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Changes in fine structure of the rabbit sperm head during passage through the epididymis

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INTRODUCTION

Although the functional role of the acrosome cap of mammalian spermatozoa is not yet clearly understood, it seems certain that this structure plays an essential part in fertilization. Recently Fawcett & Hollenberg (1963) reported that in passage of spermatozoa through the epididymis of the guinea-pig there occurs a progressive morphological differentiation of the acrosome cap in which both the shape and internal structure of this organelle undergo considerable modification. While the significance of such changes is perhaps not wholly clear, this discovery is interesting as it is known also that the fertilizing ability of guinea-pig spermatozoa is acquired during epididymal passage (Young, 1931). In discussing their findings, Fawcett & Hollenberg (1963) draw attention to the fact that the changes which occur in the guinea-pig acrosome within the epididymis primarily involve the area derived from the outer zone of the acrosomal vesicle of the spermatid. As an outer acrosomal zone apparently does not exist in the spermatids of species which possess a discrete homogeneous acrosome (Burgos & Fawcett, 1955), it is considered unlikely that the acrosome of such species will show changes in form such as occur in the guinea-pig during the passage of spermatozoa through the epididymis. In the domestic rabbit, however, in which the content of the mature acrosome is homogeneous (Hadek, 1963*b*; Bedford, 1964*a*), the longitudinal and lateral dimensions of the acrosome cap appear to diminish significantly during passage through the epididymis; likewise, the fertilizing ability of rabbit spermatozoa is acquired during passage through the epididymis (Bedford, 1963*a*). As the measurements and observations on the rabbit epididymal spermatozoa were made with the light microscope, no understanding was gained of the fine structural changes in the sperm head which occur as a concomitant of the diminution in acrosomal size. For this reason studies have been carried out on spermatozoa in different parts of the rabbit epididymis using an electron microscope.

The cell membrane which overlies the acrosome cap has also been examined in the present investigation. When observed in the electron microscope the plasma membrane appears swollen over the region of the acrosome cap in most ejaculated rabbit spermatozoa and in most spermatozoa released from the cauda epididymidis. The discovery in this present study that such swelling does not occur in a majority of spermatozoa released from the caput epididymidis points to the existence of other, non-structural, changes in the sperm head during epididymal maturation.

MATERIALS AND METHODS

Nineteen fertile male rabbits of not more than 2 years of age were used in this study. In each of eight animals small pieces of tissue were taken from epididymal regions 1, 2, 3, 7 and 8 (and from regions 4, 5 and 6 in two of these animals), shown in Fig. 1, and were fixed as described below. In each of eleven animals, spermatozoa were released from the caput (regions 1–3), corpus (proximal half of region 7), and flexure of the cauda epididymidis respectively, by incising and compressing these tissues in a medium of Ringer's solution containing 20 % heated serum. Each sperm suspension was then centrifuged for about 15 min at 3000 rev/min, the precipitated material being recovered in pellet form. The pellets and intact epididymal tissues were fixed for 2 h at 5 °C in 1 % osmium tetroxide in veronal acetate buffer, pH 7·4

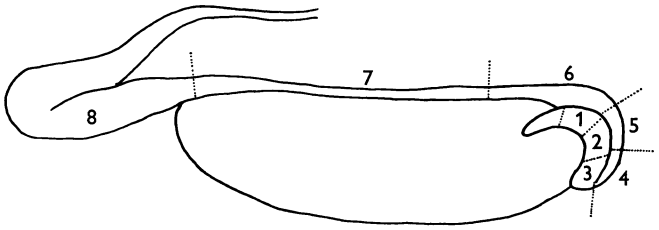


Fig. 1. Diagrammatic representation of the rabbit testis and epididymis: the epididymis has been divided into segments as referred to in the text.

(Palade, 1952), with the addition of 0·25 M sucrose (Caulfield, 1957). After fixation, the tissues and pellets were dehydrated in ascending concentrations of alcohol (from 30 to 100 %), for 1 h, then transferred to acetone for 2 h, and subsequently embedded in araldite (Glauert & Glauert, 1958) polymerized at 60 °C in gelatin capsules. Sections of approximately 0·08 μm in thickness were cut with glass knives on a Cambridge (Huxley) microtome, and were stained either with lead hydroxide (Millonig, 1961) or with uranyl acetate and potassium permanganate (A. M. Lawn, 1964, personal communication). The sections were examined with an R.C.A. EMU 3F electron microscope.

OBSERVATIONS

In region 1 of the epididymis the concentration of spermatozoa was relatively low, and the contents of the tubule did not possess the granular quality seen in regions 2 and 3 (Fig. 7). Compared with the acrosome of ejaculated spermatozoa or of spermatozoa from the cauda epididymidis, the margin of the acrosome cap was clearly elongated to a variable degree in a majority of spermatozoa in the tubules of region 1. The acrosome margin was elongated to the greatest degree in the antero-lateral area of the acrosome (Fig. 2*a*, *b*) but was distinctly protracted also in the lateral (Fig. 3) and rostral parts (Fig. 17) of the acrosome cap. Sections through the planar dimension of the sperm head clearly demonstrate the relatively greater increase in width rostral to the 'equatorial' region in the immature acrosome cap (Fig. 4) compared with that in the mature acrosome (Fig. 5). The posterior region of the acrosome cap was always constricted, even in the immature condition.

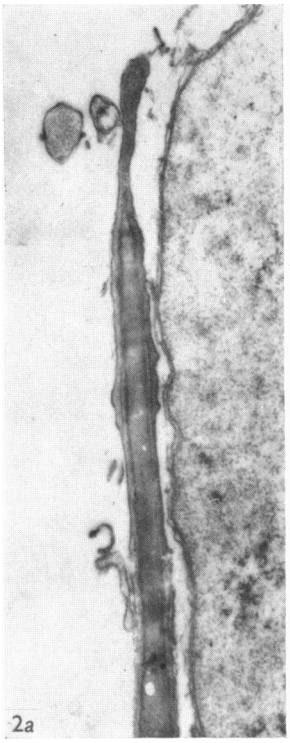
Although most sperm sections in region 1 did variously display the form of the immature acrosome described above, a few which possessed the attenuated form of the mature acrosome were interspersed among the immature forms in this region.

In region 2 and in the proximal part of region 3 spermatozoa were present in relatively greater concentration, and generally were surrounded by granular material in the lumen of the tubule (Fig. 7). An erect extension of the acrosome margin was present in some spermatozoa in region 2, but in many this protraction of the acrosome had become bent away from the flat nuclear plane to varying degrees (Fig. 7). Sections of the folded or collapsed margin of the acrosome cap are shown in greater detail in Figs. 8–10. The form of the collapsed acrosome flange varied considerably, and in some transverse sections of the rostral region of the head both lateral extensions of the acrosome had become bent towards opposite faces of the head, producing a modified S shape (Fig. 11). This configuration, in conjunction with sections of the immature acrosome cap in the flat plane of the sperm head (Fig. 12), indicates that the marginal protrusion of the acrosome may sometimes collapse on to either face as a series of fragmentary or ragged extensions, rather than as a continuous veil, as suggested in Fig. 10. The appearance of less extreme forms than those in Figs. 8–10 suggests that after collapse the acrosome extension is gradually reduced within the head plasma membrane (Fig. 13) until the form of the mature acrosome is attained. Although most spermatozoa in region 2 possessed structurally immature acrosomes, the immature forms were interspersed also with others which apparently possessed the acrosome form of mature spermatozoa.

