A comparative study of Bowman's layer in some mammals: Relationships with other constituent corneal structures

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SUMMARY

Existing morphological and morphometric studies of mammalian cornea offer little information about the morphology and morphometry of Bowman's layer. Furthermore, no data regarding the relationship between Bowman's layer and other corneal structures are currently available. It is for this reason that we have decided to carry out a comparative study of the main features of Bowman's layer in 40 species of mammals (carnivores, primates and herbivores) and to determine its relationship with other corneal layers.

The results pointed out the existence of Bowman's layer in nearly all the primates studied. The only exception was the lemur (*Lepilemur mustelinus*). Bowman's layer was absent in all the carnivores in the study but was present in some herbivores (deer, sambar deer, giraffe, ox, zebu and eland). In addition, there appears to be a certain relationship between the presence of Bowman's layer and the thickness of the epithelium and Descemet's membrane.

Key Words: Bowman's layer – Mammals – Corneal epithelium – Descemet's membrane – Morphometry

INTRODUCTION

Bowman's layer was described in humans by William Bowman (1947) at the Royal London Moorfield Ophthalmic Hospital (Jacobsen et al., 1984). The author defined it as an lusterless but perfectly transparent, continuous, homogenous, elastic lamina that did not appear to have any internal structure. Later, other authors were to study its presence in other species. Wislocki (1952) noted Bowman's layer in *Macaca mulata* (rhesus macaque). Calmettes et al. (1956) studied the corneas of several species of domestic and laboratory animals and came to the conclusion that Bowman's layer was not present in all species. In some cases, in its place there was a simple condensation of the stroma. This superficial stroma condensation appeared to have both considerable thickness and homogeneity in certain species and was thus considered to be a rudimentary Bowman's layer.

For many years, Bowman's layer was thought to be present only in the cornea of primates. However, Winquist and Rehbinder (1973) confirmed its presence in the cornea of the reindeer, and Rhebinder et al. (1977) did likewise in the case of the elk and the deer. However, Rahi et al. (1980) found no trace of it in the cornea of the

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camel. Camber (1987) found that in the case of the pig, Bowman's layer was unequal and corrugated in form, thus making it difficult to distinguish it from the surrounding fine collagenous fibres.

Lantzing and Wright (1981) identified Bowman's layer in a few species of bony fishes and Collin and Collin (1998), reported that Bowman's layer was not present in many of the teleost species examined. However, Bowman's layer is easily reconogizable in the sea lamprey and in cartilaginous fishes (Keller and Pouliquen, 1988)

With the exception of the above mentioned cases, very little is known about the existence of Bowman's layer in other mammals.

The purpose of this study was thus to investigate whether Bowman's layer is present in a variety of mammal species and to relate its presence to other corneal structures.

MATERIAL AND METHODS

We analyzed the corneas of 40 species of mammals (10 carnivores, 13 primates and 17 herbivores) all of which came from the Zoological Park of Barcelona. The human cornea was obtained from the organ donation service of the Department of Human Anatomy of the Faculty of Medicine of the University of Barcelona. The specific nomenclature for each species is given in Table 1.

All eyeballs were enucleated after a postmortem delay that in no case exceeded 12 hours. One single animal of each species studied was included in the study. Immediately after procurement, the eyeballs were shipped to our laboratory. Corneas were excised with a 3 mm scleral rim using surgical scissors. Fixation of the corneoscleral buttons was accomplished in 4% paraformaldehyde solution for 12 hours. Them, the corneas were washed in balanced saline solution, progressively dehydrated in ethanol, embedded in paraffine and sectioned with a microtome. Sections of 8 µm thickness including the whole cornea and the surrounding scleral rim were obtained and stained with hematoxylin-eosin.

The histological sections were observed and described using an OPTIPHOT-2 Nikon microscope together with UFX-DX Microflex microphotography equipment and an EVI-1011 video-camera connected to a KX-14 CPI Sony Black Triniton monitor. The morphometric study was performed on 10x15 cm micrographs after calculating the constants of unit transformation for each enlargement. The variables studied were the thickness of the cornea at the center (ccen), the thickness of the central epithelium (epic), the thickness of Bowman's layer (BL), and the thickness of Descemet's membrane (des).

Table 1.- Specific name and common name of the species under study.

