THE STRUCTURE OF THE MACRONUCLEUS OF PARAMECIUM AURELIA*

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Two types of bodies can be seen in the macronucleus of living *Paramecium aurelia* by phase-microscope observations.[1] One consists of spherical granules, about 1 μ in diameter, scattered more or less at random. The other type is seen only when the macronucleus is broken by compression. It consists of what appear to be many fine filaments, almost completely filling the nucleus. However, the filaments are so near the limits of resolution that their form cannot be determined with certainty.

Paramecia exposed to lethal doses of ultraviolet radiation show a typical series of changes in the larger granules over the course of the several hours before death.[2] The granules clump together and fuse into one or a few large vacuolated masses. All stages in this fusion process can be followed in living material. The filaments are not visibly altered and remain uniformly distributed even after the granules have clumped into one large body. On the basis of the similarity of this behavior of the granules to the known reaction of nucleoli of other organisms to radiation, Kimball[1] tentatively concluded that they were nucleoli and the filaments were chromosomes.

Confirmation has been obtained for this interpretation by staining reactions. Animals of stock 90 of variety 1 were exposed to ultra-violet from a germicidal lamp and fixed in one per cent osmic acid after observations on living animals showed that the granules had fused into large masses Controls were also fixed at this time. Osmic acid was used as the fixative since observations under the phase microscope showed that, of all the fixatives tried, only osmic acid did not seriously alter the vacuolated fusion masses. The fixed material was dehydrated and embedded in paraffin. Two-micron-thick sections were cut and stained either with the Feulgen-light green procedure following Rafalco[3] and Conn and Darrow[4] or the pyronin-methyl green procedure according to Lee.[5]

Examination of the stained sections of the irradiated animals showed that the fusion masses were stained with light green or pyronin, while the rest of the macronucleus was uniformly stained with Feulgen or methyl green. In control animals, light-green- or pyronin-stained material appeared to be scattered throughout the macronucleus, giving the Feulgen or methyl-green staining a spongy appearance quite different from the uniform texture in the irradiated macronucleus. However, it was not possible to recognize with certainty distinct granules stained with the acid dye. Thus

the granules, which were so clearly visible in the living macronucleus, were no longer clear in the fixed nucleus, and the only evidence for them was scattered distribution of the acid staining. However, this scattered distribution and the results from the observations on living and on irradiated animals combine to make it clear that there are many small nucleoli scattered throughout the macronucleus. Apparently, the rest of its volume is largely occupied by many small chromosomes.

It is of interest that an essentially identical interpretation of the structure of the macronucleus was reached by Breitschneider[6] on the basis of electron-microscope observations of sections of *Paramecium caudatum*. He saw many irregularly spherical bodies, connected by thin strands, and fewer larger bodies approximately 1 μ in diameter. He interpreted the former as chromosomes, and the latter as nucleoli. No proof was offered for these identifications.

The macronucleus has been considered polyploid (see Sonneborn[7] for review) or a multiple structure consisting of a number of subunits each of which is, perhaps, diploid.[8] The latter interpretation was based upon evidence from macronuclear regeneration taken in conjunction with genetic considerations. It is supported by Moses' evidence[9] that there is only about 40× as much DNA in the macronucleus as in the micronucleus of *Paramecium caudatum* and by Nanney's work on mating types as given by Sonneborn.[10] In the present work, no evidence was found for subunits, and all the observations were consistent with the hypothesis of polyploidy with no subunits. It is not clear whether this means that the connections between the chromosomes of a subunit are too fine and diffuse to be observed with the techniques which have been used or whether the evidence which suggests subunits must be reinterpreted.

The published observations on the ciliate macronucleus suggest at least two kinds of structure. In a number of cases, scattered acid-stained granules are reported more or less as in the present paper. However, another group of observations indicate that large Feulgen-positive bodies are scattered throughout the nucleus, e.g., Turner[11] for *Euplotes*. These large Feulgen-positive bodies are not readily homologized with the structures seen in the vegetative macronucleus of Paramecium. Commonly used fixatives, such as Schaudinn's, produce considerable alteration in the structure of the macronucleus. Thus final judgment about Feulgen-positive bodies must be reserved until careful comparisons have been made between living and fixed material. Nevertheless, it seems probable that such bodies exist and must be taken into account in any generalized scheme of the structure and function of the macronucleus. The writer is unable to offer a satisfactory hypothesis to account for them.

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