As most spermatozoa in the distal part of region 3 and in region 4 displayed the attenuated form of the mature acrosome cap which is shown in Fig. 20, it is clear from the foregoing description that major changes in the form of the acrosome occur predominantly in regions 2 and 3. Nevertheless, one must emphasize the fact that spermatozoa which possessed variant forms of the collapsed acrosome margin were observed occasionally in all regions of the epididymal duct, though the frequency of occurrence of these immature forms was low in the distal regions 7 and 8. While a great majority of spermatozoa in the tubules of regions 4–8 displayed the smoothly rounded form of the mature acrosome, in regions 3 and 4 many showed some difference in the density or staining characteristics of the acrosomal material around the region of the perforatorium (Figs. 14–16). Such a difference in staining properties of the acrosomal material was rarely seen in spermatozoa resident in the more distal regions of the epididymis. The granular nature of the extra-spermatozoal content of the tubule lumen characteristic of regions 2 and 3 (Fig. 7) was reduced in the part of the epididymal duct distal to region 3, as is evident in Fig. 20.

The appearance of the immature and mature sperm heads is shown diagrammatically in Figs. 18 and 19 respectively.

The cytoplasmic droplet was usually located in the neck region within the cell membrane, in spermatozoa resident in regions 1–3. In spermatozoa situated in the more distal epididymal regions, the droplet was located at various points along the mid-piece of the tail; in several spermatozoa in region 7, however, the droplet still remained closely applied to the neck region in those which showed the attenuated acrosome typical of mature spermatozoa (Fig. 20). In longitudinal sections of spermatozoa observed in region 8, the droplets were almost invariably located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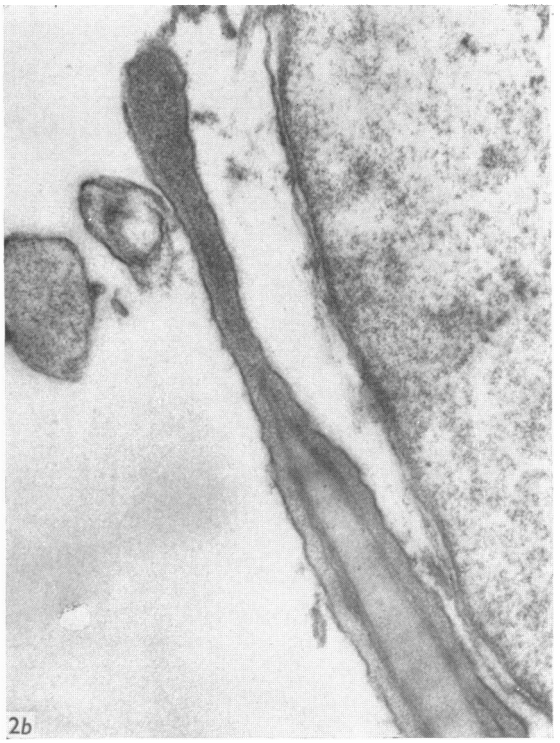
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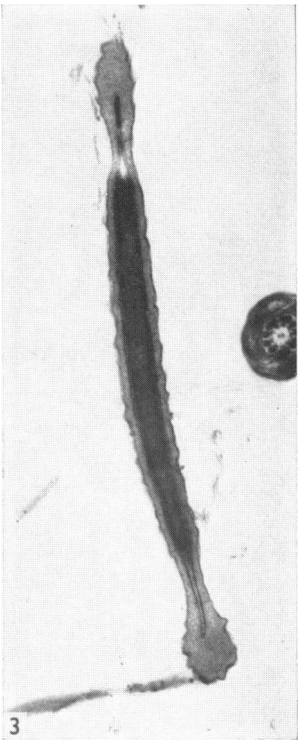
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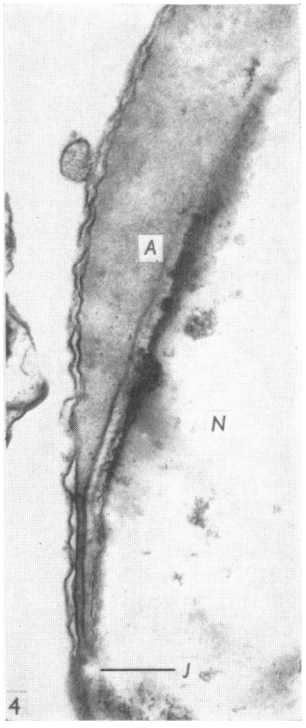

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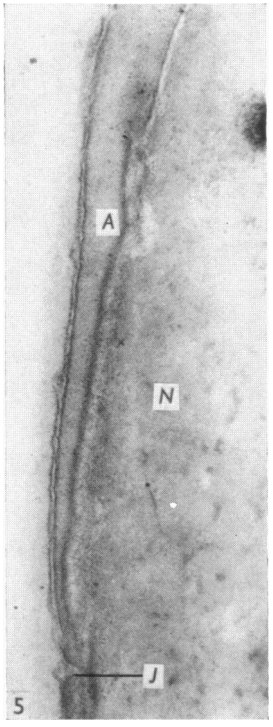

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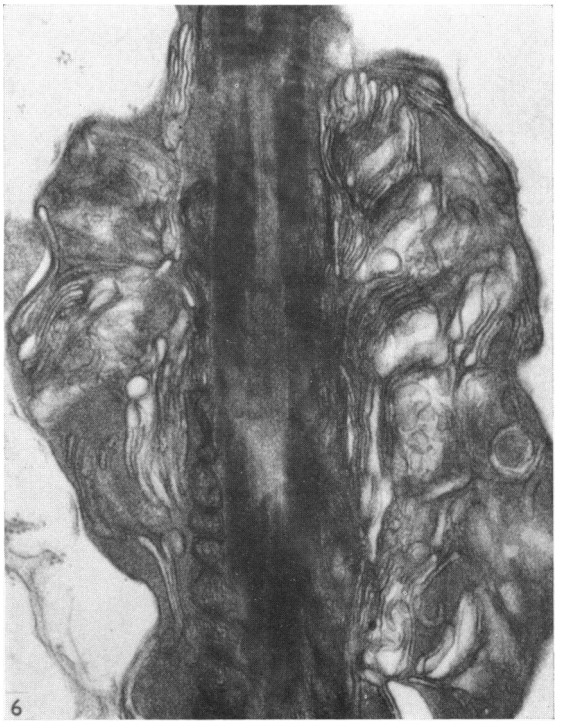
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distally, or were absent. The 'neck' droplet generally possessed a complex internal arrangement of groups of fine lamellae interspersed with vesicles (Fig. 6), as described by Bloom & Nicander (1961). Within the distally situated droplet, however, the differentiated elements had diminished and tended to be located peripherally (Fig. 21).

