DISCUSSION

THE CENTRAL BODIES AGAIN

THE nature of the central bodies or "centrosomes" has previously been discussed in these columns[1] with reference to the question whether they have any existence in the living cell or are merely random granules or artificial products of cytological technique. We will here report briefly the results of studies on the development of the insect egg (*Drosophila*) which, we believe, provide crucial evidence on the question. This object, unexpectedly enough, has been found to offer a spectacular demonstration of the phenomena on a large scale. The cogency of the evidence is due in part to the technical excellence of the preparations, but even more to the prodigious wealth of material which they display. Throughout the syncytial period of development (2,000 nuclei or more), all the nuclear divisions are almost exactly synchronous throughout the egg. At every step, accordingly, all the division-figures (or nuclei) are very nearly at the same stage, so that they may be studied in great numbers, again and again, and in all positions. The evidence thus offered leaves, we think, nothing to be desired in point of either quality or quantity.

It is proper to state that the facts were originally worked out in full by the junior author, who subsequently submitted his extensive series of preparations and photographs to the senior author for further examination. The latter has added only an independent judgment based on critical study of the material, together with the preparation of a large additional series of photomicrographs in which all the essential facts are clearly to be seen. Those facts are, in brief, as follows:

(1) In the metaphase there is at each pole a single, sharply defined central body surrounded by a single aster. In the late metaphase or early anaphase the central body at each pole becomes dumb-bell shaped and divides into two equal parts. Slightly separating, but remaining side by side, the members of each pair pass together during the anaphases to the corresponding pole; and here the pair may lie at any angle, from 90 to 0 degrees with respect to the mitotic axis, precisely as described by a number of the earlier observers. From this point forward the two may easily be followed, without loss of their identity and always clearly separate, throughout the telophases up to the end of the nuclear reconstruction.

(2) During the foregoing stages the pair of central bodies at each pole is surrounded by a conspicuous aster which at every stage remains single, showing no trace of division or the formation of small daughter-asters within the old one. In this respect mitosis in this egg differs from that of such forms as *Chaetopterus, Cerebratulus* or *Thalassema* and is more like that of *Ascaris*.

(3) After the reconstruction both spindle and asters disappear, leaving the two central bodies at each pole lying near the nucleus, and still conspicuously separate. Pictures of this kind, to be seen by hundreds in good preparations at the right stage, exclude even the smallest possibility of confusion with random granules. A little later the central bodies lie very near, or directly upon, the nuclear membrane and now move more widely apart in various degrees. Whatever their final position (whether at opposite poles or less widely separated), each as the prophases approach becomes surrounded by a small clear area and finally by a growing aster.

(4) In later prophases the central bodies move somewhat away from the nucleus, the nuclear wall fades at the nearest points, and here the spindle-fibers are clearly seen growing from the centers into the nuclear cavity. Owing to the varying degrees of divergence of the centers at this time, the incipient spindle is often flexed more or less sharply at its middle point, thus offering interesting V-shaped figures in the later prophases. In the end, however, the spindle straightens out completely and becomes perfectly symmetrical, with a single central body at each pole. The history of these bodies from metaphase to metaphase is thus completed without the slightest breach of continuity at any point.

(5) The foregoing cycle is displayed with unmistakable clearness in the division of great numbers of cells; and every stage, without a gap, has repeatedly been photographed successfully, using the Zeiss 1.5 oil immersion apochromatic and Wratten panchromatic plates.

(6) After formation of the cellular blastoderm, archenteron and mesoblast the mitoses continue to be precisely like those of the preceding syncytial cleavage except that they are smaller and no longer synchronous.

These findings, we believe, establish decisively the following conclusions:

(a) In this object the central bodies are neither random granules, nor artificial products of coagulation of the astral rays, nor staining artifacts; and obviously they do not here serve as blepharoplasts. They are not products of the asters, but, on the contrary, are themselves causally concerned in the formation of the asters.

(b) In this object the central bodies are genetically continuous by division, without the smallest interruption from one cell-generation to another. In this

[1] See the issues of SCIENCE for June 27, 1930, and April 17, 1931.

respect their history in the *Drosophila* embryo, up to a late period of development, is completely in accordance with the classical view maintained by Van Beneden, Boveri, Flemming, Heidenhain, Meves and other early leaders of cytology.

