THE AMPHIBIAN SPERM

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THE Amphibia with its three existing orders, Urodela, Anura and Apoda, offers three different types of sperm structure. The Urodelan and Anuran sperms have been the subject of a number of memoirs which have dealt with their structure and development while the Apodan sperm has not been studied till now. Recently the author (1943*a*) has been able to work out in detail the sperm development in an Apodan example, *Ichthyophis glutinosus* and so it is possible for the first time to offer a comparative account of the sperms of the three groups of Amphibia.

The work of Meves (1897), MacGregor (1899), Broman (1900, 1901), Retzius (1906), King (1907), Terni (1911), Ballowitz (1913), Champy (1913, 1923), Morita (1928) and Gatenby (1931) has thrown considerable light on Urodelan and Anuran spermateleosis. This work has established one fact of importance, *i.e.*, that the sperms of these two groups are thoroughly unlike each other, and while anatomically these two great Amphibian orders are closely related, their spermatozoa fall under two entirely different categories.

The Anuran sperm is of uniform structure with a small acrosome surmounting an elongated nucleus behind which the two centrioles are lodged close together, and from the distal centriole the tail filament issues either as a simple flagellum (*Rana, Hyla, Pelobates*) or as one with an undulating membrane (*Alytes, Bufo, Bombina, Discoglossus*). The base of the flagellum is ensheathed by mitochondria which may or may not form a spiral investment and this mitochondrial sheath is often referred to as the 'middle piece' of the sperm (Fig. 1 *B*). In the case of *Hyla* (Retzius, 1906) there is a slight space separating the two centrioles and this space with its mitochondrial aggregation is called the 'middle piece'. The spermatozoon of *Bombinator* (Broman, 1900) is very peculiar in that the centrioles are situated, not behind the nucleus, but to a side of it anteriorly so that the flagellum runs parallel to the elongate nucleus during much of its course.

Compared with this simple sperm of Anura, that of the Urodela has a very complex structure. The acrosome and nucleus occupy the anterior end of the spermatozoon and are liable to considerable variation in size and shape. Closely following the nucleus is a conspicuous solid body of large size (Fig. 1 *A*). This is the proximal centriole which has become greatly enlarged and forms one of the constant features of the urodele sperm. Behind this trails the axial filament. The distal centriole undergoes a curious modification. It becomes converted into a ring which elongates greatly and lies on the side of the axial filament for most of its length. The mitochondria are generally absent from the adult urodele sperm.

As already observed, the Apodan sperm has not been studied at all and the only observation regarding it is that by the Sarasins (1890) who have provided a very meagre account of the sperm of *Ichthyophis glutinosus*. Its structure and development have been studied by me (1943*a*) recently and as a result, it is possible for the first time to compare it with the sperms of Urodela and Anura. In the Apodan sperm (Fig. 1 *C*) the acrosome and

