ELECTRON MICROSCOPIC STUDY OF THE BULL SPERMATOZOON II

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II STRUCTURE OF THE HEAD

RESULTS

In suspension materials, the head showed a slightly elongated ovoid shape, consisting of nuclear substance with high electron density (fig. 1). The anterior half of the head was covered by the head cap which consisted of a membraneous substance with low electron density. The posterior half was slightly elongated and the basal part revealed a depression which was associated with the articulation mechanism between the head and neck (fig. 2). A pair of incisions on each side of the head at the posterior margin of the head cap was apparent in sperm treated by washing with distilled water. When washing treatment was repeated, the equatorial zone became apparent (fig. 4). In the same cells, the connection between the head and neck was relax. After further strong treatment, the head cap became separated from the head proper. The anterior half of the head without the head cap showed increased electron permeability and frequent granular structures (fig. 4). Regardless of whether the head cap existed or not, the head apex contained the acrosome corpuscle which was arc-like in shape, having comparatively high electron density (figs. 1 & 4). When a collodion membrane was destroyed by the electron beam, a lateral view of the head showed a lanceolate form, of which one side was almost flat and the other was slightly expanded. Based upon the stated observations, it may be said that the head of the bull spermatozoon is symmetrical in its broad aspect, but not in sagittal sections. A separated head cap was semi-spherical in shape and appeared as a homogeneous membraneous structure with comparatively low electron density (fig. 3).

In replica preparations, the surface of the head was smoother than other parts. The head cap, a border line between the head cap and the nuclear portion, and the posterior margin of the equatorial zone were clearly shown. In some good preparations, detailed structures such as the anterior margin of the equatorial zone and the connection between the head and the neck, were also distinctive (fig. 5).

In sectioned materials, the head was covered loosely by a triple-layered cell membrane (figs. 6~9). On the dorsal surface, the cell membrane adhered to the outer layer of the external acrosome membrane, while on the ventral surface, it touched the acrosome in only two areas (figs. 10 & 11). In section preparations on flattened view, these adhering regions

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became deltoid shape on the ventral surface (fig. 12). At the posterior half of the head, the cell membrane adhered closely to the nuclear sheath.

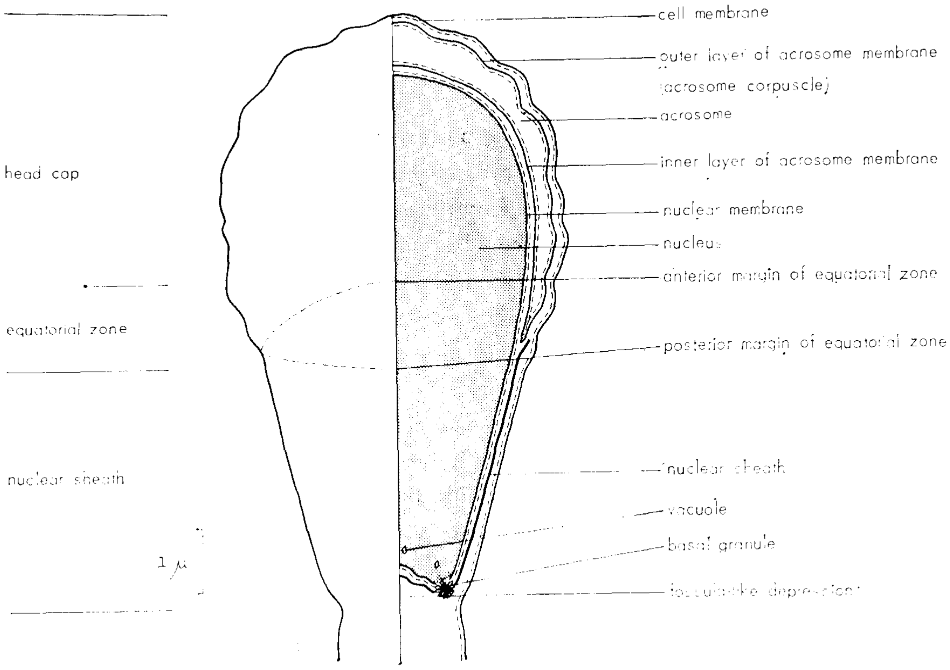
The nucleus consisted mainly of homogeneously substance with high electron density and a few vacuoles which were more prevalent near the base of the nucleus (figs. 7 & 8). The position, form, size and electron permeability of these vacuoles were variable. The head, in a flattened section, was slightly elongated ovoid as in the case of suspension material (figs. 6 & 7), but in sagittal and oblique sections the head was similar to a sword or lanceolate in shape (figs. 8 & 9). In cross section, the head showed a flat elliptical form (figs. 10 & 11).

