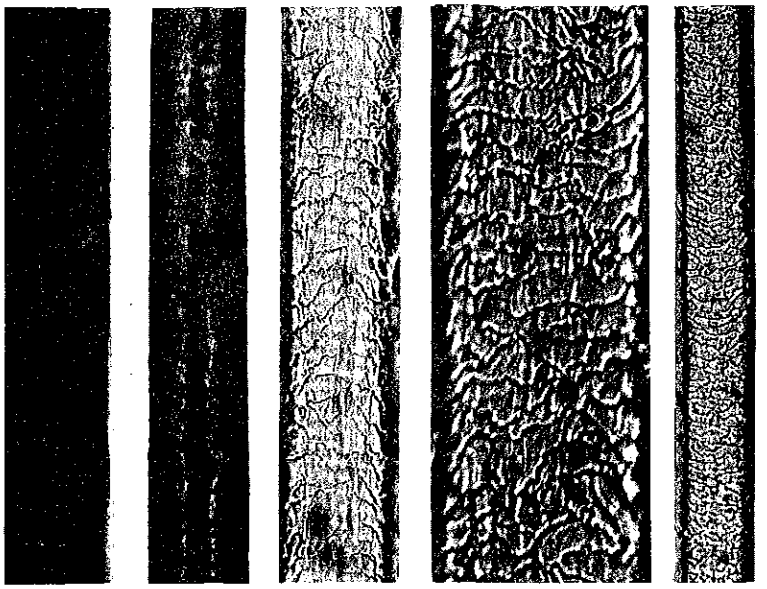


Fig. 29

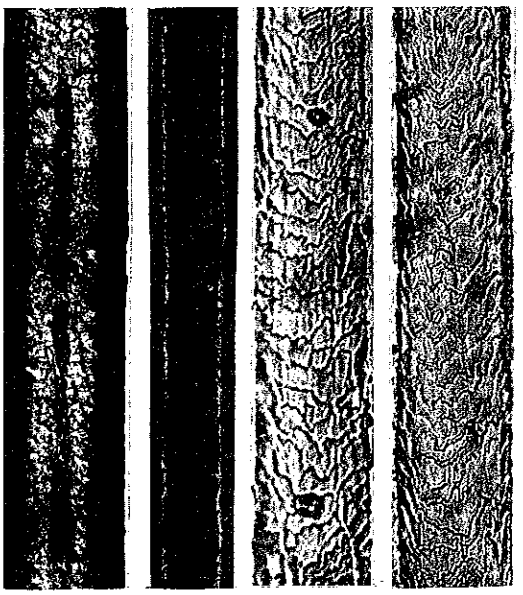


Fig. 30

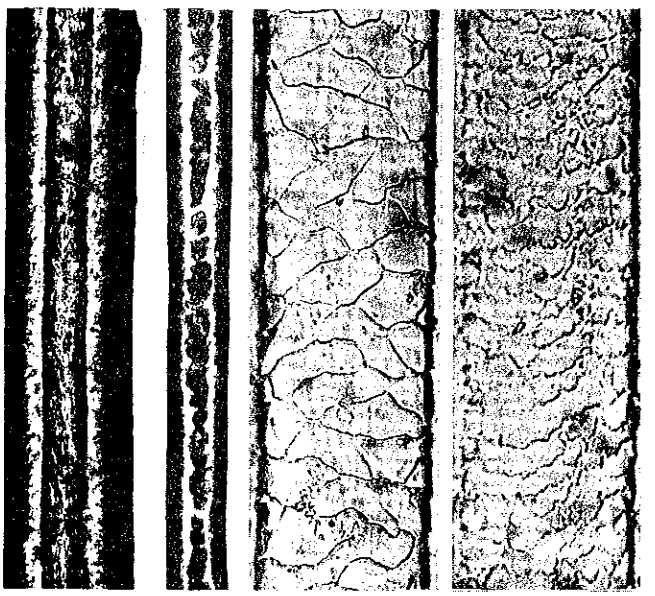
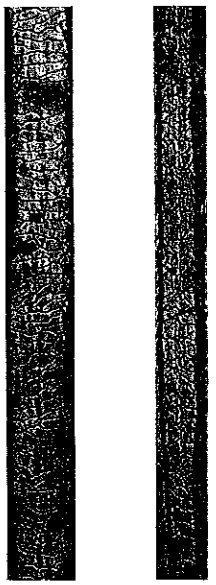
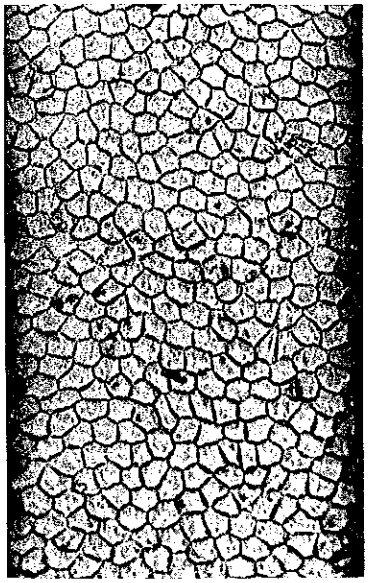