Little distortion or swelling of the plasma membrane overlying the acrosome cap is seen in a majority of spermatozoa fixed *in situ* in the rabbit epididymis. Yet it is a common experience of investigators who study the fine structure of the mammalian sperm head that the plasma membrane in the anterior region of the head becomes swollen to a greater or less degree in ejaculated spermatozoa, or in spermatozoa released from the cauda epididymidis. In the present study, distinct and consistent variations in the disposition of the head plasma membrane have been found between sperm populations freed from different epididymal regions (Table 1). This membrane had swollen over the dorsal and ventral surface of the acrosome in only a few freed caput spermatozoa (Fig. 16), and remained in close apposition to the underlying acrosome in a majority of spermatozoa released from caput regions 1–3 (Fig. 17). In most spermatozoa released from the corpus and cauda regions, however, the cell membrane had swollen away from the underlying acrosome to varying degrees.

A further difference in disposition of the plasma membrane was seen often, though not consistently, between spermatozoa released from the corpus and cauda regions respectively. The plasma membrane of ejaculated spermatozoa and of spermatozoa released from the cauda epididymidis usually appeared swollen over the flat surface of the acrosome, but almost invariably adhered to the edge of the acrosome cap. In spermatozoa released from the corpus (region 7), however, the intact plasma membrane was often completely divorced from the whole surface of the acrosome,

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Fig. 2. (*a*). Para-sagittal section of sperm head released from regions 1/2 of the epididymis. Note the elongated rostral border of the acrosome cap. Lead hydroxide. ×14000. (*b*) Enlargement of Fig. 2*a* to show the rostral elongation of the acrosome border. In this sperm head the cell membrane has been detached from the surface of the acrosome. Lead hydroxide. ×40000.

Fig. 3. Transverse section of sperm head released from region 1/2 of the epididymis. Note the narrow elongated lateral borders of the acrosome cap. The cell membrane is absent. Uranyl acetate and potassium permanganate. ×18300.

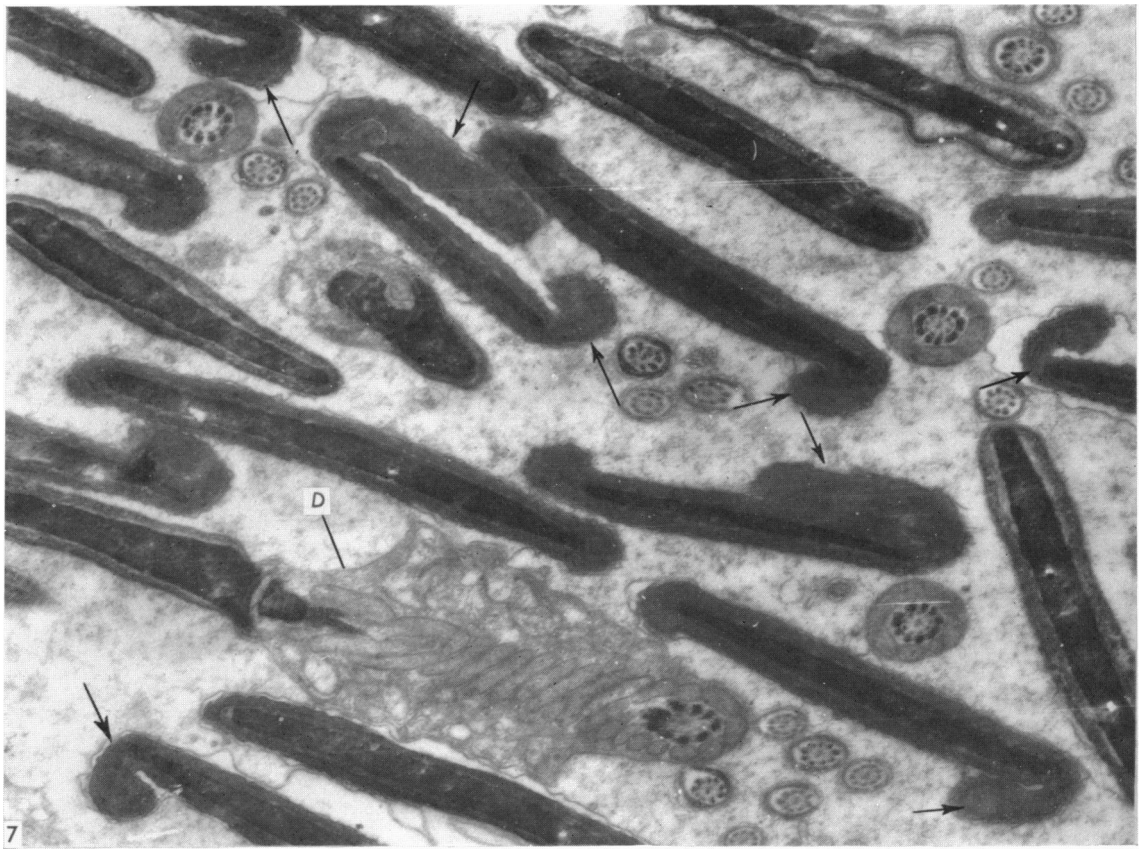
Fig. 4. Longitudinal section through the flat plane of an immature sperm head released from region 1/2 of the epididymis. The constricted posterior region of the acrosome cap shows an abrupt increase in width, rostrally. Lead hydroxide. ×32000.

Fig. 5. Longitudinal section through the flat plane of a sperm head released from region 7 of the epididymis. Note, in contrast to Fig. 4, the gradual increase in the width of the acrosome cap from the posterior to rostral region. Lead hydroxide. ×31000.

Fig. 6. Central longitudinal section through a cytoplasmic droplet located at the neck of a sperm in region 3. Note the numerous groups of lamellae, interspersed with vacuoles. Uranyl acetate and potassium permanganate. ×40500.

Key to lettering.

A Acrosome cap
C Cell membrane
D Droplet in the neck region
E Marginal extension of acrosome cap
J Junction of acrosome and post-nuclear cap
M Mitochondria of sperm mid-piece
N Nucleus
S Stereocilia
T Cross-section of sperm tail



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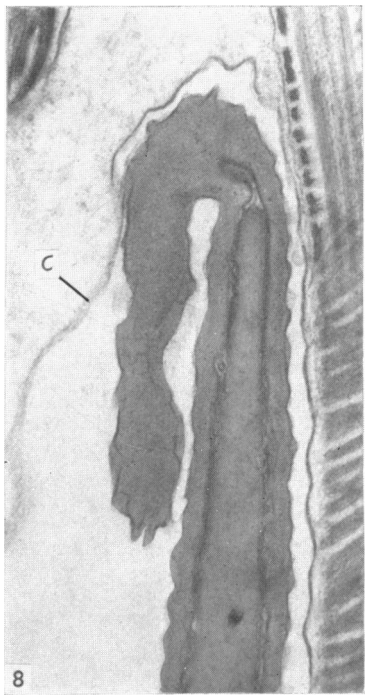

