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

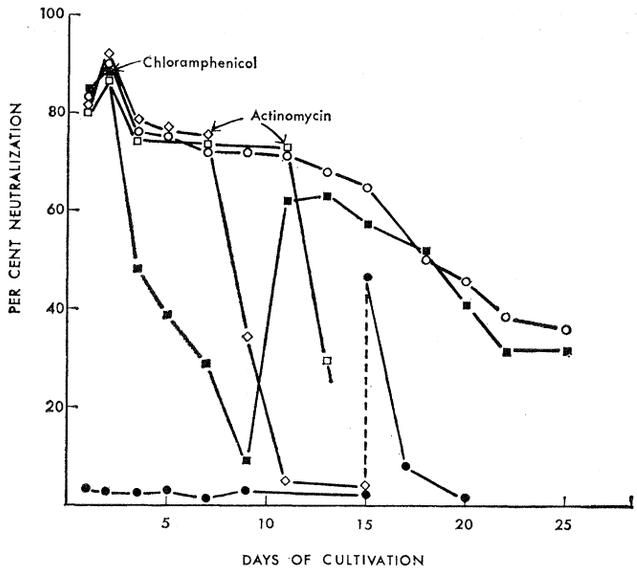
Abstract. *The formation of 19S antibody to poliovirus by rabbit spleen cells in vitro was interrupted by adding actinomycin D (1 to 10 micrograms per milliliter) for 30 minutes or longer. Shorter treatment (5 to 15 minutes) caused partial suppression. Antibody formation was slowly renewed upon removal 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



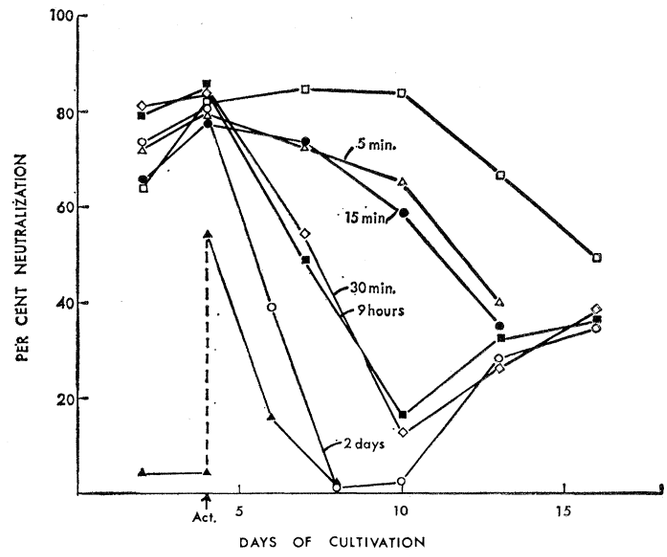


Fig. 1. Poliovirus neutralizing activity of culture medium from membrane cultures (spleen fragments). Each point is the average of three cultures. Nontreated cultures ○; chloramphenicol (50 μg/ml) added to the medium for 3 days starting on day 2 ■; actinomycin D (8 μg/ml) added for 2 days starting on day 7 ◇ or on day 11 □; nonimmune spleen fragments, 19*S* antibody added on day 15 ●. Fig. 2. Poliovirus neutralizing activity of culture medium from membrane cultures (separated spleen cells). Nontreated cultures □; actinomycin D (8 μg/ml) added for 2 days ○, for 9 hours ■, for 30 minutes ◇, for 15 minutes ●, for 5 minutes △; nonimmune spleen cells, 19*S* antibody added on day 4 ▲. For actinomycin-treated cultures each point is the average of three cultures.