Fig. 31



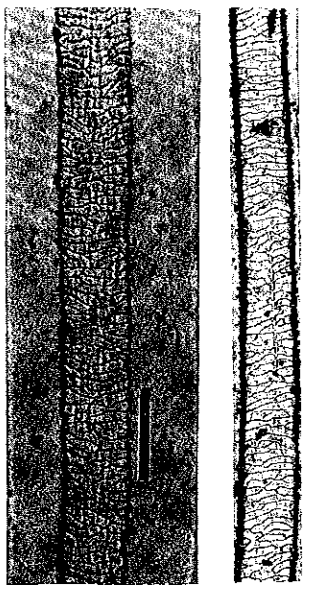
A B

Fig. 32



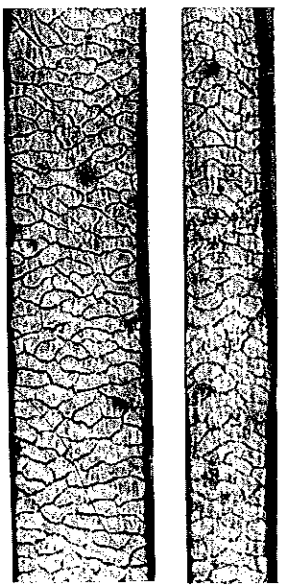
A

Fig. 33



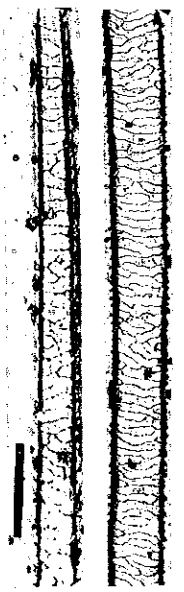