	SPECIFIC NAME	COMMON NAME
Carnívores		
1	*Panthera leo*	Lion
2	*Panthera tigris*	Tiger
3	*Panthera pardus*	Leopard
4	*Acininyx jubatus*	Cheatah
5	*Felix concolor*	Puma
6	*Felix aurata*	African golden cat
7	*Canis lupus*	Wolf
8	*Canis dingo*	Dingo
9	*Canis familiaris*	Dog
10	*Vulpes corsac*	Fox
Primates		
11	*Lepilemur mustelinus*	Lemur
12	*Callithrix jacchus*	Common marmoset
13	*Alouata caraya*	Howler
14	*Ateles paniscus*	Black spider monkey
15	*Lagotrix lagotrica*	Woolly monkey
16	*Cercocebus torquatus*	Mangabey
17	*Cercopithecus aethiops*	Vervet monkey
18	*Cercopithecus cephus*	Moustached monkey
19	*Eritrocebus patas*	Patas
20	*Macaca irus*	Macaque
21	*Pan troglodytes*	Chimpanzee
22	*Gorilla gorilla*	Gorilla
23	*Homo sapiens sapiens*	Human
Herbívores		
24	*Tapirus terrestris*	Tapir
25	*Equus caballus*	Horse
26	*Equus perzewalskii*	Perzewalskii horse
27	*Dama dama*	Deer
28	*Cervus unicolor*	Sambar deer
29	*Cer vus elaphus*	Red-deer
30	*Ranjifer tarandus*	Reindeer
31	*Giraffa camelopardalis*	Giraffe
32	*Bos primigenius*	Ox
33	*Bos indicus*	Zebu
34	*Antilope cervicapra*	Blackbuck
35	*Taurotragus oryx*	Eland
36	*Ovis musimón*	Mouflon
37	*Ovis aries*	Mutton
38	*Sus scrofa domesticus*	Pig
39	*Sus scrofa*	Wild boar
40	*Loxodonta africana*	Elephant

For the description of the data contained in Table 5 of the final results, elementary describers such as mean, standard deviation, sample maxima and minima were used for all the samples and for the groups of individuals within the same taxonomic grouping.

RESUTS

Morphological Results

Our results indicated that the corneas of the carnivores studied lack a BL. Nor did they reveal the presence any special stroma condensation which might indicate the presence of a rudimentary BL (Fig. 1). The corneal stroma in these species extended to the basal membrane of the epithelium and maintained the typical structure

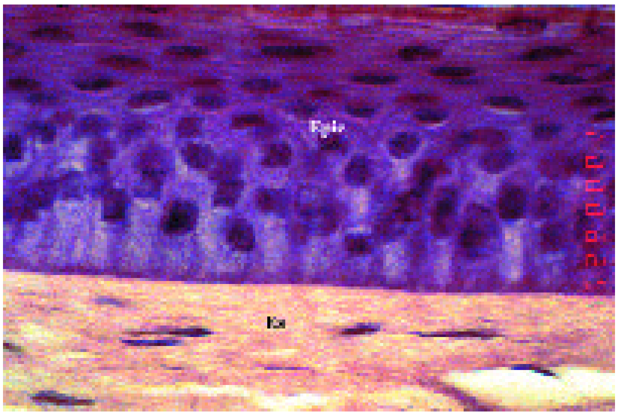

Figure 1.- Anterior cornea of the lion (*Panthera leo*). Central corneal epithelium (Epic) and corneal strome (St). Hem-Eos. x1000.

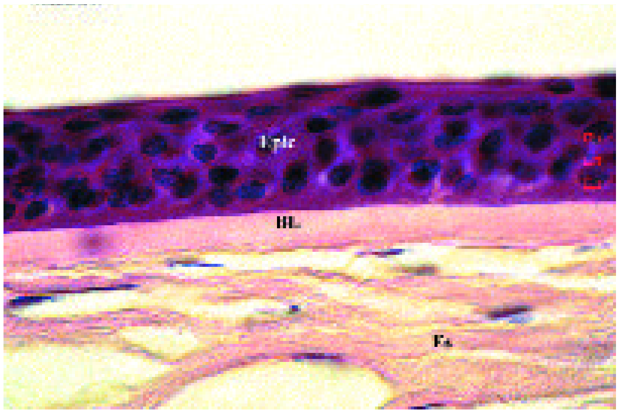

Figure 3.- Anterior cornea of the deer (*Dama dama*). Central corneal epithelium (Epic), corneal strome (St) and Bowman layer (BL). Hem-Eos. x1000.

of a dense, orientated conjunctive tissue made up of interwoven bundles.