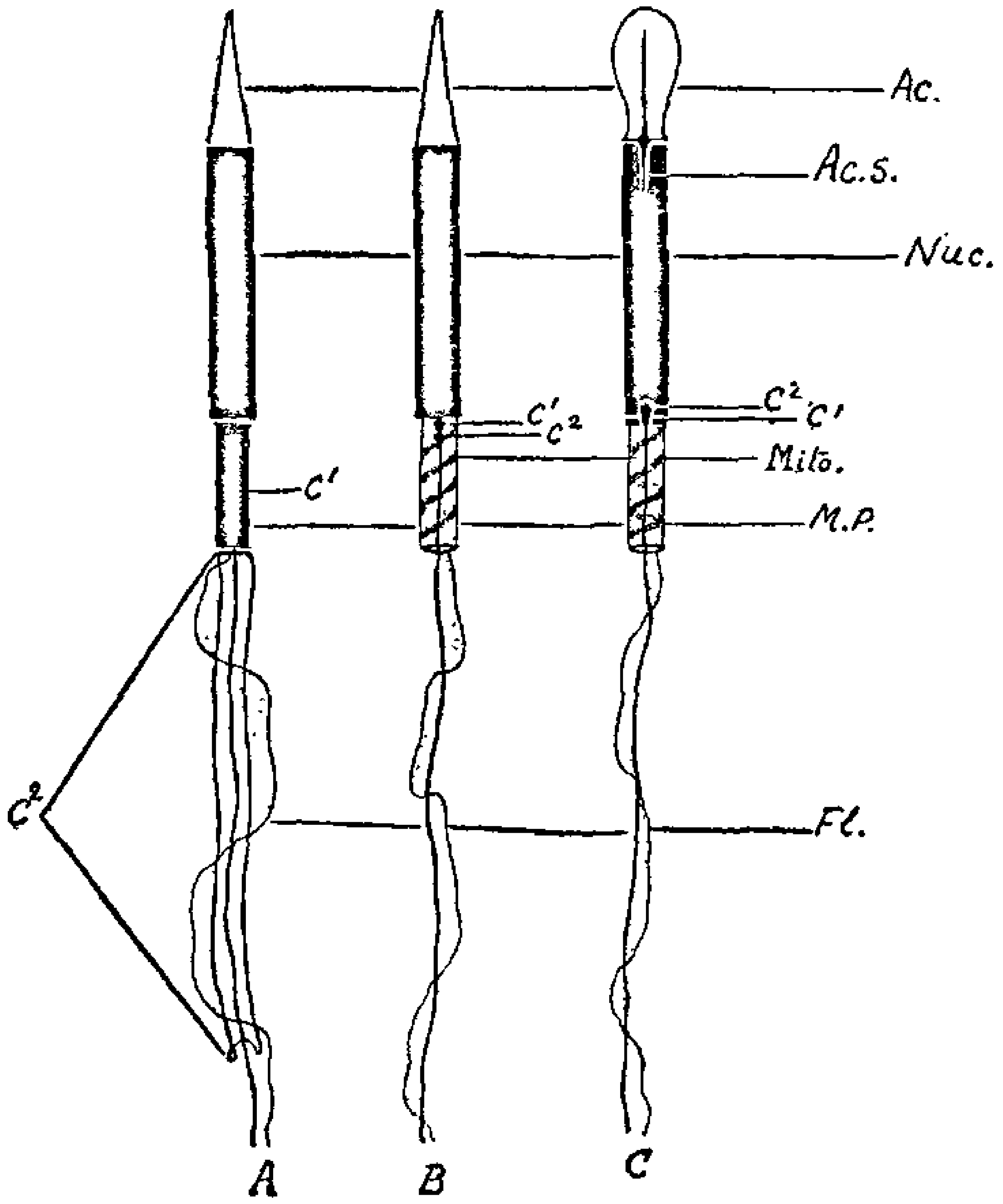

FIG 1. Diagram showing the structure of the sperms of Amphibia. *A*. Urodele sperm; *B*. Anuran sperm; *C*. Apodan sperm.

Ac. Acrosome; *Ac. s.* Acrosome seat; C^1. Proximal centriole; C^2. Distal centriole; *Fl.* Flagellum; *Mito.* Mitochondria; *M.P.* Middle piece; *Nuc.* Nucleus.

nucleus are followed by a short 'middle piece' which is a spiral aggregation of mitochondria around the base of the flagellum. The two centrioles are in close and intimate relation with the nucleus, the proximal fused with it posteriorly as a flat disc with a single perforation. The distal centriole becomes an elongated granule, which, passing through the orifice of the disc-shaped proximal centriole, becomes embedded in the nucleus. The flagellum of the axial filament is provided with an undulating membrane which extends over a great part of its length.

The Acrosome and Its Seat.—The acrosome in Amphibia varies greatly in size, form and appearance. In most Anura (*Rana, Hyla, Bufo, Alytes*) it is a pointed lance-shaped body surmounting the nucleus. In *Pelobates* (Broman, 1901) it is of great length and twisted in a more or less cork-screw fashion. In *Discoglossus* (Champy, 1923) also it is extremely long and filiform and makes up half the length of the sperm. In urodeles, it is more variable. The acrosome of *Pleurodeles* and *Triton* resembles that of the Anura in being a lance-shaped pointed structure but in *Molge* it is bent in the form of a fish-hook (Ballowitz, 1913). All variations between these two forms may occur. The acrosome of the Apodan sperm is a bulb-shaped structure with a narrow stalk planted on the nucleus. In the five species of Apoda examined by me (*Ichthyophis glutinosus, Uræotyphlus narayani, Dermophis gregorii, Siphonops annulatus* and *Gegenophis carnosus*) the acrosome has this shape. The pointed acrosome in many animals has led to the belief that it helps in the penetration of the egg membranes during fertilisation and hence has been called the perforatorium. More recently with the discovery in a number of animal sperms of an acrosome which is of such a shape that an act of perforation is difficult if not impossible, this view has been given up in favour of the one whereby, the egg itself, by means of the changes taking place in its outer constitution, becomes capable of engulfing the sperm, so that penetration of the sperm into the egg is an act largely of the egg rather than of the sperm (Wilson, 1928). The Apodan sperm is a case in point, as here the sperm with its blunt and rounded acrosome could not perforate the egg membranes.