A B

Fig. 34



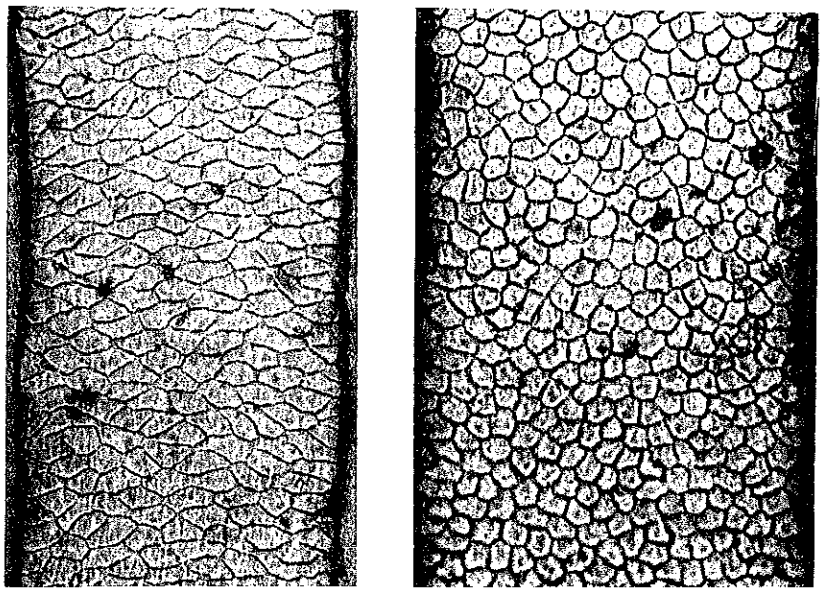
A B

Fig. 35



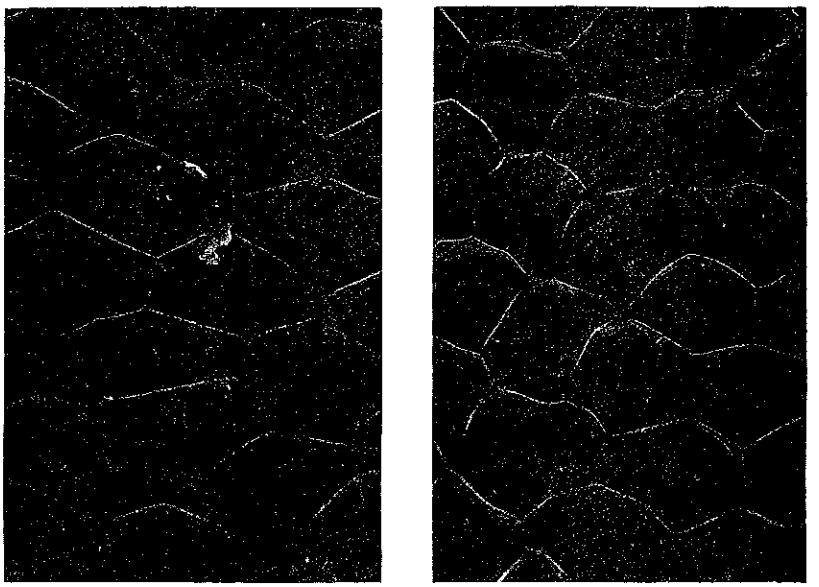
A B

Fig. 36