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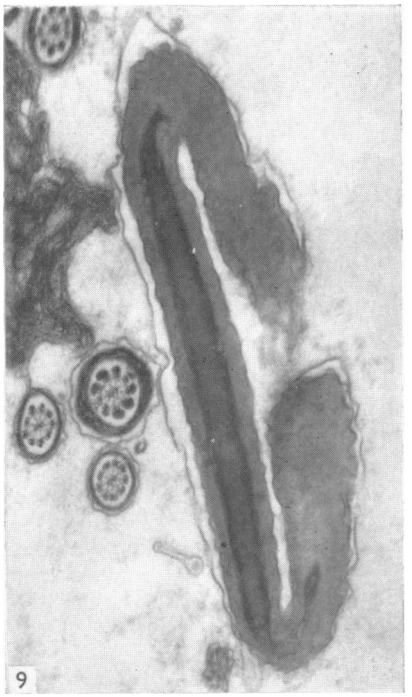

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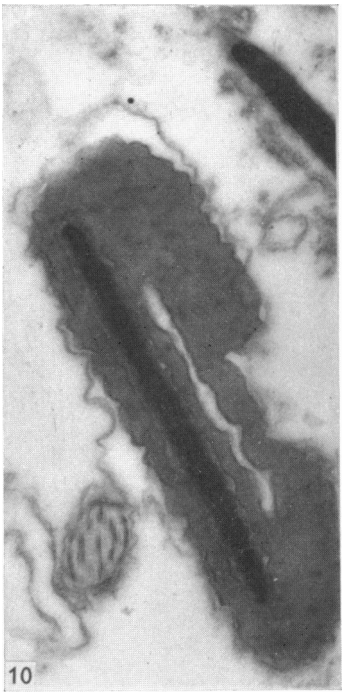

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and remained attached only at its junction with the post-nuclear cap. As the samples from the corpus and cauda regions were treated in an identical manner, it seems that the complete separation of the membrane in spermatozoa from the corpus epididymidis may reflect some transient difference in the character of the head membrane of spermatozoa resident in the corpus epididymidis.

Table 1. *Percentage spermatozoa showing a swollen plasma membrane (Fig. 16) in groups liberated from different epididymal regions*

	> 200 spermatozoa in each group.		
Rabbit no.	Caput	Corpus	Cauda
1	49	92	91
2	37	88	86
3	45	89	95
4	18	80	97
5	23	88	95
6	31	97	91
7	21	79	87
8	14	59	79
9	20	74	91
10	26	84	94
11	29	84	82

The findings presented here are necessarily based on observations made only on representative samples of tissue from various parts of the epididymis. In the areas that have been examined, no evidence has been obtained to suggest extensive disintegration and resorption of spermatozoa in any part of the normal rabbit epididymis. The acrosome had ruptured or had been lost in occasional spermatozoa, and now and then 'scavenger' cells which contained sperm fragments were found in the tubular lumen, both in the caput (Fig. 22) and in the vas deferens (Fig. 23*a*, *b*). Cells bearing a resemblance to plasma cells were also seen occasionally in the tubular lumen in the caput epididymidis, but none of these cells appeared to contain sperm remnants.

DISCUSSION

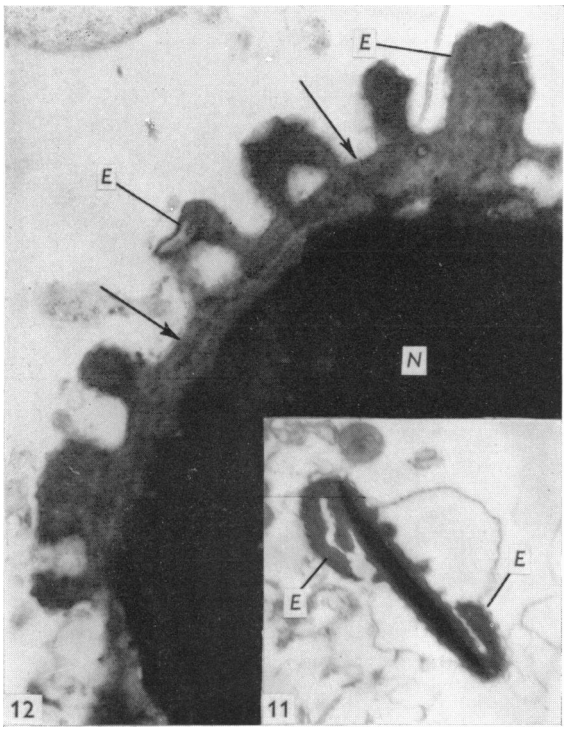
The reduction in dimensions of the rabbit acrosome during epididymal maturation results from an absolute decrease in width of that part of the acrosome which extends both rostrally and laterally beyond the edge of the nucleus. Clearly no

Fig. 7. Section through the lumen of a tubule in region 2 of the epididymis. The spermatozoa are surrounded by granular material in the tubule lumen. Note that the spermatozoa are not oriented in any particular way, and that many of the sectioned sperm heads possess the 'hooked' acrosome flange (arrowed) to a varying degree. Lead hydroxide. ×14000.

Fig. 8. Sagittal section of the rostral third of an immature sperm head *in situ* in region 2 of the epididymis. Note the collapsed extension of the acrosome cap. The cell membrane is swollen and separate from the sperm surface. Lead hydroxide. ×44000.

Fig. 9. Transverse section of sperm head *in situ* in region 2. This section is cut approximately through the rostral third of the acrosome cap, and shows the collapsed acrosome flange at each lateral border. Lead hydroxide. ×30500.

Fig. 10. Transverse section through sperm head in region 2. This section is cut through a region rostral to that in Fig. 9. The collapsed acrosome flange meets centrally. Lead hydroxide. ×30500.



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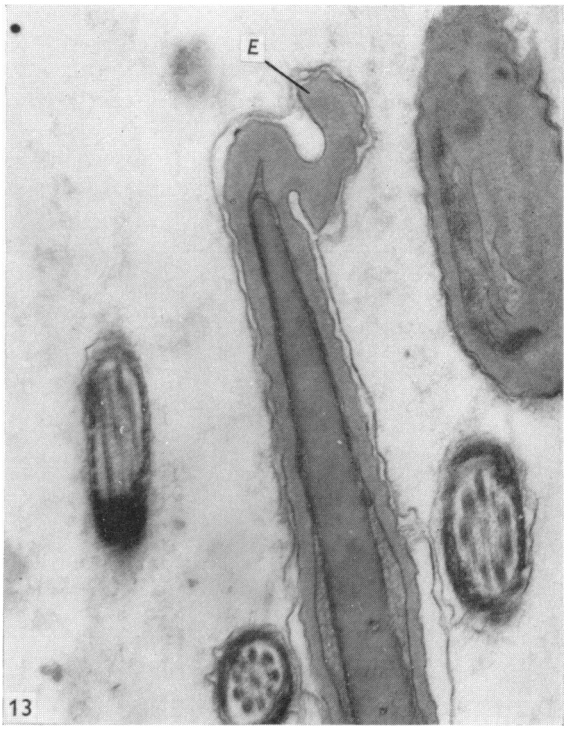