Three fossula-like depressions were observed at the base of the nucleus. The central one was twice to three times larger than the other two (figs. 7 & 13). In oblique sections these structures appeared as 3 or 2, but in sagittal sections, they appeared as only one (figs. 8 & 9).

The so-called nucleus was covered with a nuclear membrane with high electron density. The nuclear membrane consisted of inner- and outer-layers. The inner-layer was thin and attached closely to the nuclear substance, while the outer-layer was composed of many fine granules with high electron density, forming a mono-layer arrangement (figs. 8 & 9).

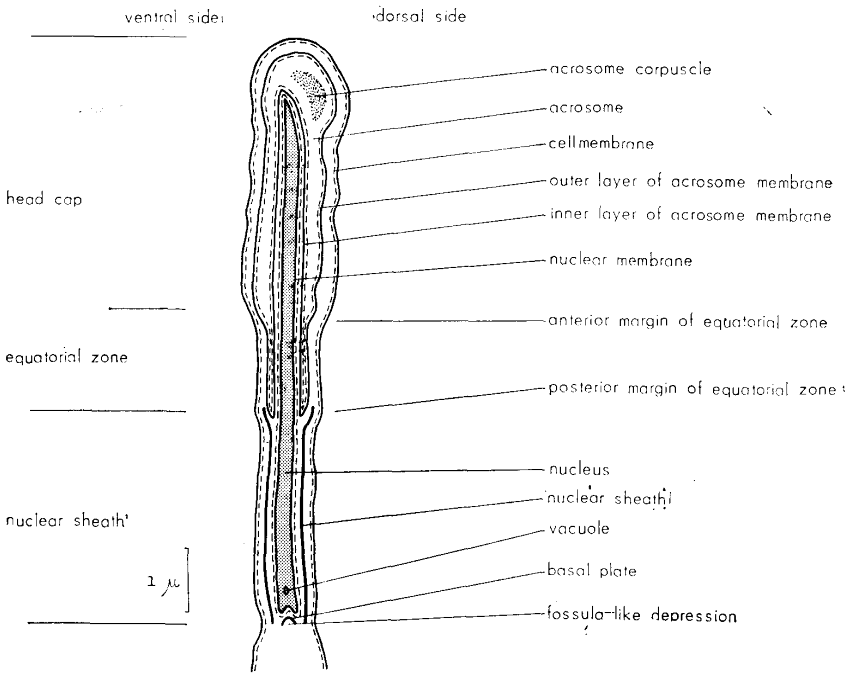
The head cap covering the anterior half of the head consisted of an external and internal acrosome membranes and an acrosomic substance between these membranes. Each acrosome membrane is a unit membrane system. The inner-layer of the internal acrosome membrane contacted the outer-layer of the nuclear membrane. Both acrosome membranes formed an acrosomic vessel which contained the acrosomic substance (text figs. 1 & 2). The electron density of the acrosomic substance was variable. There was a high electron dense mass

TEXT FIGURE 1 *Schematic diagram of head (flattened view)*



N. B.: Left half means surface and right half shows inner structures located in the apical region of the head which has been named the acrosome corpuscle, at the dorsal side. The external and internal acrosome membranes were continuous with each other at the posterior margin of the equatorial zone, where the acrosome sack became very narrow and almost empty of the acrosome substance (figs. 8 & 9). Thus, the equatorial zone consisted mainly of the cell membrane, external and internal acrosome membranes, and surrounded as a belt on the middle part of the head.

TEXT FIGURE 2 *Schematic diagram of head (sagittal view)*



The posterior half of the head, from the posterior margin of the equatorial zone to the posterior end of the head, was covered with a nuclear sheath under the cell membrane (figs. 8, 9 & 13). The nuclear sheath extended from the posterior end of the head cap, that is, the posterior margin of the equatorial zone, descending closely along with the outer-layer of nuclear membrane, terminating at the basal granules located to the base of the head. The nuclear sheath was characterized by an electron density.