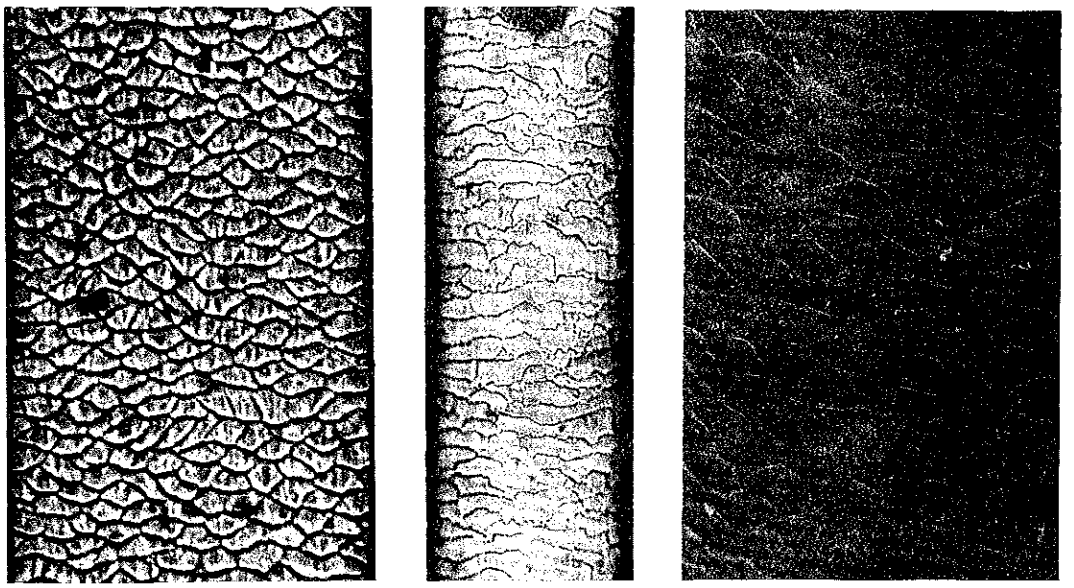
A B

Fig. 37



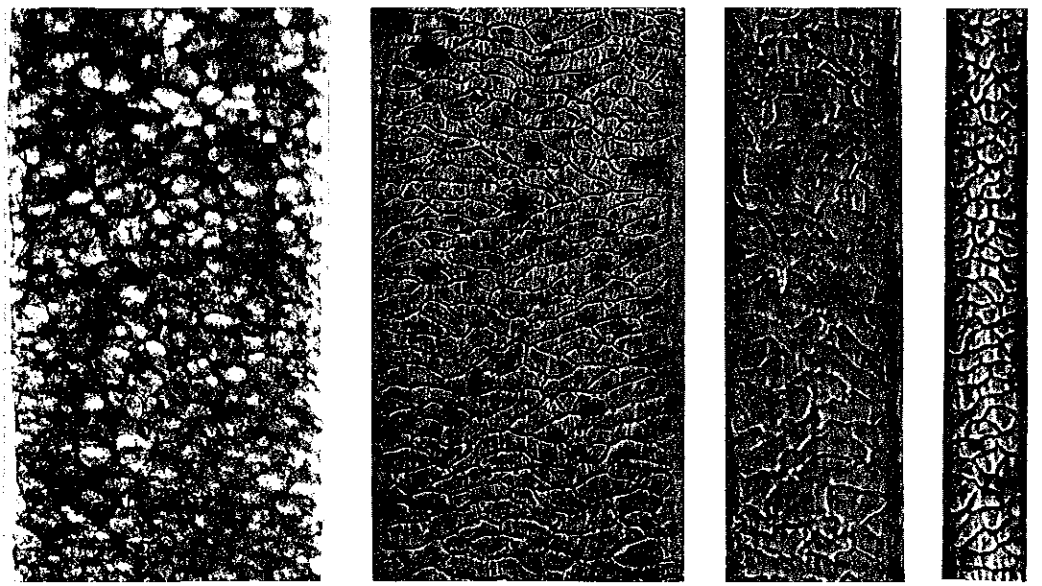
A B

Fig. 38



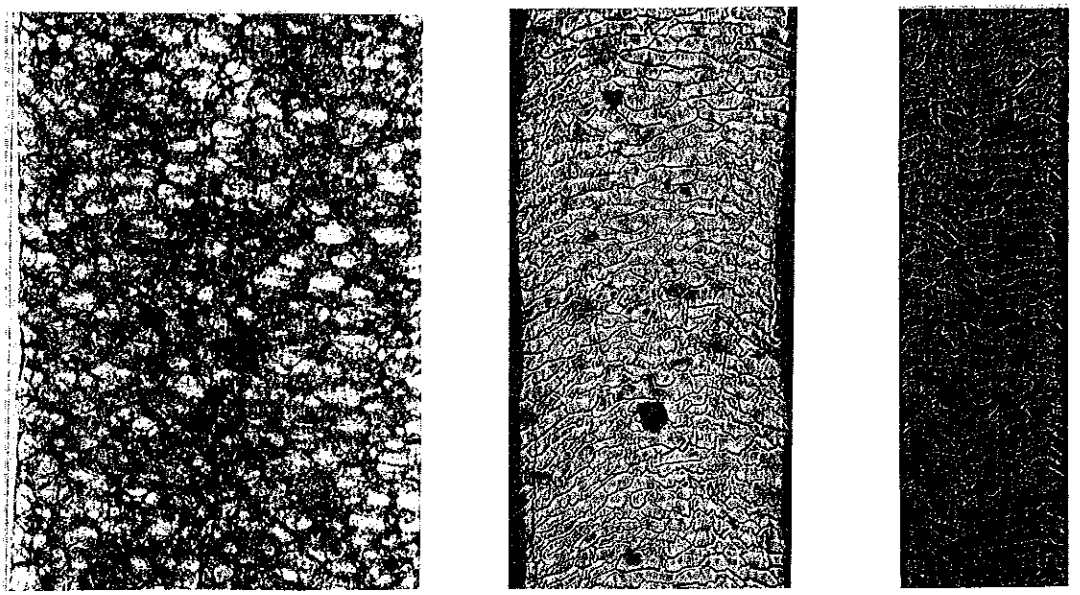