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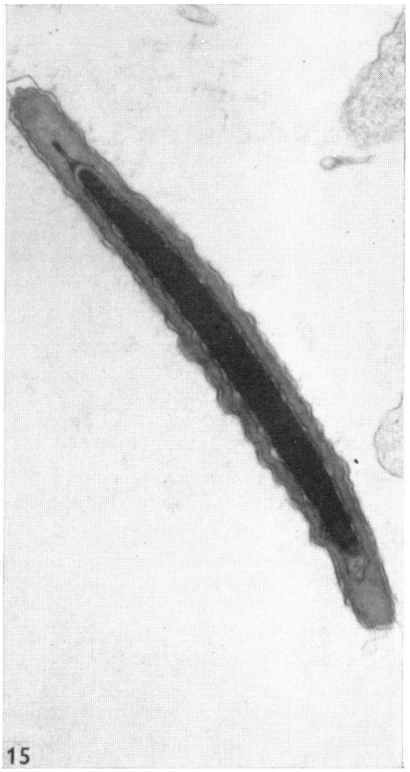

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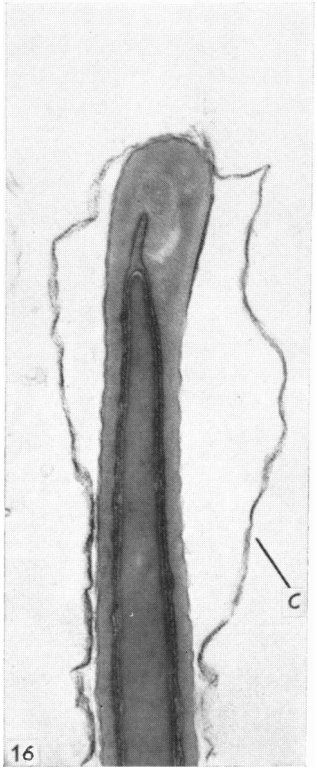

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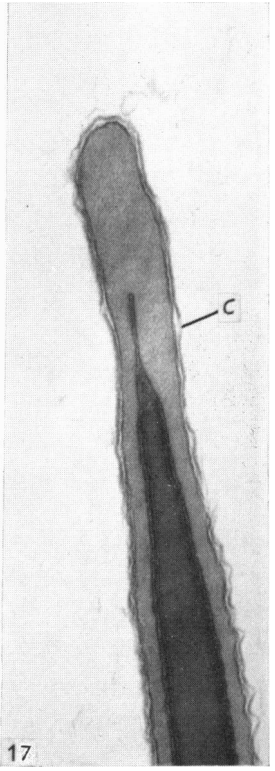

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change takes place in the posterior region of the acrosome, which can be seen to be markedly constricted in thin sections of immature spermatozoa (Fig. 4), and in entire stained immature spermatozoa (Bedford, 1963*a*).

Comparison of the appearances of spermatozoa in different epididymal regions makes it seem probable that the earliest stage of maturation, represented in the acrosome by an erect peripheral extension of variable length, is often followed by collapse of the marginal extension, thus conferring a 'hooked' profile on many sectioned sperm heads. From the appearance of forms showing less extreme protrusion of the acrosome margin it is assumed that, thereafter, the acrosome border is gradually reduced until the attenuated bulbous border of the mature acrosome is achieved. The different staining characteristics of the acrosomal material around the perforatorium (Figs. 14–16) in many spermatozoa in regions 3/4 only may well be a reflexion of some transient maturation change in the material of the acrosome; this phenomenon cannot, however, be compared with the consistent zonal differentiation which occurs in the acrosome of the guinea-pig.

The acrosome cap probably facilitates the passage of spermatozoa through the zona pellucida; electron microscopy has shown that most of the rabbit acrosome is absent from spermatozoa lying within the zona pellucida (Moricard, 1960; Austin, 1963; Hadek, 1963*a*), and more recently it has been claimed that enzymes extracted from the acrosome will dissolve the zona pellucida in some rabbit ova (Srivastava, Adams & Hartree, 1965). Although, at present, we have no clear appreciation of the

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Fig. 11. Transverse section through a sperm head released from region 1/2 of the epididymis. This section shows collapsed lateral extensions of the immature acrosome, which face opposite sides of the head. Uranyl acetate and potassium permanganate. × 13500.

Fig. 12. Section of sperm head released from region 1/2 of the epididymis, cut through the flat plane in the rostral region of the sperm head. Note the ragged extensions which protrude from the border of the immature acrosome cap; the border between the acrosomal extensions (arrowed) is the presumed limit of the mature acrosome. Uranyl acetate and potassium permanganate. × 22000.

Fig. 13. Longitudinal section through immature sperm head *in situ* in region 3. Note the configuration of the acrosomal extension within the cell membrane. Lead hydroxide. × 30000.

Fig. 14. Longitudinal section of sperm head *in situ* in region 3 of the epididymis. There is an obvious difference in the staining properties of the material immediately surrounding the perforatorium, compared to that of the remainder of the acrosome cap. Note that the acrosome in the apical region is somewhat elongated, compared with the bulbous apex of the mature acrosome (see Fig. 20). Lead hydroxide. × 17500.

Fig. 15. Transverse section through sperm head *in situ* in region 4. Note the lightly stained area which surrounds the perforatorium. The lateral borders of the acrosome are reduced compared with those in Figs. 3 and 9–11; but this sperm has not yet acquired the slightly bulbous border seen typically in transverse sections of mature spermatozoa. Lead hydroxide. × 19200.

Fig. 16. Longitudinal section of sperm head released from region 3/4 of the epididymis. Note the swollen plasma membrane overlying the acrosome cap, and the different density of the acrosome material immediately surrounding the perforatorium. Lead hydroxide. × 35000.

Fig. 17. Central longitudinal section of sperm head released from region 1/2 of the epididymis. Note that the plasma membrane remains in close apposition to the underlying acrosome cap. The apex shows the elongated form typical of the immature acrosome cap. Lead hydroxide. × 37000.

relative significance of particular changes which are known to occur in spermatozoa while passing through the epididymis, it seems certain that changes in the acrosome must be of functional importance. It is appropriate here to emphasize, however, that virtually no spermatozoa in the proximal half of region 7 (Fig. 1), which for the most part possess mature acrosomes, are yet capable of fertilization (unpublished results). This shows that the acrosomal changes, which obviously are a facet of the maturation process, cannot, themselves, constitute the ultimate event in the maturation of spermatozoa in the rabbit epididymis.