The basal granules consisted of gathering masses of granular corpuscles or very small ring-like structures with comparatively high electron density. Though they were observed at the both sides of the basal part in a flattened section (figs. 7 & 13), they did not appear in a sagittal section. There existed basal plate along with the three fossula-like depressions (figs. 8 & 13), thus, the head was connected with the neck by the basal plate. The basal plate was approximately equivalent to the nuclear sheath in electron density.

DISCUSSION

For many years, it has been known that the head of sperm in the human and domestic animals is, in general, ovoid or spherical in shape[20,85,86,102,107].

However, in detailed morphological features, each species possesses its own characteristics, thus each species can be distinguishable by examination of its respective sperm morphology [10,53,102]. In a report by RETZIUS [86] who drew schematic figures of spermatozoa of many vertebrates species, figures of the bull spermatozoon are also presented and have served as a reference for this work, The head of the bull sperm has an oval-like or tennis racket-like shape [11], although variation in head morphology between individual animals as well as within animals has been reported [24,107]. The acceptable range in morphological variation among normal cells has not been clearly established.

Based upon many electron microscopic investigations, it is now clear that the head of spermatozoa is composed mainly of nuclear substance with high electron density. However, the fact that the nuclear substance is obtained spermatocytes has already clearly proven from results of light microscope [19,20,44,121]. According to LEUCHTENBERGER et al., the nuclear substance of bull spermatozoa consists of D N A of 43 per cent and other proteins.

There are observed three fossula-like depressions at the base of the head in flatten section preparations. In most of the previous reports [14,17,26,48], schemata with one depression are listed, while BISHOP & AUSTIN in the bull and RANDALL & FRIEDLAENDER in the ram report three depressions. The present author has observed that the central one of the three depressions is larger than the others and that they are arranged abreast, since only one depression appears in sagittal section preparations (figs. 8 & 9) and two or three in oblique section preparations. In human spermatozoa, the number of the depression is not yet decided [3,87,95]. Therefore, whether some species difference may exist between the bull and the human is not known.

Although FRIEDLAENDER states that the head of the ram spermatozoon is flattened toward the top with an angle of 60°, in the bull sperm, the angle seems sharper. RAHLMANN also mentions that the head of the bull sperm is thickest at the base and gradually flattened toward the anterior direction. Moreover, the actual thickness of the head seems slightly thinner than those previously reported as 0.5 to 1 μ (figs. 8 & 9).

It is well known that the nuclear substance has generally a strong resistance to physical treatments and is stained with basic dyes, such as acridine orange [10]. There were many opinions as to whether the nuclear substance occupies the whole head or not, especially in human spermatozoa. That is, most of workers considered that the anterior and posterior halves of the head are composed of different substances in staining property with each other [16,106], and also other investigators stressed that it is reasonable to distinguish three portions with different properties; the anterior portion covered by the head cap, the equatorial zone and the posterior portion[7,92,93]. On the basis of electron microscopic studies it is obvious that the head is principally homologous, and consists of nuclear substance[3,15] with the exception of the acrosome and other tightly adhering membranes.

SCHULTZ-LARSEN states that a large vacuole observed at the top of the head of human spermatozoa is an inversion of the acrosome substance of the head cap. At the time of his study when the suspension method was used for electron microscopic technique, the nuclear substance was considered to be a mass of granular structures[111,118]. According to many findings obtained from sectioned preparations of ejaculated sperm, it may be said that such granular structures are likely to appear in immature spermatozoa[30,31,56,65] and in cells destroyed physically[81] or chemically[24,29]. Similarly, SCHULTZ-LARSEN considers some granules seen in the nuclear substance of human spermatozoa as a degenerative product of the cell. YASUZUMI and co-workers found a hexagonal tubular arrangement in the nucleus of the spermatid of the grasshopper (*Gelastorrhinus bicoler* DE HAAN)[119] and strip-like structures in the snail spermatid (*Cipangopludina malleata*)[120]. Similar structures are observed in immature sperm cells in Mammalia[30,39,64]. However, in mature mammalian spermatozoa such structures have not been reported. Based upon the present results it is clear that the nucleus of the bull spermatozoon consists of a homogeneous substance with high electron density. This finding is in accord with those of BRADFIELD[23], BLOM & BIRCH-ANDERSEN[15] and RAHLMANN, but in these reports, no data from section preparations on flattened view is presented.