A B C

Fig. 39



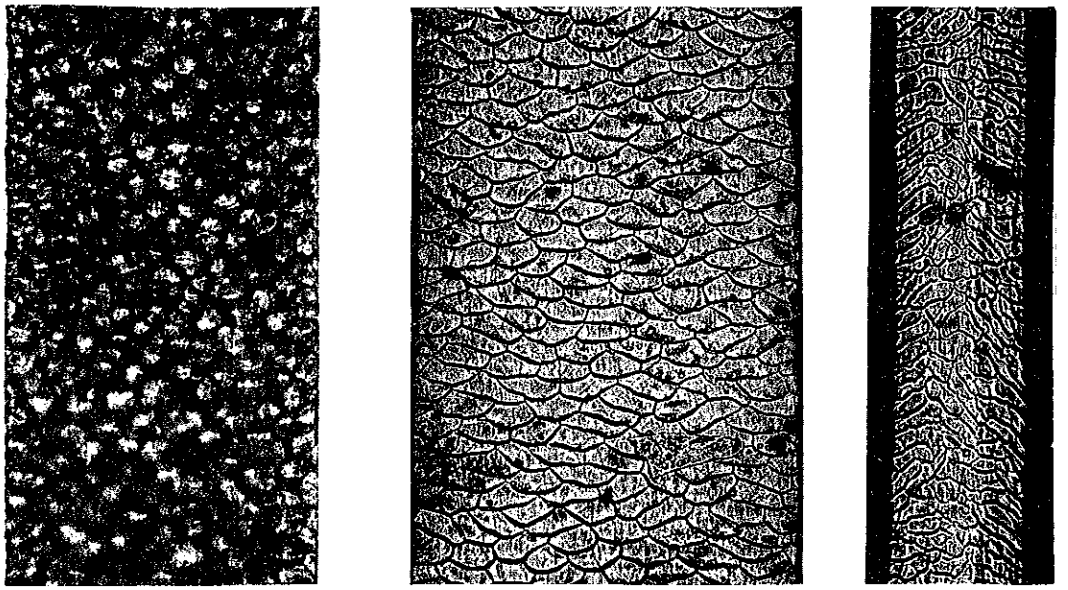
A B C D

Fig. 40



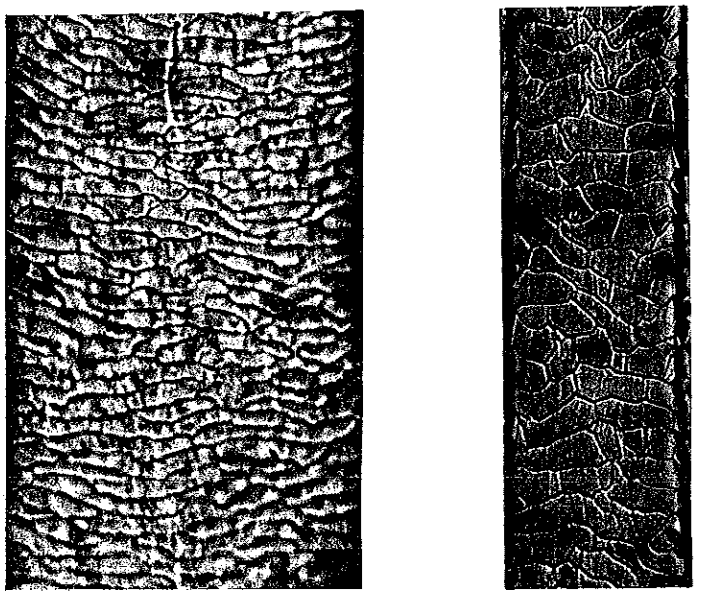
A B C

Fig. 41



A B C