In the case of the primates, all the cornea had a more or less developed BL. The only exception was the cornea of the lemur, the only representative of the sub-order of the pro-apes and the most primitive of all the primates studied.

Some of the New World monkeys, had a well defined BL as was the case of the howler monkey or the black spider monkey, while others, such as the marmoset, had a hardly discernible BL. In Old World monkeys, BL was poorly defined and of very reduced thickness. The mangabey was striking because its BL was similar to that observed in higher primates.

Within the hominid group (Fig. 2) the morphological characteristics of the BL was similar to those of its human counterpart. In both species (chimpanzee and gorilla), it was well defined and adopted the form of a denser, more homogenous and more hyaline layer than the stroma.

Regarding the herbivores the existence of BL was detected in the corneas of six species (deer, sambar deer, giraffe, zebu, ox, and eland). The morphological characteristics of these membranes (Figs. 3 to 8) confirm to those established by Jacobsen et al. (1984) for humans.

In the case of the reindeer, contrary to the opinion of Winquist (1973) and Rehbinder (1977), we failed to establish the existence of a

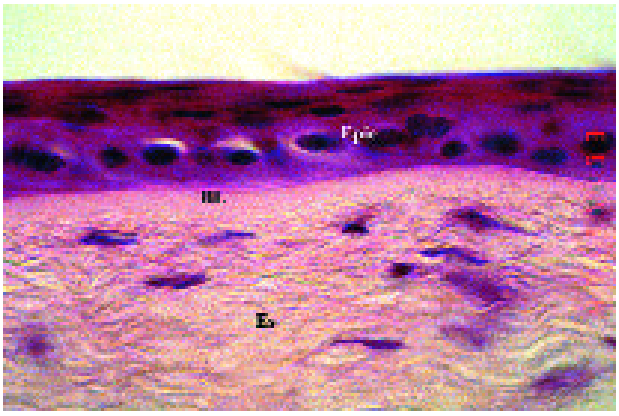

Figure 4.- Anterior cornea of the sambar deer (*Cervus unicolor*). Central corneal epithelium (Epic), corneal strome (St) and Bowman layer (BL). Hem-Eos. x1000.

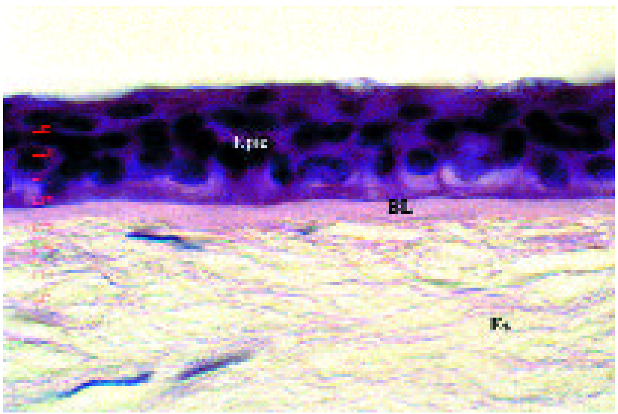

Figure 2.- Anterior cornea of the chimpanzee (*Pan troglodytes*). Central corneal epithelium (Epic), corneal strome (St) and Bowman layer (BL). Hem-Eos. x1000.

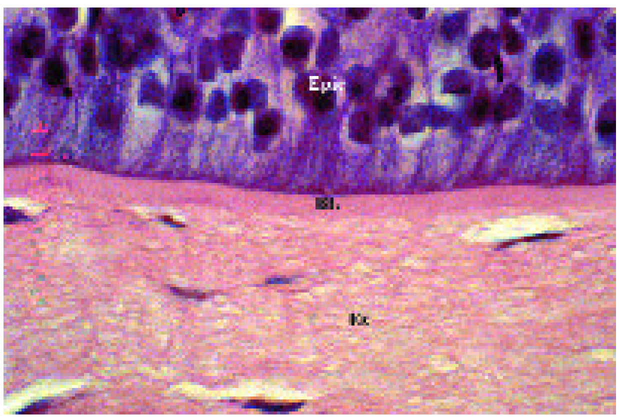

Figure 5.- Anterior cornea of the giraffe (*Giraffa camelopardalis*). Central corneal epithelium (Epic), corneal strome (St) and Bowman layer (BL). Hem-Eos. x1000.

