of poisoning depend on the binding of DDT to a protein in nerve by Van der Waals' force.

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Endogenous Circadian Rhythm in Cytoplasm of Acetabularia: Influence of the Nucleus

Abstract. *It was shown by three different methods that in the unicellular and uninuclear green alga* Acetabularia *the nucleus is capable of determining the phase of the circadian rhythm of the oxygen balance in the cytoplasm.*

In the green alga, *Acetabularia*, there is a circadian rhythm of oxygen balance, and it has been demonstrated that this periodicity continues in the absence of an exogenous "Zeitgeber," or synchronizer (*1*, *2*). Even 40 days after removal of their nuclei, anucleate cells retained their rhythmic diurnal variations. These results seemed to indicate that the circadian rhythm under investigation was independent of the nucleus. On the basis of this evidence alone, however, involvement of the nucleus in the periodicity could not be definitely excluded.

In an attempt to solve this problem, we decided to combine the nucleus with cytoplasm in different phases, and thus to find out which of these two parts governs the rhythm under conditions of constant light.

The technique used for the determination of the oxygen balance was essentially the same as that described previously (*2*). The treatment of the plants and the experimental conditions were varied to achieve three types of experiments.

In experiment 1, the rhizoids were transplanted. At least 14 days before the experiments were started, plants of one culture were divided into two groups. One group was illuminated from 8 a.m. to 8 p.m. and the other from 8 p.m. to 8 a.m. At the beginning of each experiment, the rhizoids in the plants of one group were replaced by the rhizoids of plants of the other group (*3*). In addition, the tips of the acceptor stalks were amputated to remove the metabolically most active part of the plants (*4*). The transplantations resulted in combinations of rhizoids (containing the nuclei) and stalks with opposite phases of their periodicities. After transplantation, the plants were kept under constant conditions to avoid any exogenous zeitgeber. The oxygen balance was determined every 12 hours (at 8 a.m. and 8 p.m.), starting 3 days after transplantation.

In experiment 2 only the isolated nuclei were transferred (*5*). As in experiment 1, one group of plants was illuminated from 8 a.m. to 8 p.m., and the other from 8 p.m. to 8 a.m., for at least 14 days before implantation of the nuclei. The isolated nuclei were practically free of cytoplasm prior to implantation, so that cytoplasmic effects were presumably excluded.

In experiment 3, two different parts of the same plant were exposed to op-

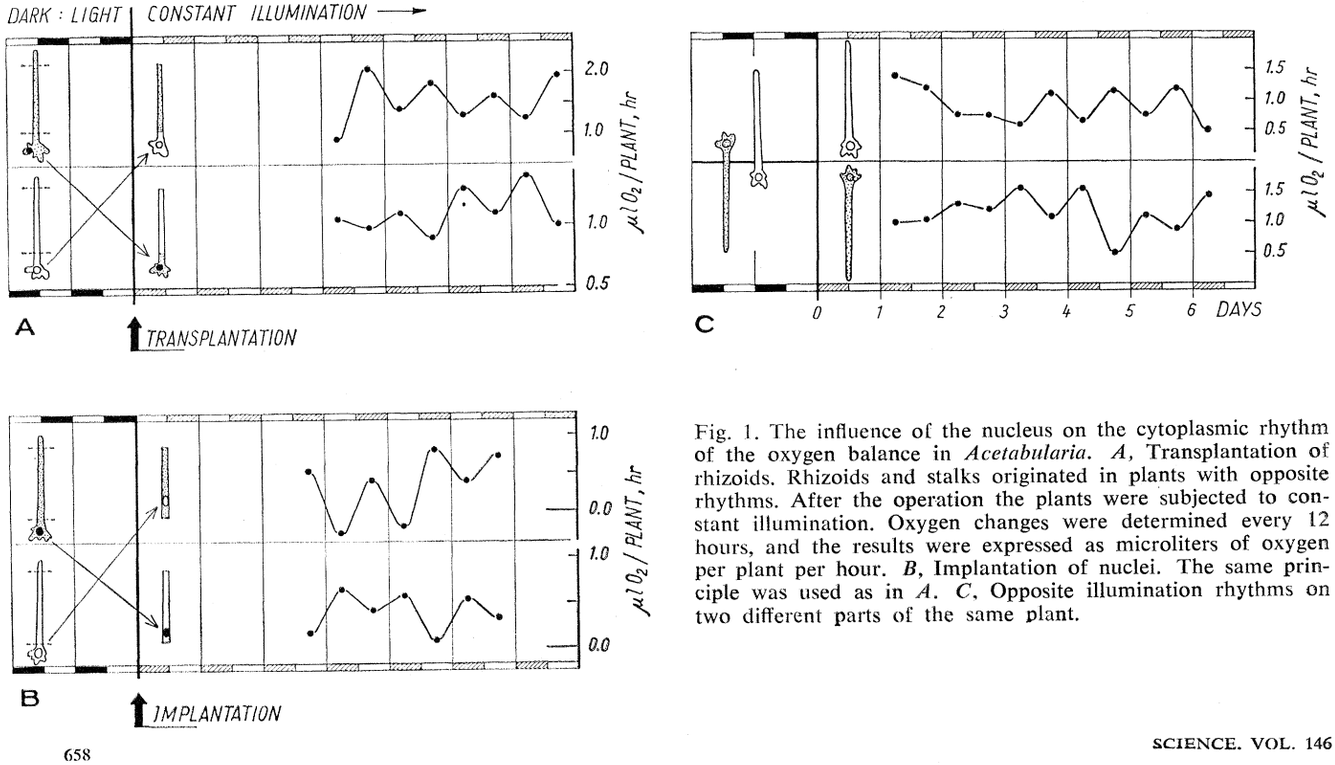

Fig. 1. The influence of the nucleus on the cytoplasmic rhythm of the oxygen balance in *Acetabularia*. *A*, Transplantation of rhizoids. Rhizoids and stalks originated in plants with opposite rhythms. After the operation the plants were subjected to constant illumination. Oxygen changes were determined every 12 hours, and the results were expressed as microliters of oxygen per plant per hour. *B*, Implantation of nuclei. The same principle was used as in *A*. *C*, Opposite illumination rhythms on two different parts of the same plant.