Although vacuoles have been observed in the head of spermatozoa using even the light microscope[86,106], the position, number, size, shape, contents and physiological significance are not yet clear. Some researchers using the electron microscope also reported the presence of vacuoles, and some of them alluded to regularity in arrangement of the vacuoles[41,47]. BRETSCHNEIDER[26] states that in bull sperm, the vacuoles appear most frequently in the anterior portion of the head and the region below the equatorial zone. In human spermatozoa, it is generally recognized that the frequency in appearance of the vacuoles is highest in the anterior half[3,38,41]. However. SCHULTZ-LARSEN believes that a so-called large vacuole in the anterior half of the head of human spermatozoon is actually not a vacuole, but a special part of the acrosome inserted into the nucleus, having lower electron density than nuclear substance. In the present report, there was no such insertion observed, and the vacuoles appeared mainly in the posterior half of the head. They were round in shape with a diameter less than $0.1\,\mu$. SEYMOUR & BENMOSCHE observed vacuoles just escaping from acrosome and postulated that they served as a suction apparatus to penetrate the egg. Furthermore, some investigators considered the vacuole as karyoplasm or a final product in metabolism[26,92], while others deemed them as artifacts[5,37,60]. It may be very difficult to clarify the mechanism involved in formation of such vacuole from the standpoint of spermatogenesis[38]. According to the present author's opinion, the vacuoles observed in mature spermatozoa seem to be caused by accumulation of gassy or liquid metabolic products, which may first be produced physiologically by spermatozoon itself and then may become large in size and number by various physical and chemical affections.

It seems that some workers who studied sperm morphology by the light microscope could not definitely understand the nuclear membrane as a membraneous structure, and so the conception of the nuclear membrane presented by them seems to mean only an outer margin of nucleus[86,108]. By means of electron microscopy, the existence of the nuclear membrane is clearly established, but there are only a few morphologists who have defined the fine structure of the nuclear membrane. Most workers think of the membrane as a mono-layered structure[26,81,87,95]. The electron microscopic photographs of the present author demonstrate evidently a two-layered structure of the nuclear membrane covering whole part of the nucleus (figs. 8 & 9). The inner layer of the nuclear membrane is, in general, thin, contacting directly with the nuclear substance, while the outer one consists of fine granules, and both run parallel with each other around the nuclear substance. According to the present author's opinion, it is not considered that the outer layer of nuclear membrane has a net work fibrous structure, as has so far been said in somatic cells[29,41,63].

The basal granule which was found commonly in most of the flattened sections of the head (figs. 7 & 13) corresponds with the structure which is called the "anterior end knob" or "nuclear ring" in the study of human spermatozoa by the light microscope[10,57,106]. The existence of this structure in bull spermatozoa has already been suggested by some workers[17,26]. The same is true for boar spermatozoa[18]. BISHOP & AUSTIN were successful in demonstrating the basal granule using Giemsa stain and fluorescence microscopy. RANDALL & FRIEDLAENDER measured the width of the basal granule of ram spermatozoa at 25~30 mμ. It is not decisive that disaccord in opinion concerning the morphological features or number of the basal granule may be due either to species difference or to difference in research procedures. Further investigation will be needed to clarify these problems. As for the developing process of the basal granule, only a little informations are available. ZLOTNIK found that the nuclear sheath was formed by extending backward of the nuclear ring, in the course of the histological investigation of spermatogenesis in the bull, dog and cat, and they contradicted GATENBY's theory that the nuclear sheath originates by the forward extension of the nuclear ring itself. Furthermore, ultrastructural studies of BURGOS & FAWCETT on cat spermatids and ISHIBASHI and KOISHI on spermatogenesis in the monkey, noticed the nuclear ring. From their findings, it is suggested that the origin of the basal granule may be the nuclear ring, because of the resemblance in the morphological characteristics and of the correlation with the circumferential structures. However, many more electron microscope studies of the process of spermatogenesis are needed before clarifying the functional significance of the nuclear granule.