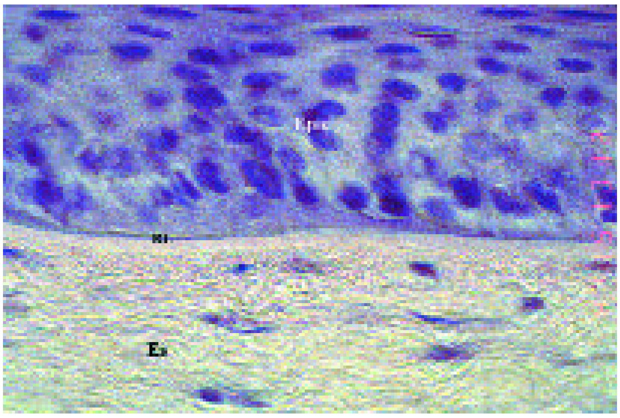

Figure 6.- Anterior cornea of the zebu (*Bos indicus*). Central corneal epithelium (Epic), corneal strome (St)and Bowman layer (BL). Hem-Eos. x1000.

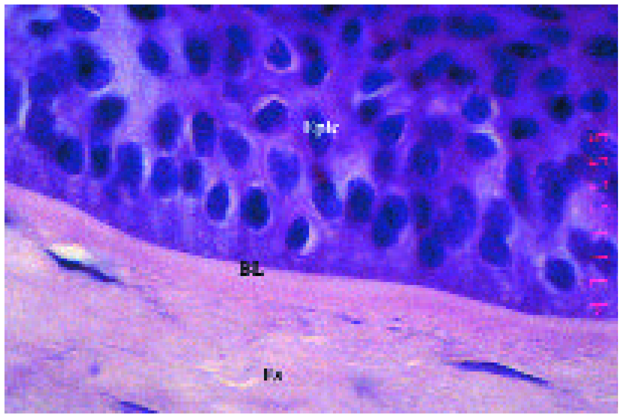

Figure 7.- Anterior cornea of the ox (*Bos primigenius*). Central corneal epithelium (Epic), corneal strome (St) and Bowman layer (BL). Hem-Eos. x1000.

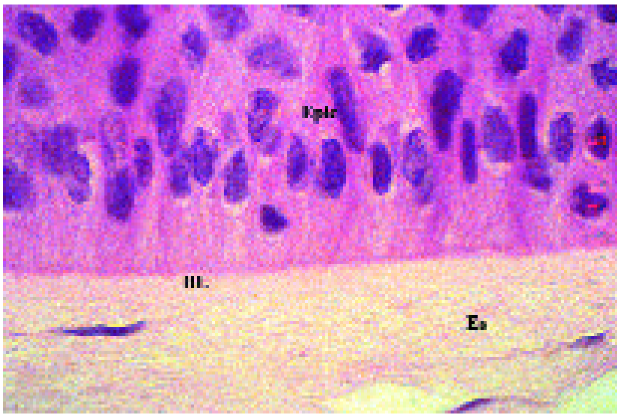

Figure 8.- Anterior cornea of the eland (*Taurotragus oryx*). Central corneal epithelium (Epic), corneal strome (St) and Bowman layer (BL). Hem-Eos. x1000.

clearly defined BL. At best, it could be defined as a rudimentary membrane closer in definition to that of Calmettes et al. (1956) for other species. The same can be said of the blackbuck.

Morphometric Results

Table 2 shows that all the carnivores appeared to lake a BL. The BL of the primates (Table 3), with the only exception of the lemur, had a thickness varying between 1.4 µm and 5.0 µm, the mean value being 2.9 µm. Within the group of herbivores (Table 4), BL was well defined in 6 species, with thickness varying between 3.2 µm and 5.9 µm and a mean value of 4.6 µm.