We are convinced that the phenomena in *Drosophila* are in no way exceptional in amphiastral mitosis save in respect to the clearness and profusion of the evidence; and we are confident that intensive and impartial study, using an adequate technique, will demonstrate essentially similar conditions in amphiastral mitoses generally.

The foregoing observations will later be set forth in full, with suitable figures, by the junior author.

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A NEW POSTERIOR PITUITARY PREPARATION

DURING July and August, 1930, the following method was developed and tested. The resulting material proved to be so different in chemical and pharmacological properties, that a preliminary report was given at the chemistry section of the Cleveland A. A. A. S. meeting.

Fresh beef posterior pituitary lobes are finely ground with a small quantity of sand and transferred to a flask containing about ten volumes of neutral high grade acetone. It is placed in the ice-box and occasionally shaken. New fresh portions of glands are added as obtained from slaughter house, keeping the same acetone ratio. When enough has been obtained to make a convenient batch (100 grams) the material is filtered and fresh acetone is added, shaken frequently and kept at ice-box temperature. Again it is filtered, washed with acetone and once more suspended in 10 volumes of acetone, shaken and cooled as before. The residue from this last acetone treatment is nearly white and dry. Three treatments (each at least 24 hours) with the best grade ether are now used, the procedure being the same as for acetone. Then three additional treatments with high grade petroleum ether. After the last petroleum ether extraction the material is spread out and the occluded solvent evaporated, then returned to the original flask and extracted with ten volumes of a mixture containing methyl alcohol, 70 per cent.; water, 25 per cent.; acetic acid, 5 per cent. This treatment is much like the preceding ones. The above process is repeated two times more. These three acid alcohol extracts contain the active material. They are evaporated in shallow dishes at low temperature with the aid of a fan. The residue is dissolved in a small volume of acid alcohol and precipitated with acetone and ether. The solution and precipitation is repeated. It is further purified by solution in water containing enough pyridin to dissolve the material and then precipitated with acetone and ether. The yield is very satisfactory.

The use of acetic acid in the above extracting medium is the least objectionable, though the other acids in low concentration are also very effective. In place of methyl alcohol, ethyl or propyl can be used. Sixty per cent. acetone and acetic acid is also a very satisfactory solvent.

The final product is not very soluble in distilled water, though moderately soluble in boiled distilled water. It has a rather sharp iso-electric point at about pH 5. It easily dissolves in dilute acids or dilute alkalies. It is precipitated by copper and zinc salts, by many of the acid protein precipitants and by salting out with ammonium chloride and other salts. The biuret is pale violet. Trypsin, as well as strong acids, destroy the activity and hydrolyze the substance. It is unstable in weak alkali. It seems to be a polypeptide. It contains labile sulfur. It gives strong reaction on blood vessels and isolated uterus, but has no effect on frogs' melanofores.

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THE OCCURRENCE OF FILTERABLE FORMS OF BACTERIA IN NATURE

FOR a number of years many bacteriologists have refused to follow the conventional view that the bacteria are limited in their morphology to the typical cells with which we are familiar in the laboratory. Increasing evidence of pleomorphism and life cycles, which may include ultramicroscopic and filterable forms, has accumulated. Throughout the world the number of workers capitulating to the more "radical" school of bacteriologists has increased during recent years. In America, among others, have been such outstanding investigators as Drs. Mellon, Löhnis, Henrici, Rosenow, Hadley and Alice Evans, who have vigorously supported the newer view in one or more of its several aspects.

To Hadley and his coworkers belongs the honor of having proved beyond reasonable doubt the existence of filterable forms of several of the well-known bacteria. A careful reading of the work of Hadley, Delves and Klimek[1] should be sufficient to convince fair-minded skeptics.

While knowledge of "filterable viruses" as the causes of certain diseases is old, our knowledge of such organisms has been limited to a few obligate parasites. That there exist free-living saprophytes of such a nature has been denied. Thus Barthel and Bengtsson,[2] in a work addressed specifically to this problem, found no evidence of filterable microorganisms in soil. From the work of Hadley and his asso-

[1] *J. Infect. Diseases*, 48, 1–153, 1931.
[2] Meddelande No. 341, Centralanstalten försöks. jordbruk., Bakteriologiska avdelningen No. 47 (1928).