brain rhythms, but an occasional isolated snore may start a train.

(7) When asleep sounds of a certain character, such as rustling paper or coughing by a person in the bedroom, closing a door some distance from the subject or low conversation, which does not wake the sleeper, will quite regularly initiate a train of waves which may last for from 5 to 8 seconds (frequency 9 to 10/seconds) and then die out. Fig. 1 A illustrates this effect from the repeated closing of a door at one-minute intervals and allows comparison with regions where no sound stimuli were sent in. The depth of sleep and the noise level in the room determine whether this "sound response" will appear. One deep sleeper gave no response on closing the door but responded regularly on slamming the door.

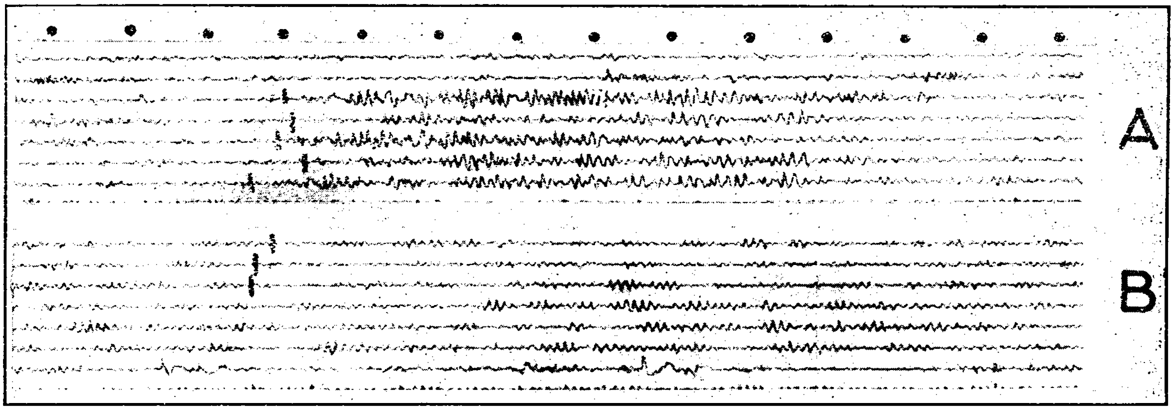

FIG. 1. Sections of brain potential records each taken one minute apart. Read from left to right. At vertical mark sound stimuli sent to subject. Note marked trains of brain rhythms in A when subject asleep but none in B when subject awake, although stimulated by same sound. Time in seconds given by dots at top.

(8) When awake, the same sounds that during sleep initiate a train of waves no longer give rise to them. Fig. 1 B clearly shows this.

(9) During sleep trains of waves appear which can not be correlated with any detectable external stimulus, but which may be connected with internal disturbances of unknown origin. The cause of these very regular bursts is now under investigation.

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THE CENTRIOLE AND ITS ROLE IN MITOSIS AS SEEN IN LIVING CELLS

THE centrioles in the various genera and families of hypermastigote flagellates[1] range in length from 2 or 3 microns to 80 or more and may be seen easily in living organisms, since they are 1 to 3 microns in width. The achromatic figure and the manner in which it arises from the centrioles also may be seen very clearly in living cells. These protozoa, then, furnish ideal cytological material. Unfortunately, however, there appears to be a tendency among some cytologists to disregard cytological observations on protozoa, although there is no justification for such a tendency, because protozoa are cells, and observations made on them furnish as valuable a basis for generalizations as those made on *Ascaris* eggs, grasshopper testes or other types of classical material. Indeed, most of the Hypermastigina show much more clearly than any other known cells the centrioles, the manner of their duplication, the formation of the achromatic figure from them and the rôle of the achromatic figure in chromosome movement. Furthermore, observations on living material of these organisms show beyond question that the observations on fixed and stained material deal with realities, not artifacts produced by fixation. And the close similarity between the behavior of these hypermastigote centrioles and the centrioles of other cells leaves no room to doubt the general application of the observations on these flagellates to mitosis in both animals and plants.