Table 2.- Carnivores thickness of the central cornea (ccen) and its different layers expressed in micrometers (µm). Central cornea (ccen), central corneal epithelium (epic), Descemet membrane (des) and Bowman layer (BL).

Carnívores	ccen	epic	des	BL
1 *Panthera leo*	1005	61	10.0	0
2 *Panthera tigris*	1341	77	15.9	0
3 *Panthera pardus*	1468	55	18.6	0
4 *Acininyx jubatus*	1028	57	14.5	0
5 *Felix concolor*	682	36	7.3	0
6 *Felix aurata*	763	56	7.7	0
7 *Canis lupus*	867	79	27.7	0
8 *Canis dingo*	820	61	7.7	0
9 *Canis familiaris*	1075	38	8.8	0
10 *Vulpes corsac*	820	46	9.1	0

Table 3.- Primates thickness of the central cornea (ccen) and its different layers expressed in micrometers (µm). Central cornea (ccen), central corneal epithelium (epic), Descemet membrane (des) and Bowman layer (BL).

Primates	ccen	epic	des	BL
11 *Lepilemur mustelinus*	385	20	13.6	0
12 *Callithrix jacchus*	390	16	1.8	1.4
13 *Alouata caraya*	371	30	1.4	3.2
14 *Ateles paniscus*	480	21	1.8	5.0
15 Lagotrix lagotrica	639	34	2.3	1.4
16 *Cercocebus torquatus*	566	23	2.7	4.1
17 *Cercopithecus aethiops*	439	20	1.8	1.4
18 *Cercopithecus cephus*	412	25	1.8	1.4
19 *Eritrocebus patas*	580	32	2.7	1.8
20 *Macaca irus*	335	17	1.4	1.8
21 *Pan troglodytes*	829	25	3.6	4.1
22 *Gorilla gorilla*	857	39	1.8	4.1
23 *Homo sapiens sapiens*	770	35	4.5	5.0

Table 4.- Herbivores thickness of the central cornea (ccen) and its different layers expressed in micrometers (µm). Central cornea (ccen), central corneal epithelium (epic), Descemet membrane (des) and Bowman layer (BL).

Herbívores	ccen	epic	des	BL
24 *Tapirus terrestris*	2046	57	15.0	0
25 *Equus caballus*	458	28	7.3	0
26 *Equus perzewalskii*	971	98	40.4	0
27 *Dama dama*	497	27	4.5	5.9
28 *Cervus unicolor*	820	16	2.7	4.1
29 *Cervus elaphus*	1040	102	55.4	0
20 *Ranjifer tarandus*	589	24	4.1	0.0
31 *Giraffa camelopardalis*	867	139	10.0	5.0
32 *Bos primigenius*	843	100	7.7	3.2
33 *Bos indicus*	959	63	3.6	4.1
34 *Antilope cervicapra*	913	109	10.5	0.0
35 *Taurotragus oryx*	890	132	26.8	5.0
36 *Ovis musimón*	725	91	9.1	0
37 *Ovis aries*	689	70	17.7	0
38 *Sus scrofa domesticus*	1063	47	4.5	0
39 *Sus scrofa*	1815	84	15.4	0
40 *Loxodonta africana*	1005	60	21.8	0

Table 5.- Thickness in μm. Mean, standard deviation, minimum and maximum for the whole sample and for each taxonomic group. Central cornea (ccen), central corneal epithelium (epic), Descemet membrane (des) and Bowman membrane (bow).

Variable	n	mean	deviation	minimum	maximum
ccen					
Global	40	817	362.9	336	2046
Carnivores	10	987	253.8	682	1468
Primates	13	543	181.7	336	857
Herbivores	17	953	411.5	458	2046
epic					
Global	40	55	33.2	16	140
Carnivores	10	57	14.3	37	80
Primates	13	26	7.4	16	39
Herbívores	17	74	37.8	16	140
des					
Global	40	10.3	11.05	1.4	55.4
Carnivores	10	12.5	6.73	6.8	27.7
Primates	13	3.2	3.27	1.4	13.6
Herbívores	17	15.1	14.33	2.7	55.4
BBL					
Global	18	3.1	1.76	0.0	5.9
Carnivores	-	-	-	-	-
Primates	12	2.9	1.44	1.4	5.0
Herbivores	6	4.6	0.87	3.4	5.9

In order to facilitate the analysis of the results within each group, we have included the basic descriptive statistics of the sample: the number of data, the mean, the standard deviation, and the maximum and minimum of each variable. The values of these statistics are shown in Table 5.