There are many reports on morphology and function of the head cap of spermatozoa in various kinds of animals[2,4,12,13,16,17,20,25,27,35,36,49,84,106]. In the recent years, infertile spermatozoa with "acrosome defect" are discussed as a very important problem[48,50,75,94,99,109]. The mammalian spermatozoon has a head cap which varies in form and size with the species. During spermiogenesis, BOWEN[19] found that this structure originated in the Golgi apparatus of the spermatid. This process has now become quite clear with the aid of the electron microscope[36,39,64]. For many years, confusion existed between the head cap and the perforatorium of rodents (WALDEYER's cutting tool theory), but now it has became clear that there are differences among them in developmental origin and chemical composition according to the investigations of BOWEN[20] and TILLMANN with the optical microscope and of CLERMONT and co-worker[34~36] with the light and the electron microscopes.

Since the head cap is very sensitive to environmental conditions, it is easily altered in an irreversible way upon injury and death of the cell[2,10,16,41,48]. The head cap is easily lost from the sperm head itself by chemical and physical treatments. Detachment of this structure has been observed by some researchers using the optical microscope[2,12] and the electron microscope[28,98]. BLOM[14,15] stated that a semen sample collected from a bull on sexual rest for several months tends to show a high percentage of sperm without head caps and a bull showing over 18 per cent detached acrosomes is generally of low fertility. HANCOCK[48,50] described "knobbed sperm" or sperm possessing an acrosomal defect in the bull. He stated that in the living sperm, the head cap adheres closely to the nucleus, but when the sperm has died, the head cap becomes swollen and loose, turning up at the top. On the contrary, some researchers have the opinion that the connection between the head cap and the nucleus is not so tight, even when the spermatozoon is alive[53,63].

On the structure of the head cap, many reports published earlier deals with this as a different structure from acrosome[2,28,106]. In addition, some authors thought that the head cap is composed of two (inner and outer) membranes which are in contact with each other without any contents[10,49]. FAWCETT and co-workers[30,31,39] who studied spermatogenesis in various animals by the electron microscope, noticed that the head cap is composed of inner and outer layers and the inner component is frequently dense at the top of the head. It seems to generally be accepted that this component is commonly found in mamalian spermatozoa, showing a strong PAS-reaction during the course of spermatogenesis[10,20,38,55]. It has been said that the head cap in the human and bull sperms is composed of one membrane containing acrosome[15,23,38,95], but ÅNBERG could show that this membrane has a two-layered structure in the human sperm. According to SCHULTZ-LARSEN, there is an invasion of the acrosome membrane in the human sperm which begins from the top of the head and terminates to the nuclear substance, forming a structure like a cavity. This finding is rather resembling with that of the abnormal spermatozoa in the bull reported by BLOM & BIRCH-ANDERSEN[123] with ultramicrotomized materials.

REED & REED found several granules measuring 0.25μ in the head cap of human and bull spermatozoa. BAYOR et al. also observed these granules and they considered them as artifacts due to aged specimens. Likewise, RANDALL & FRIEDLAENDER reported four kinds of the granules in the ram sperm. In the present study, granules were observed in suspension materials (figs. 2 & 4), but not in sectioned preparation, thus it may be said that these structures are artifacts.

The present study revealed that the head cap is composed of a two-layered acrosome membrane as same as ROBERTSON's unit membrane. ÅNBERG also found it in the human sperm. The membrane closely covers the anterior half of head, but at the equatorial zone it returns itself, forming sack-like structure containing acrosomic substance and an acrosome corpuscle. From micrographs of suspension and section materials (figs. 1 & 9), the acrosome corpuscle seems to be an anchor- or horseshoe-like shape with sharp ends on both sides, being located on the dorsal surface of the head. This concept of the acrosome corpuscle is supported by a micrograph of the detached head cap (fig. 6). According to BLOM & BIRCH-ANDERSEN[15], in most cases, the acrosome corpuscle was recognizable as a vacuole, but in the present study, it always appeared as a structure with high electron density. The thickness of the head cap of the bull sperm measures about 0.1μ according to one report[15], however, it may vary considerably by the site or the direction in measurement.