spleen fragments from a rabbit which had received $1 \times 10^{9.0}$ plaque forming units (PFU) of the Brunhilde strain (type 1) of PV intravenously 3 days prior to the splenectomy. The serum antibody, tested at the time of the splenectomy, and the antibody produced by the cultures during the first 14 days, was a γ_1-globulin and was destroyed by treatment with 2-mercaptoethanol (0.2*M*) (*8*). The culture medium displayed no neutralizing activity to Coxsackie B-4 virus. Earlier experiments had revealed that the addition of actinomycin D to "membrane cultures" during the first 4 days of cultivation caused cessation of the continued formation of primary 19*S* (γ_1-globulin) antibody. As can be seen in Fig. 1, incorporation into the medium of actinomycin D (8 μg/ml) as late as the 7th or 11th day also produced a striking reduction in antibody formation. The antibody peak on the 2nd day is typical for fragment cultures and is associated with a release of intra- and intercellularly stored antibody.

In recent work by Ambrose and Coons (*9*) and by me (*6*) chloramphenicol in concentrations of 40 to 50 μg/ml markedly suppressed antibody formation in vitro. This effect is clearly seen in Fig. 1, which also shows that the cultures renewed their antibody production 4 to 5 days after the removal of the drug. A comparison with the curve representing the disappearance of 19*S* (γ_1-globulin) antibody (added on day 15 to nonimmune spleen fragments) from successively replaced media, reveals that treatment with actinomycin D caused an abrupt and complete or nearly complete cessation of 19*S* antibody formation.

Figure 2 illustrates the results obtained with separated spleen cells from a rabbit which had received $6 \times 10^{8.0}$ PFU of PV intravenously 3 days prior to the splenectomy. Also this animal produced only or predominantly 19*S* antibody (based on zonal density-gradient centrifugation) at the time of the splenectomy. The addition of actinomycin D to cultures for 1, 2, or 4 days had earlier been found to inhibit the continued formation in vitro of primary 19*S* antibody to PV. In this experiment the cells were also incubated with the drug for much shorter periods in order to determine whether a brief inhibition of RNA synthesis would affect the production of 19*S* (γ_1-globulin) antibody. Actinomycin D (8 μg/ml) was added on the 4th day and allowed to remain in the medium for various periods of time. In order to remove all the drug, the cups containing the cultures were rinsed twice with medium before new drug-free medium was added. Figure 2 shows that the cultures exposed to actinomycin for 30 minutes or more completely or nearly completely ceased to form antibody to PV and that on the 10th day the medium exerted very little or no neutralizing activity. Even a transient incorporation of the drug for 15 or 5 minutes noticeably suppressed the antibody formation. The reduction in activity on the 10th day amounted to 30 and 23 percent, respectively.

The renewed antibody synthesis after the removal of actinomycin D (Fig. 2) supports the conclusion that the antibiotic did not cause general irreversible cell damage. But a reversible cell damage or the possible presence of a small population of cells resistant to actinomycin D cannot be ruled out. Considering the information that has been accumulated from bacterial and mammalian systems on the biological activity of actinomycin D (*2*) it is assumed that antibody formation ceased because the antibiotic inhibited the synthesis of template RNA. It can be seen in Figs. 1 and 2 that during the first 2 days after interruption of RNA synthesis by actinomycin (2 or 4 days of treatment) the antibody activity declined at a rate which approximately corresponded to the rate at which the 19*S* antibody that had been added to nonimmune cells was lost. The activity of cultures, frozen and thawed three times, also declined at a similar rate. These results indicated that the period

of continued antibody formation after the interruption of RNA synthesis was relatively short. If antibody production had continued at an undiminished rate for one or more days, no more than about 50 percent reduction in antibody activity would have been expected within 2 days. The rapid decline in activity suggested that antibody formation was halted within 1 day after the inhibition of RNA synthesis and that the average lifetime of the residual RNA was consequently less than 1 day. Additional support for the idea that the lifetime of this RNA was shorter than 1 day was provided by the finding that a relatively brief inhibition of RNA synthesis (½ hour or 9 hours) caused very nearly complete cessation of antibody production. Further, the fact that interference with the synthesis of template RNA for only 5 or 15 minutes significantly reduced antibody formation suggests an average lifetime of less than ½ day.