Regarding the existence of possible relationships between the thickness of BL and the rest of the corneal layers, analysis of the correlations revealed that such a relationship cant be considered significant. However, we did detect the existence of a certain relationship between the thickness of the epithelium and Descemet's membrane. Figure 9 shows the data concerning the thickness of the epithelium (epic) as compared with the thickness of Descemet's membrane (des) and the regression lines for both carnivores and herbivores. Although the correlation coefficients are low (0.702 and 0.470, respectively) thus rendering the fitting of the regression lines difficult, we have considered it worthwhile to include this information because of the characteristics it reveals since with the exception of the lemur, the primates appears to have the least thickness in both Descemet's membrane and the epithelium; all of them seemed to have BL and their ocular size was relatively small. By contrast, the maximum thickness of Descemet's membrane appears to be associated with medium-sized or large eyes as was the case of the carnivores or herbivores, which had thick epithelia and lack BL.

However, herbivores with the least thickness of Descemet's membrane (red deer –27–, sambar deer –28–, reindeer –30–, zebu –33–) approached the characteristics of the primates in that they appeared to have thin epithelia, relatively small eyes and a relatively well developed BL. Other cases of low thickness of Descemet's membrane, together with a more or less developed BL, were the giraffe (31), the ox (32), and the blackbuck (34). These species had thick epithelia, probably due to the fact that their eye size is large, a factor which distances them from the primates.

The pig (38) appears to be special because in spite having of a fairly thin Descemet's membrane and epithelium and a small ocular size (all of which are characteristics pertaining to primates and herbivores with BL), it does not have BL.

Discussion

The results obtained here permit us to affirm that the corneas of the carnivores included in the study (felines and canids) have no BL. Contrary to the findings of Calmettes et al. (1956), we failed to detect any condensation in the underlying fibers of the epithelium. We do coincide, however, with Shively and Epling (1970) in that the cornea of the dog lacks BL.

We have observed the presence of BL in all the primates studied with the exception of the lemur. In the mangabey, black spider monkey, chimpanzee and gorilla, the morphometric and morphological characteristics of BL are similar to human one.

With regard to the herbivores, we observed the existence of BL in the deer, sambar, giraffe, ox, zebu and eland. Its characteristics are consistent with those described by Jacobsen et al. (1984) for human beings.