Another important structure is the equatorial zone. In earlier investigations using the optical microscope, this has been reported as a special band-like structure around the head[18,85,86,121]. Its definition varies with the interpretation of the structure of the head cap[26,38,53,105,116]. It is also said that this structure is, in general, rather clear in the sperm of farm animals, while it is obscure in the human sperm[38,41]. As for the character of the equatorial zone, BISHOP & AUSTIN (in the bull and hamster) and HANCOCK[49] (in the boar) offered respectively the following opinion: the formation of this structure is based on the difference in length between two acrosome membranes, that is, the internal one is shorter than the external, thus a limited area which is lined off by the posterior margins of the internal membrane (anterior line) and of the external one (posterior line) would represent the equatorial zone. While, another opinion is that the segment is the result of a naked area between the posterior margin of the head cap and the anterior margin of the nuclear sheath[25,26,41,44,121]. In suspension materials of ram sperm, RANDALL & FRIEDLAENDER considered the equatorial zone as a triangular structure attaching to the surface of the middle portion of the nuclear surface. In human sperm, however, this structure is sometimes considered as only one line "equatorial line" which is formed by the posterior margin of the head cap, by some authors on the basis of the optical[55,102] or electron microscopic studies[3,38,95], where as other author as BAYLE thinks of it as a belt-like structure as well as those in farm animals. No one has so far demonstrated distinctly the equatorial zone in section preparations of human or farm animal's spermatozoa. In the present study, however, the equatorial zone became evident in sagittal and slightly oblique sections. The anterior margin corresponds with a portion of the acrosome membrane, in the middle part of the head, where the acrosomal substance is most scanty. The equatorial zone begins from this portion and terminates in the portion where the acrosome membrane returns anteriorly, i. e., the posterior margin, is the portion in which the nuclear sheath covers the acrosome membrane. The width of the equatorial zone measures about 1.5 μ at the central and widest part. In a flattened section through near the nuclear membrane, the outline of the equatorial zone is in accord with that of replica preparations (fig. 5). In some species of farm animals, NICANDER & BANE recently called the equatorial zone as the equatorial segment, however, they could not reveal its detailed structures, such as the anterior and posterior margins and a double-layered acrosome membrane.

Concerning the structure of the equatorial zone, the present study suggests that it is a circular band surrounding the mid-portion of the nucleus, having a variable width which is greatest at the central portion of the dorsal and ventral surfaces and becomes smaller towards both lateral sides. These findings may be sufficient to deny such an opinion that the anterior and posterior margins of the equatorial zone might be due to artifacts which would probably appear when the head cap is detaching from the head itself[53,106]. Though it is true that both margins of the equatorial zone becomes clearer, when the sperm suffers from some damages[10,18], the equatorial zone itself exists as a morphological component of the bull spermatozoon and not an artifact resulting from injury. In the presnt study, detachment of the head cap occurred at both anterior and posterior margins of the equatorial zone ; in the case of detachment at the anterior margin, the external acrosome membrane was torn and lost, while in anterior case the internal one was lost. To make clear the mode of detachment of the head cap, however, much more work will be needed.

It may probably be due to the difference in the method used that BLOM & BIRCH-ANDERSEN[15] could not demonstrate the equatorial zone in sectioned preparations of bull sperm. YAMANE noted two special granules on the anterior margin of the equatorial zone in the white rabbit. If this may be due to species difference, it is very interesting as well as of probable functional significance. Much more study will be required before the morphologenesis and functional significance of the equatorial zone can be explained.

The existence of the nuclear sheath has been proven in smear materials by special staining methods[11,121] and also supported by electron micrographs of suspended materials[6,26,41,81]. In section materials, this structure was demonstrated in the sperm of farm animals by BRADFIELD[23], RAHLMANN, and NICANDER & BANE, while in human sperm it was not yet proven. Some researchers who deny the presence of the nuclear sheath consider this structure as a part of the mitochondrial sheath of the middle piece[95], a hyperplastic tissue of the cell membrane or a part of the nuclear membrane[3,38], whereas some cytologists who are studying spermatogenesis think of it as 2 different structure[44,121] and have demonstrated electron microscopically that its origin starts from the nuclear membrane and the nuclear ring[56,64,116]. These differences in opinion may be due to morphological variability during the course of spermatogenesis. In mature spermatozoa the nuclear sheath always adheres closely to the cell membrane, thus differentiation between both is likely to be mistaken. In the present study, this structure is found to be thicker than the cell membrane, both adhering closely to each other.