Fig. 42



A B

Fig. 43

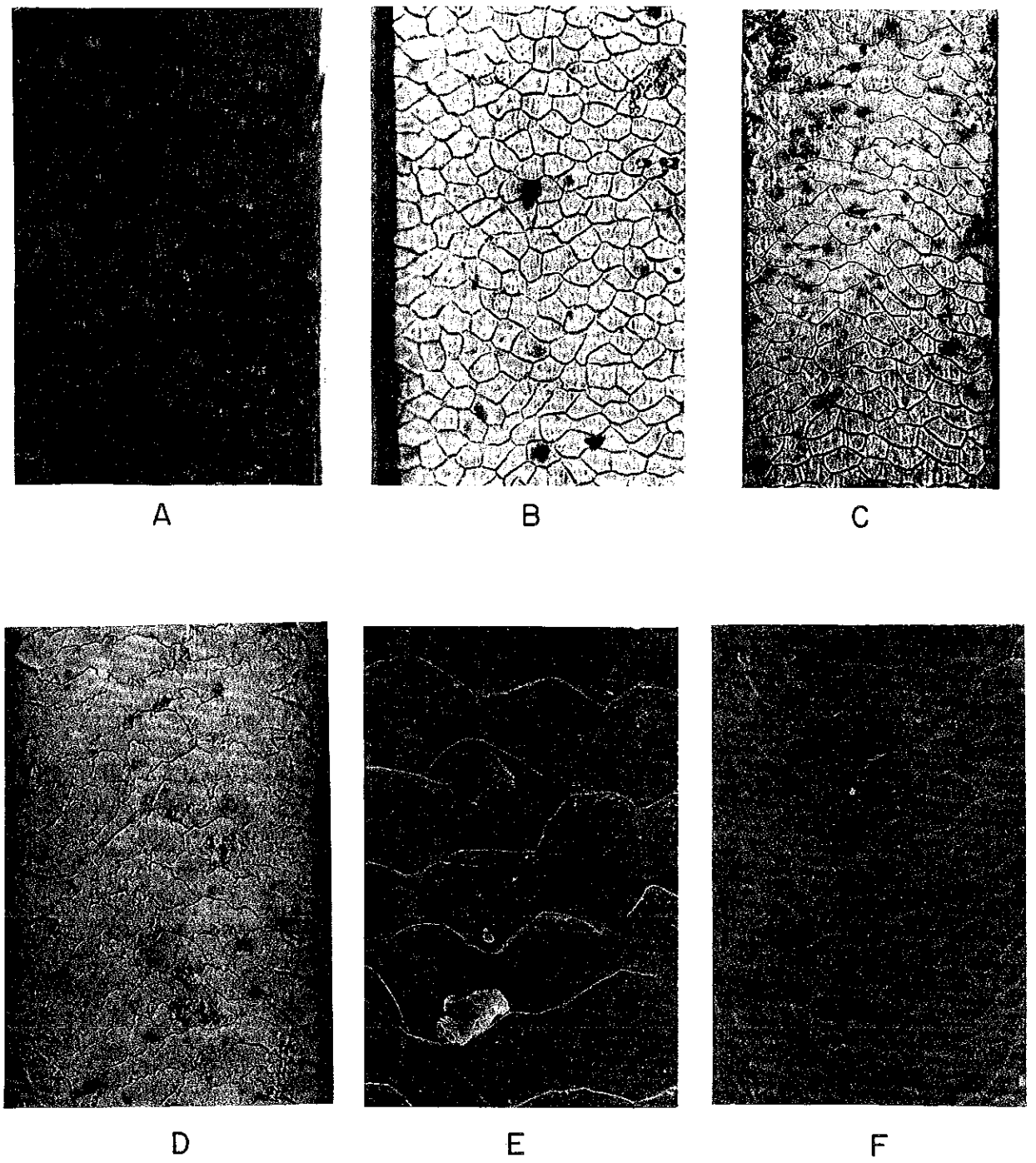
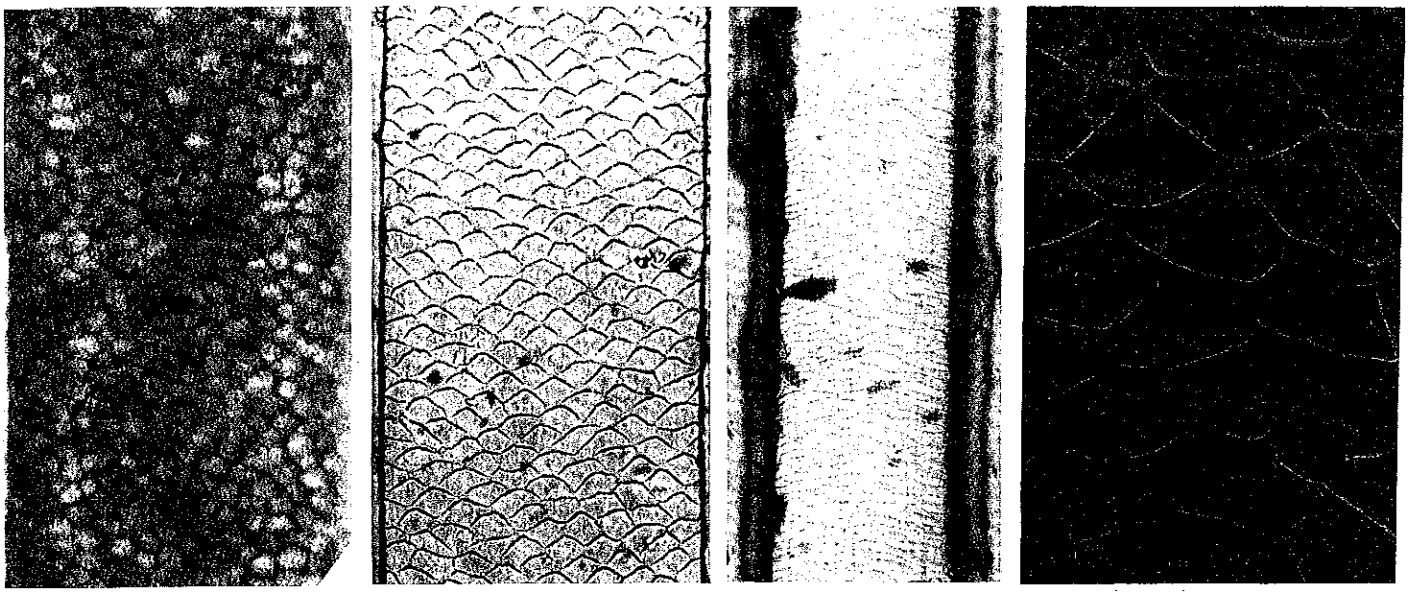
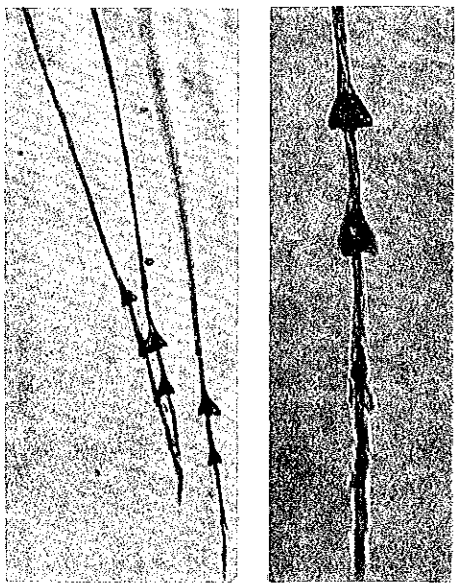