In some genera, particularly those with short centrioles as in *Joenia, Mesojoenia* and other genera of the Lophomonadidae, the achromatic figure arises from the greater portion of the centriole; in other genera, with longer centrioles, it arises only from the distal half or third of the centriole; and in those genera with elongate centrioles, it arises from only a small portion of the centriole, the distal portion. In certain genera, the distal portion of the centriole from which the achromatic figure arises is surrounded by a

[1] The names of the 29 genera and 6 families need not be given here, since they are given in a recent publication to which the reader interested in them is referred (*Mem. Amer. Acad. Arts and Sciences*, Vol. 17, No. 2, 1934).

hyaline centrosome which (in different genera) varies from 2 to 6 microns in diameter; while in other genera no portion of the centriole is surrounded by a centrosome. The fibers of the achromatic figure arise from the centriole, not from the centrosome. The latter, when present, merely serves a minor function in directing the fibers so as to make the central spindle portion of the achromatic figure less flat or band-like. This may be seen by comparing the central spindle of *Barbulanympha* with that of *Stauro-joenina*.

In the interphase in some genera the two centrioles are of equal length, either short or long; while in other genera there is one short and one elongate centriole, the short or daughter centriole elongating in the early prophase. In other genera, both centrioles are short in the interphase and elongate in the early prophase. After these centrioles have functioned in the production of the achromatic figure, cytoplasmic division occurs and each daughter cell receives one elongate centriole, which soon degenerates except for the proximal portion, so that in the interphase the parent as well as the daughter centriole is short. In brief, then, there are (1) short interphase centrioles which function without elongation; (2) short interphase centrioles which elongate in the early prophase; (3) one long and one short centriole in the interphase, the short one elongating in the early prophase; and (4) two elongate centrioles in the interphase. The centrioles are continuous from one cell generation to the next, a new one being produced from the proximal portion of an old or persisting centriole during each cell division.

In addition to the achromatic figure, all the other extranuclear organelles, such as flagella, parabasals, axostyles, etc., arise from the centrioles, so that the centriole is clearly an autonomous organelle, and the dynamic center of the cell since it reproduces itself and all the other organelles except the nucleus. (It should be noted, however, that, except in a few genera during the annual encystation generation, the production of flagella and other extranuclear organelles from the centrioles occurs one generation before the production of the achromatic figure. See footnote 1.)

The achromatic figure which arises from the centrioles is composed of astral rays, some of which join and overlap to form the central spindle, some of which become extranuclear chromosomal fibers, and some of which remain as astral rays throughout mitosis and hence perform no apparent function. It is perhaps desirable, however, to avoid the use of the long-used term spindle, since it has been used to refer to either the central spindle or the extranuclear chromosomal fibers or both. At least, there is less likelihood of confusion if the three parts of the achromatic figure are referred to as astral rays, extranuclear chromosomal fibers and central spindle. Of course, it must be realized that the fibers of the central spindle and the extranuclear chromosomal fibers are merely astral rays that are functioning in the process of nuclear division, those of the central spindle serving as a stabilizer which prevents the nucleus from being pulled in two before the proper movement and distribution of the chromosomes, and those that are attached to the chromosomes serving to move the chromosomes to the poles.

The formation of the achromatic figure is initiated by the outgrowth of astral rays from each of the interphase centrioles which, in different genera, lie from 5 to 40 microns apart in the cytoplasm. The distance from nucleus to centrioles varies in different genera from 2 to 3 microns to 50 or more. As the astral rays elongate, those arising from one centriole soon meet those arising from the other centriole; when they meet, they join, grow along one another and overlap, thus forming the central spindle, which as it develops depresses the ever intact nuclear membrane and takes up an axial position. In the meantime, chromosomes have formed and each chromosome is anchored to the nuclear membrane by an intranuclear chromosomal fiber which varies considerably in length in different genera and which should probably be considered a part of the chromosome. In most genera there is an enlargement or knob where each intranuclear chromosomal fiber joins the nuclear membrane. Presently some of the astral rays become extranuclear chromosomal fibers by connecting with the knobs or "kinetic bodies" of the intranuclear chromosomal fibers in the nuclear membrane. When such a connection is established, the chromosome is connected with the centriole. Not all the connections are made at the same time, but eventually all the chromosomes are connected with the centrioles in this manner, half being connected with one centriole and half with the other, so that as the centrioles of the dividing cell separate the daughter chromosomes are moved to the poles. The fibers composing the central spindle pull apart, and presently the achromatic figure begins to disappear, the last part to disappear being the extranuclear chromosomal fibers. No other fibers are present during mitosis, and it appears to the writer that the so-called interzonal fibers, sometimes described during mitosis, particularly in the anaphase, are either the fibers of the central spindle or connections between the two groups of daughter chromosomes which, in certain forms, pull out for a considerable distance before pulling in two.