The acrosome and the nucleus being quite separate bodies, the union between the two is by means of a mechanism whose real nature has been obscure for a long time. Gatenby (1931) was probably the first worker to draw attention to this fact and in his work on the development of the spermatozoon in Urodela has correlated the presence of certain deeply staining granules with the formation of an acrosome seat in relation with the nucleus into which the acrosome is fitted. Earlier, similar granules had been noticed by Meves (1897), MacGregor (1899), Terni (1911) and Bowen (1922) in other Urodela, without however, their real significance having been understood. Recently I have described the acrosome seat in the Apodan form *Ichthyophis*. The acrosome gives off a lance-shaped plug which fits into a pit in the centre of the nucleus so that the two are held together firmly (Fig. 1 *C, Ac. s.*). No other account of the acrosome seat exists in regard to the Amphibian sperm but it is more than likely that future work will show that in all animals the acrosome and the nucleus are incorporated with each other by means of an apparatus which, while varying in the details of its constitution, is uniform in that its main function is to cement the acrosome and the nucleus.

The Nucleus.—The nucleus which, with the acrosome, constitutes the head of the spermatozoon, is also variable in form in the Amphibia but generally within not very wide limits. In the Urodela and Anura its form and shape are generally uniform. It is a deeply staining body, either cylindrical or pointed towards the anterior end (*Rana, Bufo, Triton, Alytes*). In *Bombinator* it is elongated, and broad anteriorly and pointed posteriorly. In the Apoda too there is a great uniformity in the matter of shape and form of the sperm nucleus. In the five species the author has been able to examine (1943) it is an elongated cylinder. In the matter of the size of the nucleus, however, there is no uniformity. I have recently attempted a line of study with a view to determine the volume relationships of the sperm nucleus and the nucleus of the spermatid. It is a matter of common knowledge that one of the most important changes involving the nucleus in spermateleosis is its condensation and consolidation and it is believed that in its transformation from the spermatid to the spermatozoon, the nucleus undergoes a progressive reduction in volume, presumably due to loss of fluid from the nucleus. The nucleus of the adult sperm, therefore, is a packed mass of chromatin in which no details of structure can be made out. The question naturally arises as to what the relationship is between the volume of the nucleus of the spermatid and that of the adult spermatozoon. No work on these lines has been done in any group of animals and it has been shown for the first time that so far as the Apoda are concerned, the condensation is very considerable, the volume reduction between the spermatid nucleus and sperm nucleus varying between 80 per cent. and 95 per cent. in the different species. (Seshachar, 1943).

Centrioles.—The disposition of the centrioles in the Amphibian sperm varies very much and in this respect, the Anura and Apoda resemble each other while the Urodela stand apart. In the Anura, the centrioles lie one behind the other posterior to the nucleus (Fig. 1 *B, C¹, C²*) in the form of granules (except in *Bombinator* where they lie alongside the nucleus and not behind). They are generally distinct from the nucleus and close to each other. In *Hyla* a short distance separates the two centrioles. From the distal centriole arises the flagellum. In the Apoda too, the centrioles lie behind the nucleus but in this group they are far more deeply and intimately associated with the nucleus (Fig. 1 *C, C¹, C²*). The proximal centriole becomes a flattened disc-shaped structure adhering firmly to the nucleus behind and so intimately fused with it in the adult sperm as not to be distinguishable from it except by the use of special reagents. The centre of the disc has an orifice through which the distal centriole, becoming a spindle-shaped granule centres into the nucleus and lies embedded in it. In the adult Apodan sperm, therefore, it is not possible to distinguish the centrioles, as it is in the Anuran sperm, where the centrioles are clear and separate from the nucleus.