If actinomycin D had no direct effect on the functional integrity of polyribosomes, the results would then suggest that in rabbit spleen cells the ribosome-associated RNA fraction that directs the synthesis of polypeptide chains of the H- or L-types, or both, which compose the 19*S* γ_1-globulin, has an average lifetime of ½ day or less. The confirmation of the messenger nature of this RNA must await studies of its sedimentation behavior and a comparison of its base composition with that of spleen cell DNA.

In exponentially growing *Bacillus subtilis* an average lifetime of about 2 minutes was estimated for mRNA (*4*), but in mammalian cells, particularly those which manufacture only a few types of proteins, mRNA has been reported to be fairly long lived. Protein synthesis in mouse fibroblasts was also reported to continue for several hours after inhibition of RNA synthesis to 99.9 percent (*10*), and the mean lifetime of mRNA in rat liver cells was estimated as 4 to 8 hours (*3*). In rabbit reticulocytes both actinomycin-sensitive and actinomycin-resistant protein synthesis was described, indicating the presence of short-lived as well as stable messenger (*11*).

The addition of 20 to 50 μg/ml of chloramphenicol results in complete inhibition of protein synthesis in bacteria (*12*), and even lower concentrations markedly suppress protein synthesis in cell-free systems (*13*). In contrast, protein synthesis in mammalian cells was only partially interfered with by concentrations of chloramphenicol 15 to 20 times higher (*14*). But such amounts of the drug are capable of causing reduced cellular respiration and even cell death (*15*). Only when amino acid incorporation directed by synthetic messenger (polyU) was measured in the reticulocyte ribosome system did chloramphenicol exert an inhibitory effect, which, however, was attributed to an effect on polyU rather than on the ribosomes *per se* (*16*). Thus, a puzzling feature of the chloramphenicol activity has been its apparent specificity for the bacterial ribosomes (*17*). However, in my study and in the recent extensive work by Ambrose and Coons (*9*), chloramphenicol concentrations of 40 to 50 μg/ml markedly suppressed antibody formation in vitro. A possible explanation for the discrepancy between the results in these two studies and in earlier work, with mammalian cell systems, is—as was pointed out by Ambrose and Coons—the longer exposure of the cells to the antibiotic in the studies of antibody formation. This proposed explanation is supported by our finding that treatment for 4 hours or less with chloramphenicol failed to suppress the continued formation in vitro of primary 19*S* (γ_1-globulin) antibody to PV.

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Induction of Several Adaptive Enzymes by Actinomycin D

Abstract. *Although actinomycin D has been used to prevent protein synthesis in experiments of several hours' duration, its effects on the synthesis of adaptive enzymes which are induced over a period of several days have received less attention. Treatment of young rats with doses of actinomycin D, which permitted survival for a period of 5 days, resulted in marked increases in the activities of four hepatic enzymes known to be induced by cortisol: alanine transaminase, tyrosine transaminase, serine dehydrase, and tryptophan pyrrolase. Actinomycin D also induced responses of two of these enzymes in adrenalectomized rats.*

Actinomycin D can block protein synthesis (*1*), inhibit the response of a number of adaptive enzymes (*2*), and prevent or delay antibody formation (*3*). The fact that actinomycin D can inhibit the DNA-mediated synthesis of RNA, presumably by reacting with guanine bases of DNA (*4*), has proved useful in studying the role of newly formed RNA in protein synthesis.

When cortisol is injected intraperitoneally into young rats, induction of tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase occurs rapidly, the activity reaching a maximum within 6 hours (*5*). We have confirmed previous studies demonstrating that actinomycin D (150 μg/100 g) can block the rapid induction of tyrosine-α-ketoglutarate transaminase and tryptophan pyrrolase by cortisol (*2*). The induction of alanine-α-ketoglutarate transaminase and serine dehydrase after the administration of cortisol occurs slowly,