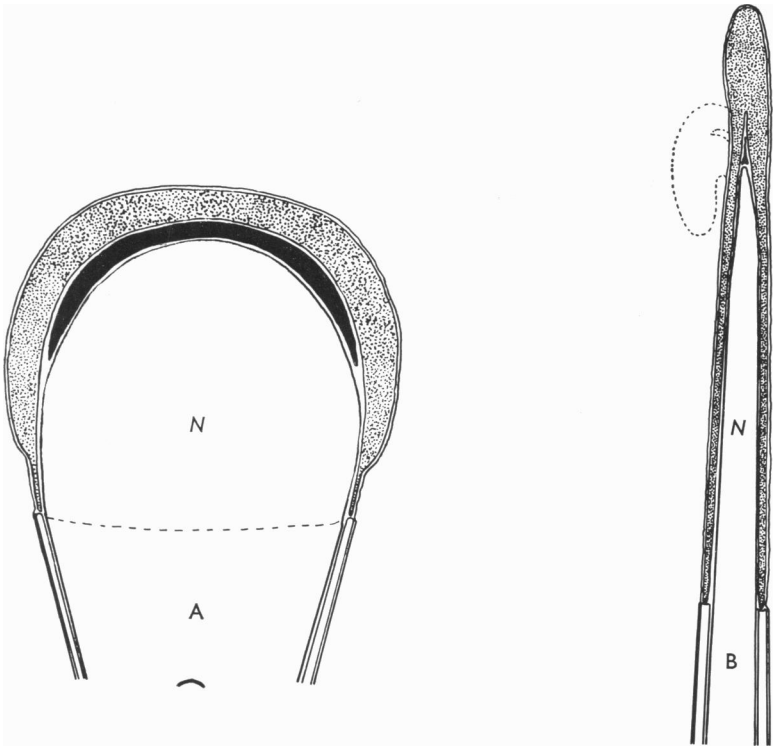


Fig. 18. Diagrammatic representation of the shape and structure of the immature sperm head in the proximal regions of the epididymis. A, Plan view; B, sagittal section. The dotted lines in B represent the outline of the acrosome margin after collapse or folding of the marginal extension. *N*, Nucleus; stippled area, acrosome cap; black area, perforatorium.

According to Fawcett & Hollenberg (1963), during the passage of spermatozoa through the guinea-pig epididymis, changes in the acrosome are closely correlated with particular regions of the epididymal duct. In the rabbit, the phase of maturation which involves acrosomal attenuation most frequently takes place in regions 2/3, but such correlation is by no means absolute in this species. Spermatozoa with acrosomes characteristic of mature spermatozoa have been observed, albeit as a low percentage of the total in regions 1 and 2, in all segments of the rabbit epididymal duct. Conversely, some immature 'hooked' spermatozoa were seen occasionally in the distal regions of the epididymis, and have been found sporadically in uterine contents obtained after natural mating (Bedford, 1964*a*). Results in a previous

light-microscope study (Bedford, 1963*a*) indicated that the marked reduction in acrosome width may be correlated with movement of the cytoplasmic droplet away from the neck region. While such a correlation may apply generally, it is apparent in electron micrographs that spermatozoa with neck droplets may possess a mature acrosome (Fig. 20); it seems unlikely, therefore, that there can be a direct physiological relationship between acrosome maturation and the state of the droplet. Nicander (1958) considers that movement of the droplet away from the neck always occurs while the spermatozoon is in region 4. It is clear, however, that no absolute

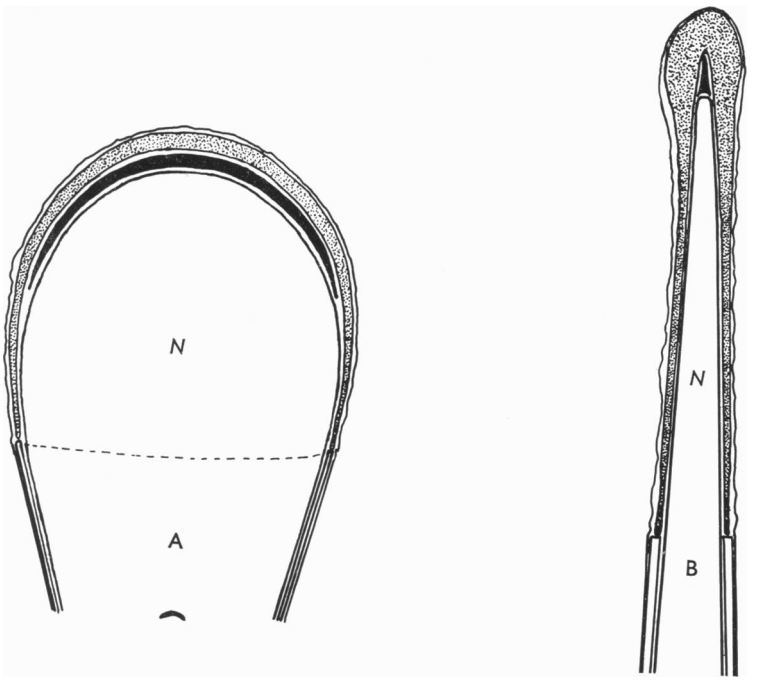
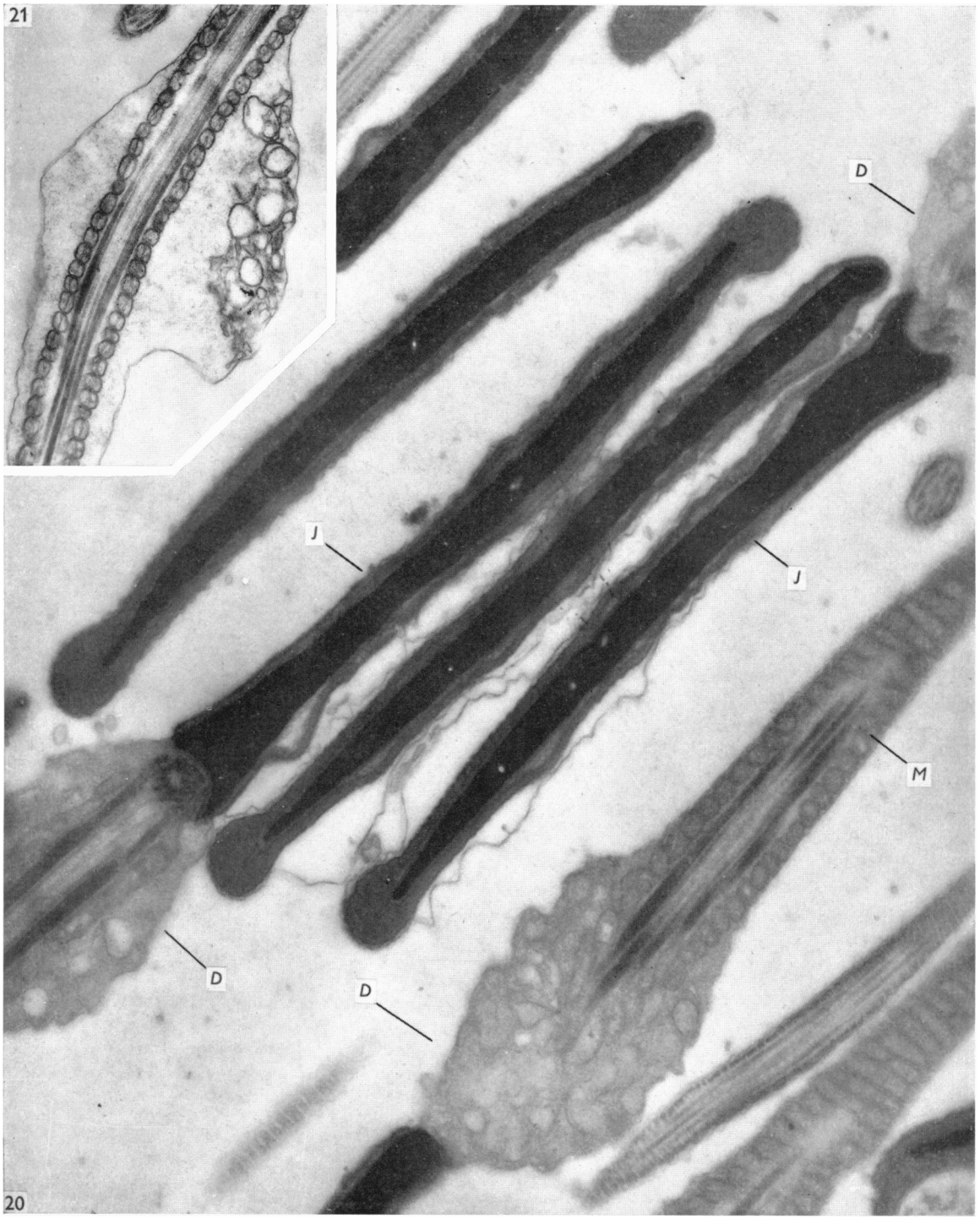