posite illumination rhythms. The plants were threaded through small holes of an opaque plastic membrane so that 15 to 35 percent of the length of the plants, together with the rhizoid, were on one side, and the remaining 65 to 85 percent, including the tips of the plants, were on the other. The two compartments were subjected to opposite day-night cycles. After a period of 14 days of such treatment, the plants were transferred to reaction vessels (*2*), and the oxygen balance was determined under constant light conditions, as in experiments 1 and 2.

In all three experiments, the cytoplasmic rhythm of the oxygen balance could be shifted in accordance with the phase of the exogenous light-dark rhythm to which the nucleus had been exposed. Examples are shown in Fig. 1, *A*, *B*, and *C*. However, some plants retained their original cytoplasmic rhythm. This was true for 9, 7, and 4 plants of total numbers of 20, 19, and 11 studied in experiments 1, 2, and 3, respectively. In most of the plants which retained their original cytoplasmic rhythm, microscopic observations revealed that the nuclei had degenerated or were obviously separated from the host cytoplasm by wide gaps (6, 6, and 4 plants out of the 9, 7, and 4, respectively).

It seems very improbable that these results may be explained by the small amounts of cytoplasm transferred along with the nuclei. Thus, if the nuclei exerted no influence on the rhythm, then the transplanted and implanted *Acetabularia* should behave like anucleate cells. In other words, considering the relative quantities of cytoplasmic material involved in the regulation of the rhythm, the rhythm should be governed by the acceptor cytoplasm. In addition, in plants whose different parts were subjected to different conditions of illumination, the rhythm would be expected to be that of the greater part of the plant. This is not in agreement with our experimental results. It follows that the nucleus is capable of determining the phase of the diurnal rhythm of the oxygen balance, and we believe that this is the first clear demonstration of participation of the nucleus in the mechanism of a circadian rhythm.

It appears that this regulation depends on the exchange of material between the nucleus and the cytoplasm. From this one may conclude that it is possible to change the phase of an endogenous rhythm by means of certain metabolites, at least at the cell level. Thus, such experiments might provide a tool for investigating the biochemical mechanism of the circadian rhythms.

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Antibody Formation in vitro by Separated Spleen Cells: Inhibition by Actinomycin or Chloramphenicol

Abstract. *The formation of 19S antibody to poliovirus by rabbit spleen cells in vitro was interrupted by adding actinomycin D (1 to 10 micrograms per milliliter) for 30 minutes or longer. Shorter treatment (5 to 15 minutes) caused partial suppression. Antibody formation was slowly renewed upon removal of the drug. These results suggest that 19S antibody formation is contingent upon DNA-dependent RNA synthesis and that the genetic messenger has an average lifetime of one-half day or less. Treatment with chloramphenicol (20 to 50 micrograms per milliliter) for 3 days caused the cessation of 19S antibody formation.*

Primary and secondary antibody formation to poliovirus (PV) by separated rabbit spleen cells has been studied in vitro. The continued primary 19*S* antibody formation in vitro could be interrupted by actinomycin D (C_3) or chloramphenicol. The formation of antibody to bacteriophage T_2 in vitro was also inhibited by low concentrations of actinomycin D (see *1*).

This antibiotic combines with guanine residues in the DNA primers and hence, in concentrations of 1 to 10 μg/ml, inhibits nuclear RNA synthesis 90 to 100 percent (*2*). If the ribosome-associated messenger RNA (mRNA), which governs the synthesis of a certain protein, is short lived, the inhibition of mRNA synthesis will deplete the ribosomes of their templates and cause the breakdown of polyribosomes and the interruption of protein synthesis. By determining the shortest period of actinomycin treatment that will cause polyribosome breakdown (*3*) or inhibit the formation of cell proteins (*4*) one has estimated the average turnover rate of the mRNA in a particular cell type. In this study, the average lifetime of ribosome-associated RNA presumed to direct the formation in rabbit spleen cells of the two kinds of polypeptide chains (H and L) of which the 19*S* (γ_1-globulin) antibody is composed (*5*), was roughly estimated by a similar approach.

Rabbits were anesthesized and their spleens were removed aseptically and placed in a previously described medium (*6*). The spleens were cut into fragments (1 × 2 mm) which were washed in buffer and broken up with a 5-ml syringe and 18-, 20-, and 24-gauge needles. The cells were filtered through gauze, packed by low-speed centrifugation, resuspended in medium, and seeded on membrane filters (about 3×10^7 cells on each filter). The filters had been glued to the top of lucite cylinders which were placed in a sterilized disposable plastic tray (*6*). Each membrane culture was covered with a circular (about 17 mm in diameter) piece of sterilized lens paper, and 0.5 ml of medium was added. Spleen fragments were used in a few experiments but the technique of cultivation was the same as for the separated spleen cells. Control cultures consisted of spleen cells from a nonimmunized rabbit. The cultures were incubated in a humidified incubator supplied with air containing 6 percent CO_2, and the culture medium was replaced every 2nd or 3rd day. The medium was titrated for neutralizing activity to PV on HeLa cell monolayer cultures by the plaque assay technique (*7*).

Figure 1 shows results obtained with