Fig. 44



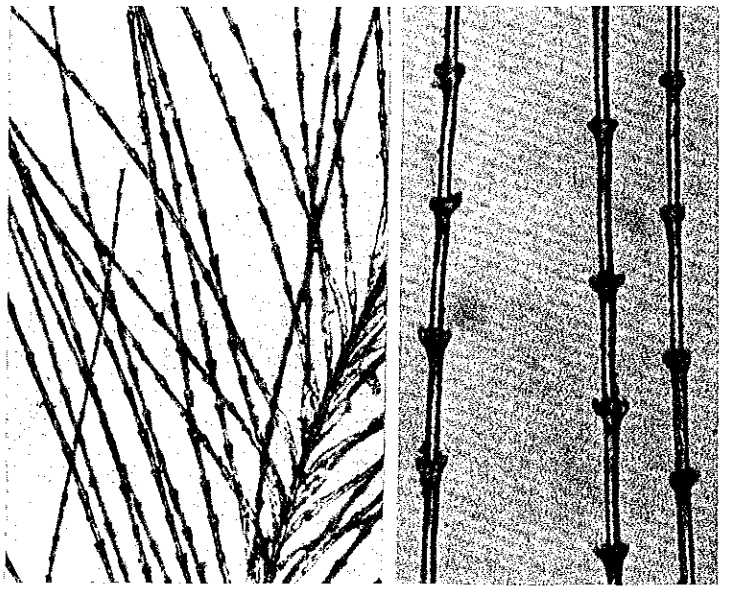
A B C D

Fig. 45



A B

Fig. 46



A B

Fig. 47

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