Fig. 19. Diagrammatic representation of the shape and structure of the mature sperm head. A, Plan view; B, sagittal section. *N*, Nucleus; stippled area, acrosome cap; black area, perforatorium.

regional correlation exists with respect to movement of the droplet along the midpiece, as spermatozoa with neck droplets have been found not uncommonly in region 7 (Fig. 20) and occasionally in region 8 (Bedford, 1963*a*) It is not yet known whether the cytoplasmic droplet contributes to the process of sperm maturation.

It is a matter of some interest that sections of the developing acrosome in rabbit spermatids embedded in the Sertoli cell apparently do not generally show evidence of a marginal extension (L. Nicander, 1964, personal communication); this suggests that the acrosome margin may become protracted at the time of, or soon after, the spermatozoon 'backs away' from the surrounding Sertoli cell. At present one cannot offer a satisfactory explanation for the presence of both immature and mature stages of acrosome development in one limited region of the rabbit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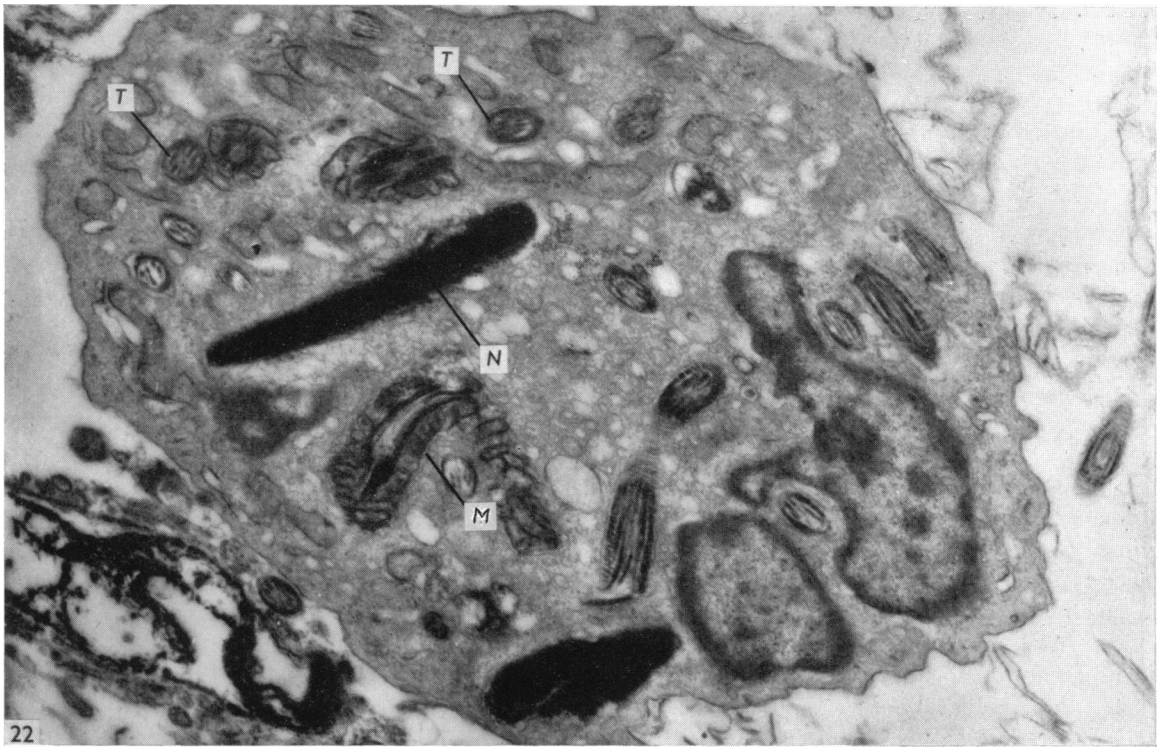
eipdidymis, but it seems possible that in the caput epididymidis such a situation may arise for one or more of the following reasons. First, those spermatozoa which apparently possessed the typical form of the mature acrosome may have been sectioned through immature heads in areas between acrosomal protrusions, at the points marked with arrows in Fig. 12. Secondly, if time is the main factor which determines the rate of maturation of the acrosome, it seems possible that in some spermatozoa formed in the polar region of the testis distant from the rete attenuation of the acrosome margin may occur in the seminiferous tubules. Thirdly, rabbit spermatozoa may not all possess the same degree of elongation of the acrosome margin at the time of their release from the Sertoli cell. It is hoped that these questions will ultimately be resolved by detailed studies of the process of spermateliosis in this species.

The marked and consistent difference in disposition of the head cell membrane between spermatozoa released from the caput and more distal parts of the epididymis respectively (Table 1) points to the existence of other, non-structural, changes in the sperm head, which must occur mainly in regions 4–6 of the epididymal duct. Recent studies using fixatives of increasing osmolality indicate that swelling of the sperm-head cell membrane is essentially osmotic in nature (Bedford, 1964*b*). The difference in reaction of the head membrane in spermatozoa from different regions of the epididymis may therefore reasonably be interpreted as reflecting some change in osmotic status of many sperm heads during epididymal passage. Such a change might be brought about by alteration in the cell membrane *per se*; this is suggested both by the increased capacity for uptake of stain by heads of sperm from the corpus region of the ram, bull (Ortavant, 1953) and rabbit (Glover, 1962), and also by the change in electrophoretic (Bedford, 1963*b*) and iso-agglutination behaviour (Bedford, 1965) of rabbit spermatozoa during their epididymal passage. On the other hand, the increase in specific gravity of ripening bull spermatozoa (Lindahl & Kihlstrom, 1952) suggests that the differences in osmotic status of maturing sperm heads might result from a relative increase in osmolality of the materials within the sperm-head membrane. It may be that both factors are implicated.