But the condition in the Urodela is very striking. The proximal centriole becomes greatly enlarged as a deeply staining block adhering to the nucleus behind it, while the distal centriole becomes a ring and is drawn out along the flagellum as a long pessary-shaped structure extending over a considerable length of the tail filament. No granular centrioles are found in the adult spermatozoon of any Urodele.

The 'Middle piece'.—The term 'middle piece' is used to designate any region, however different in structure, provided it is immediately posterior to the nucleus. This is more clear in Amphibia than in any other group, for the 'middle piece' is different in the three orders of Amphibia. In the Anura, the centrioles are, as has been observed, behind the nucleus and the mitochondria invest the base of the axial filament as a sheath. The mitochondrial sheath with the two centrioles is called the 'middle piece'. In *Hyla* (Retzius, 1906), the centrioles are separated by a short space and this space with the mitochondrial aggregation is called the 'middle piece'. In the Apoda, since both the centrioles are more closely associated with the nucleus than in the Anura, the 'middle piece' is purely a mitochondrial aggregation behind the nucleus. In the Urodela on the other hand, the conditions are quite different. There are generally no mitochondria in the adult urodele sperm and the term 'middle piece' is, therefore, applied to the conspicuously large proximal centriole which is just behind the nucleus (Fig. 1 *A*). So, while the term 'middle piece' is applied to the mitochondrial aggregation in the Anuran and Apodan sperms, it is the proximal centriole itself that is termed 'middle piece' in the urodele sperm.

The mitochondria in the 'middle piece' of the Apodan sperm (presumably also in the Anuran) occur inside a cylindrical transparent tube fitted to the posterior end of the nucleus. The origin of this tubular sheath I have been able to trace in *Ichthyophis* (1943 *a*). Around the posterior end, and at the sides of the transforming spermatid nucleus there appears a space which is gradually pushed backwards and becomes the tubular sheath in which mitochondria aggregate and arrange themselves in a spiral fashion around the axial filament. The presence of three different structures in the 'middle piece' of the Apodan sperm has to be recognised: (1) The axial filament which arises in relation with one of the centrioles, (2) the mitochondrial aggregation which is a spiral investment around the base of the axial filament, and (3) the transparent tubular sheath which holds the mitochondria in place. While the earlier workers had described the first two of these, the presence of the third has not been explained so far.

From the foregoing account of the spermatozoa of the three groups of Amphibia, it is clear that they fall under three entirely different categories. Of these, the Anuran sperm is the simplest. The Apodan sperm resembles it greatly, especially in the close association of the two centrioles with the nucleus as well as in the mitochondrial 'middle piece'. The Urodelan sperm on the other hand, in the great enlargement of the proximal centriole to form a solid body and the curious elongation of the distal centriole to form an accessory structure running along the tail filament, provides a condition unique in the animal kingdom.

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OBITUARY

SIR EDWIN BUTLER, C.M.G., C.I.E., F.R.S.

As it must to all living beings, death came to Sir Edwin Butler on April 4, 1943, following an attack of influenza. That he was ill was known for some time but no one had realised that his end was so near.

Edwin John Butler was born on August 13, 1874, in Co. Clare, Ireland and received his early education at the Queen's College, Cork. He took the Bachelor's degree in medicine from the Royal University, Ireland, in 1898, but having come under the influence of Professor M. M. Hartog at the Queen's College, he preferred the study of fungi to the practice of medicine. That aquatic Phycomycetes should attract him in Hartog's laboratory is natural and obtaining a travelling fellowship in 1899, he spent two years on the continent where the study of fungi had made great strides. He studied under Cornu and van Tieghem at Paris and Poirault at Antibes and spent some time at Freiburg where a few decades previously de Bary studied and taught mycology and attracted students from all over Europe.

In 1901 Butler was appointed Cryptogamic Botanist to the Government of India with headquarters at the Royal Botanic Gardens, Calcutta. In India fungi had been collected by Koenig, a student of Linnaeus and the first