In the interphase the centrioles (and the centrosomes too in those genera where they are present) may be moved for a considerable distance by mechanical means without altering their appearance in the least. In fact, it is only when the cell is completely destroyed that they disappear. And when the achromatic figure has been formed from the centrioles, it is possible not only to see that the daughter chromosomes are connected to the centrioles, but also to demonstrate such a connection by pulling either of the centrioles away from the nucleus, the chromosomes moving with the centriole as it is pulled. Such a procedure also demonstrates the elasticity of the extranuclear chromosomal fibers and those of the central spindle, for unless the centriole is pulled a considerable distance (far enough to break the fibers) from the nucleus, it and the chromosomes pulled with it immediately spring back into position when the tension is released. Thus, in these organisms, there is not the slightest doubt regarding the existence of the centrioles, the formation of the achromatic figure from the centrioles, the fibrillar nature of the achromatic figure, and the rôle of the achromatic figure in nuclear division.

The question naturally arises: Are all centrioles like those of hypermastigote flagellates and do they function in the same manner? As already noted hypermastigote centrioles vary considerably in size and in the type of achromatic figure that arises from them. In some genera the central spindle is flat and band-like, in some it is cylindrical, in some it is compact, and in some it is dispersed. In certain genera the astral rays are fine and can not be seen so readily as in others, and in those with fine astral rays the extranuclear chromosomal fibers are more difficult to see. In brief, there is every gradation beginning with genera having large centrioles and a large achromatic figure which may be seen with a 16 mm objective and a 10X ocular, and ending with those where the centrioles and achromatic figure may be seen only faintly with oil immersion objectives. So that it is only a short step from hypermastigotes of the last category to the cells of other forms of life where, in fixed and stained material, the centrioles and the achromatic figure have the same appearance as those of hypermastigotes in living material. In this connection it should be noted that in the polymastigotes *Saccinobaculus* and *Pyrsonympha* the intranuclear achromatic figure may be seen in living cells, but the centrioles from which it arises can not be seen, and in fixed and stained material the centrioles can be seen in only one of the three species of *Saccinobaculus*. There are evidently all gradations of centrioles, from the large, dense ones of certain hypermastigotes to the less dense and diffuse ones of other cells, and whether a centriole can be seen in living or in fixed and stained material depends on its nature and that of the cytoplasm or nucleoplasm in which it lies. The same is also true of the achromatic figure. But the ability to demonstrate a centriole only under certain conditions of fixation and staining does not indicate that it is an artifact; nor does the inability to demonstrate it at all indicate that it is not present. It merely means that its nature is such that it can only be seen under certain conditions or that it can not be seen at all with the aid of any known technique. In any cell —and this includes practically all cells—where some type of an achromatic figure is formed, centriolic material must be present; it may be congregated into a large, dense, extranuclear body as in some hypermastigotes or, on the other hand, it may be rather generally scattered through the nucleus as in the cells of many vascular plants. There is no reason why the centriole and the achromatic figure should be less variable in different types of cells than other organelles. And the fact that the centriole in certain animal and plant cells give rise to flagella, as well as to the achromatic figure, does not appear to be sufficient reason for regarding it as another organelle, since in some generations (cell divisions), both in animals and plants, the centriole gives rise only to the achromatic figure, while in other generations it gives rise to flagella and the achromatic figure. In such organisms, then, which are by no means few in number, the same body sometimes would be considered a centriole and at other times something else. What appears to be the best explanation of the situation is that in certain forms the centriole still possesses the ability to give rise to locomotor organelles in addition to the achromatic figure, while in other forms either it has never performed this dual function or this ability has been lost, or there is no longer any need for the centriole to produce locomotor organelles. If there are cells where the locomotor organelles arise from a body that does not produce the achromatic figure, the term blepharoplast appears applicable to this body.

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