Using the light microscope, the basal plate at the basal part of the head could not be resolved. ÅNBERG was first to show this structure in the human sperm by using the electron microscope, however, later researchers could not recognize it in the human sperm[38,87,95]. The present study revealed its presence in bull sperm as a structure with similar electron density as the nuclear sheath. It is located the fossula-like depressions of the basal part (text figs. 1 & 2). This finding is in confirmation of that of BLOM & BIRCH-ANDERSEN[15], however they failed to demonstrate it in sagittal sections. The basal plate has a similar density as that of the nuclear sheath, however in view of the findings obtained from flattened and sagittal sections, the present author cannot agree with the opinion that both structures form a continual structure; they seem to be completely separate.

The author yet has no right to say so much for the findings of cross section of the head, because of insufficiency in number of such photographs and of difficulty to determine their sectioned portion (figs. 10 & 11). More investigation is necessary before this problem can be discussed.

From many years, there existed the conception that the sperm is entirely covered with a cell membrane, and many illustrations have been reported. Most of these illustrations were only based on imagination arising from the opinion that the spermatozoon is also a cell. In this connection, some authors have the opinion that the cell membrane covers wholly a sperm[32,53,81,92], while the others believe that it covers partially[17,26,37,55]. This disagreement may be due to characteristics of the sperm cell membrane, especially its resistance against chemical and physical treatments. None of researchers who are studying ultramicroscopically the human sperm by means of the section preparation deny the presence of the cell membrane. The detailed structure of the membrane, however, is not always in accordance.; some authors consider it as a two-layered structure[95], but the others as a single-layered one[3,38]. In the bull sperm, a unit membrane system with two-layers has been reported[14]. In the present study also this is true; a triple-layered cell membrane covers the entirely of spermatozoon, the greater part of which the proximity to the cell is large.

Some microphotographs obtained from section preparations show very interesting features, regarding the mode of attachment of the cell membrane to the ventral surface of the head (fig. 21). The membrane conforming to the head consists of eight opaque layers at the anterior half and five at the posterior half. According to YASUZUMI[116], late spermatids of various species are covered by seven-layered membranes, while the Sertoli cell has only two-layered membranes.

Though BRETSCHNEIDER[26] and BRETSCHNEIDER & ITERSON[29] reported that the head of the bull sperm is slightly asymmetric based upon results from suspended materials, the present study using suspended and sectioned preparations clearly demonstrates that the head is quite symmetrical. In the review of literature on optical microscopy and electron microscopy of human sperm and sperm of domestic animals, there is no apparent difference in structure between the dorsal and ventral surfaces of the head. In the present study, the difference in the dorsal and ventral surfaces of the head of the bull sperm were distinguished.

CONCLUSION

Concerning the detailed structure of the head of bull spermatozoa, much remains to be unknown. The present author has tried to clarify these unknown structures by the aid of an electron microscope, using together with various physical or chemical treatment.

The results obtained are as follows:

1) The head cap which has so far been thought as a simple membraneous

structure consists actually of a pouch-like structure named acrosome membrane which contains acrosome and an acrosome corpuscle. Accordingly, the name of "head cap" should be used as an inclusive nomenclature of all these structures (text figs. 1 & 2).

2) The acrosome corpuscle is located on the dorsal surface of the anterior half of the head as an anchor or horseshoe-like shape with both sharp ends. The acrosome membrane shows a unit membrane system.

3) The equatorial zone is a part of the head cap. Its anterior margin corresponds with a portion of the acrosome membrane, where the acrosomal substance is most scanty, while its posterior margin does with a portion where the acrosome membrane returns upwards. The equatorial zone is a circular band surrounding the mid-portion of the head, having a variable width is greatest as the central portion of the dorsal and ventral surfaces and becomes smaller towards both lateral sides.

4) The nuclear sheath begins at the portion of the posterior margin of the equatorial zone, where the former covers the latter. Then, it covers around the posterior half of the head and terminates in the basal granules.

5) A pair of basal granules are located one at each lateral side of the basal portion of the head.

6) Three fossula-like depressions are observed at the basal portion; the central one is larger than the others. Along these depressions, there is a basal plate which connects with the neck.

7) The nucleus which contains homogenous nuclear substance with high electron density is flat and oval in shape, but it tapers away at the dorsal side of the anterior margin.

8) The head is quite symmetrical on flattened view, but not on lateral view.