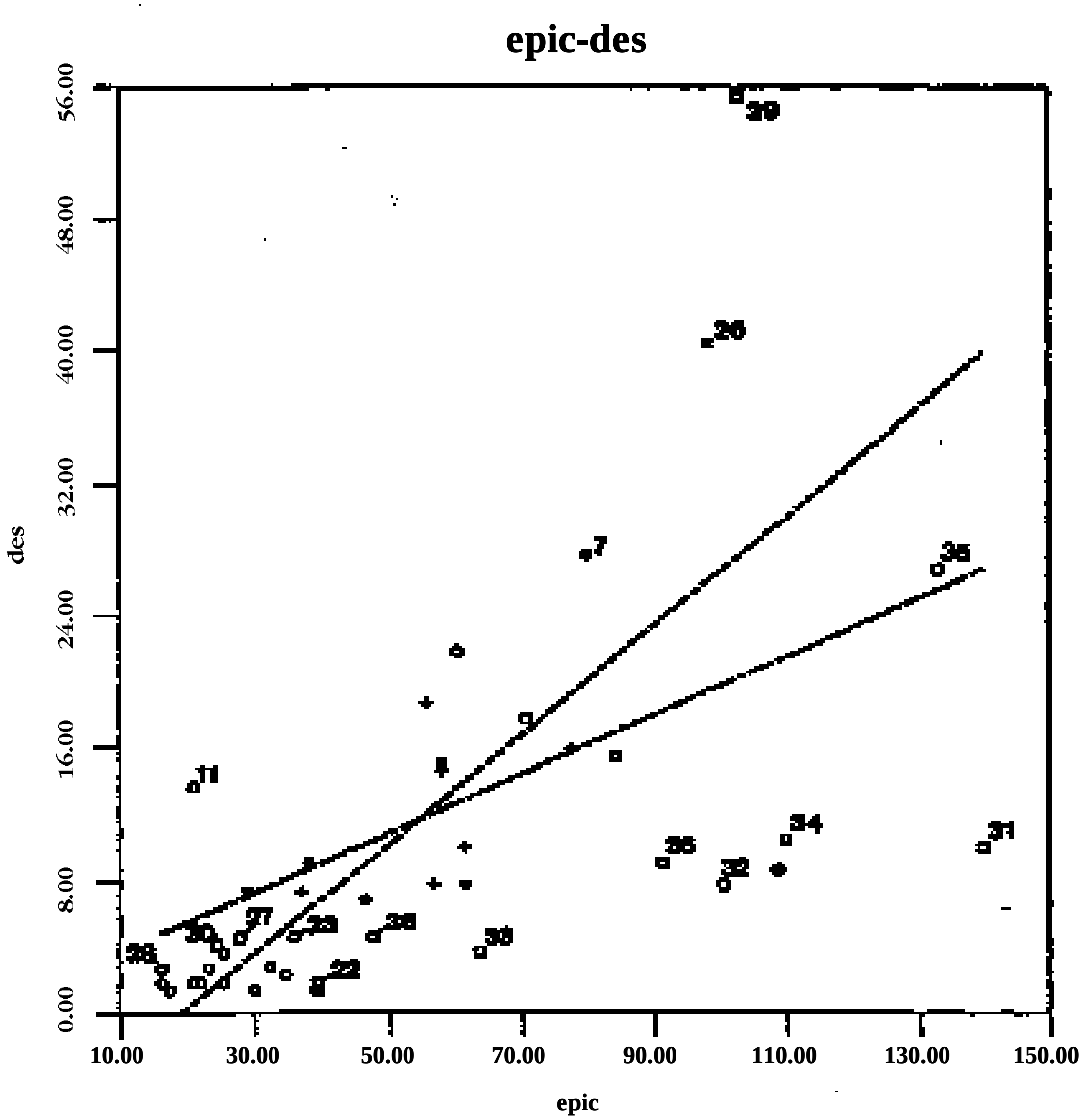

Figure 9.- Central corneal epithelium thickness (epic) againts Descemet's membrane thickness for all species. Felines (plus sign); Canides (black circle). Primates (circle); Hervibores perisodactyles (times sign), Hervibores artiodactyles (square). Regression lines are also shown for carnivores (dashed) and herbivores (dotted-dashed).

Our findings are in agreement with those of Calmettes et al. (1953) in that BL is a supportive structure for the cornea. Our data, however, lead us doubt whether the presence of the membrane could be associated with eyes with a thin corneal structure. We base this claim on the observation that within the group of herbivores some species appeared to have a well defined BL as well as thick corneal and epithelial layers (the giraffe is a good example)

Regarding the relationship between BL and the rest of the corneal layers, we feel that we have established that this relationship does exist between the epithelium and Descemet's membrane. In this sense, our data show that, in animals with small ocular size. BL appears to be accompanied by both a thin epithelium and a thin Descemet's membrane. The only exception is that of the reindeer in which, even thought the above characteristics are present, there appears to be no trace of BL in its cornea. Accordingly, taking into account that both Winquist (1973) and Rehbinder (1977) describe BL in the reindeer we feel that this species merits further study

Our study also shows that when ocular size increases, the species having BL show a low thickness of Descemet's membrane and a high thickness of the epithelium. However if BL is not present, the thickness of both Descemet's membrane and the epithelium is increased.

These considerations appear to confirm that BL and Descemet's membrane, together with the epithelium, play a mechanical role in the maintenance of the curvature of the cornea. Thus, large or medium sized eyes, whose corneas lack BL, compensate its absence with greater epithelial thicknesses and with Descemet's membrane.

Finally we would like to mention that our study of the morphological parameters was done with a view to offering a simple description of the data, without claiming any type of statistical inference. The reason for avoiding any inference is because only one individual of each species was studied. Therefore, although the individuals studied are thought to be representative of their species, this in no way would allow us to study possible intra or interspecific variability. Study of this variability would explain the exceptions shown by our results and, in this sense, we believe that further studies would be required to clear up some of the doubts arising from our work.

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