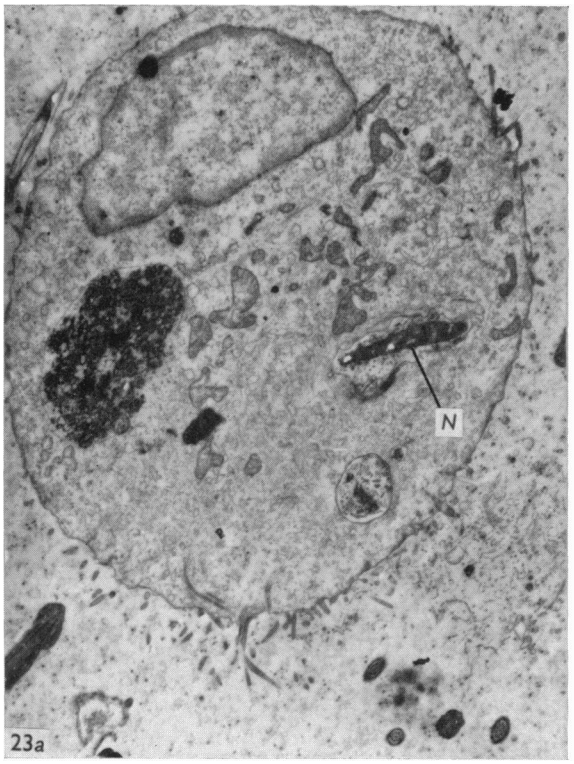
The quantitative aspects of the production and fate of spermatozoa are not well understood. Spermiophagia in the epididymis, in mainly abnormal states, has been described by earlier workers (Wegelin, 1921; Akiyoshi, 1924; Morgenstern, 1924), and more recently by Phadke (1964). Nicander (1963) reported the occasional presence of sperm fragments within the epithelium of the vas efferens and epididymis in the bull and rabbit respectively. Nevertheless, since the claim by Simeone & Young (1931) that massive disintegration of guinea-pig spermatozoa occurs normally in the distal region of the male tract, no convincing morphological evidence has appeared, as yet, to show that spermiophagia occurs to any significant

Fig. 20. Spermatozoa *in situ* in region 7. The acrosome shows the attenuated bulbous apex seen typically in mature spermatozoa. The cell membrane has become distorted away from the acrosome surface in two spermatozoa, but remains closely adherent to the acrosome in the others. Note the presence of cytoplasmic droplets in the neck region in three spermatozoa. Uranyl acetate and $KMnO_4$. ×22000.

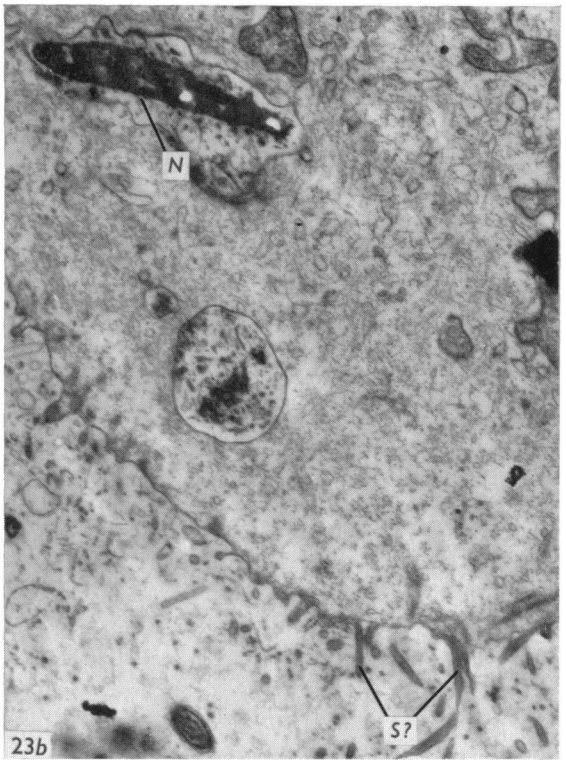
Fig. 21. Cytoplasmic droplet in the distal region of the sperm mid-piece *in situ* in region 8. Note the homogeneous appearance of the cytoplasm, and the confinement of the tubular and vesicular elements to the periphery of the droplet. Lead hydroxide. ×20500.



22



23a



23b

degree in the lumen of the normal excurrent duct. In the present study, cells were observed occasionally in the lumen of the epididymal duct, particularly so in the caput region; only rarely, however, did any of these cells contain sperm fragments, as seen in Figs. 22–23. The luminal cells were not necessarily of an identical type as judged by the appearance of the cytoplasmic organelles. While the origin of these cells is not known, the remnants of stereocilia on the lower surface of the cell in Figs. 23*a* and *b* suggest that this may have come from the duct epithelium.

SUMMARY

1. Electron-microscope studies have been carried out on rabbit epididymal spermatozoa, *in situ* and after release from specific regions of the epididymis.

2. Observations in the initial segment of the caput region have shown that, in comparison with mature spermatozoa, most spermatozoa in this region possess an elongated margin in the rostral part of the acrosome cap.

3. During passage through the caput, the extended margin of the acrosome becomes folded dorso-ventrally in many spermatozoa and, subsequently, is reduced in size. In the regions distal to the flexure of the caput epididymidis most spermatozoa display the attenuated bulbous acrosome characteristic of mature spermatozoa.

4. Unlike the situation in the guinea-pig, there is no absolute correlation of certain stages of maturation with any particular segment of the epididymal duct. A few spermatozoa possessing apparently mature acrosomes were seen in all segments of the caput epididymidis, and somei mmature forms were seen occasionally in the distal regions of the epididymis.

5. A marked difference in the disposition of the sperm-head cell membrane occurs between spermatozoa released from different regions of the epididymis. This finding, in conjunction with other evidence, indicates that some change in osmotic status of the sperm head takes place during maturation in the rabbit epididymis.

6. During movement of the cytoplasmic droplet down the mid-piece, the tubular and vesicular content of the droplet is considerably reduced. Although movement of the droplet occurs most commonly in the proximal part of the epididymis, such movement cannot be correlated absolutely with any one limited region of the duct.

7. Cells of unknown origin were seen occasionally in the epididymal lumen, particularly so in the caput region. Occasional examples of spermiophagia were observed, but this phenomenon seems to occur only rarely in the normal rabbit epididymis.

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Fig. 22. Section of cell found in the lumen of a tubule in region 3. Note the presence of sperm nuclei, mid-piece and tails within the substance of the cell. Uranyl acetate and potassium permanganate. × 12250.

Fig. 23. (*a*) Section of cell found lying in the vas deferens. Note the sperm nucleus lying within a vacuole. Uranyl acetate and potassium permanganate. × 5850. (*b*) Enlargement of (*a*). Note the surface projections which resemble stereocilia; it appears that the sperm nucleus may be undergoing digestion within the vacuole. Uranyl acetate and potassium permanganate. × 12000.

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