dendritic membrane. Among these ions, Ca^{++} was considered as the most likely one to be affected by ATP, since there are numerous data establishing its importance in determining membrane permeability (*8*). Therefore, we studied the action of various compounds which could affect membrane-bound Ca^{++}, such as chelating agents [ethylenediaminetetraacetic acid (EDTA), dipicolinic acid (DPA)], or sodium oxalate, which forms an insoluble salt with Ca^{++}. A slight stimulatory effect was obtained with EDTA and DPA (Table 1) but sodium oxalate was not effective.

Our results suggest that the depolarization of the dendritic membrane depends on the removal of Ca^{++} ions from their sites of binding on the membrane. Adenosine triphosphate could act at this stage because of its chelating properties. In about one quarter of the mosquito population, Ca^{++} removal is sufficient to depolarize the nerve membrane, as shown by the results with the nonelectrolyte solutions. In the bulk of the population, however, the influx of Na^{+}, made possible by the removal of Ca^{++}, is necessary to lower the potential and to depolarize the membrane. It could be assumed that the nucleotide is bound through the adenine moiety to a specific receptor site on the surface of the membrane. The geometry of the ATP molecule may be such that the terminal phosphate groups of the bound nucleotide are brought in juxtaposition to a second site on the surface where the Ca^{++} ion is located. This may explain why ATP is superior to other polyphosphates as a stimulant and, despite its lower formation constant with Ca^{++} ions, is also superior to stronger chelating agents such as EDTA (*9*).

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Ribosomes and Ribonucleic Acids in Three Morphological States of Neurospora

Abstract. *Dormant ascospores contain the same ribosomal population as growing hyphae and resting conidia. This conclusion is based on analyses of sedimentation coefficients and of nucleotide composition of ribosomes, ribosomal RNA, and soluble RNA. Thus dormancy is not characterized by the absence of the important parts of the protein-synthesizing apparatus.*

Changes in the physiological state are accompanied by variations in the ribosomal population, affecting class distribution or physicochemical properties, of bacterial (*1*), yeast (*2*), and plant and animal cells (*3*); variations also occur in the nucleotide composition of ribonucleic acids (RNA) of protozoan cells (*4*). We have, therefore, compared the properties of the ribosomes and RNA present in three morphological states of the fungus *Neurospora crassa*.

Ribosomes present in extracts of both resting conidia and growing hyphae from *N. crassa* have similar physicochemical properties (*5*). These include sedimentation coefficients of ribosomes and ribosomal RNA (rRNA) and nucleotide composition of rRNA. Also, the nucleotide composition of total RNA and soluble RNA (sRNA) from conidia and hyphae is the same. Perhaps these results could have been predicted since conidia might be considered merely as segments of hyphae (*6*). They can be regarded as the product of a simple morphological change, are of asexual origin, and can germinate readily in nutrient medium without an activating treatment.

In contrast, ascospores of *N. crassa* have a sexual origin and are the result of a relatively complex mycelial differentiation. They require heat or chemical activation, and then only water and oxygen, in order to germinate. Nonactivated *Neurospora* ascospores can be stored for years in the presence of water without loss of viability (*7*). In addition, their respiratory activity is at a minimum, and it has been suggested that they do not contain all the enzymes present in hyphae (*7*). Ascospores are thus dormant and, in this respect, analogous to bacterial endospores. Endospores of *Bacillus subtilis* contain only two of the four classes of ribosomes present in the vegetative cells (*8*); the same study also suggested that rRNA is not the major portion of the total spore RNA.

Ascospores were obtained from the mating of wild-type strains (*9*) on Westergaard and Mitchell's synthetic medium (*10*). They were harvested and freed of contaminating conidia and hyphal fragments by flotation and sedimentation (*11*). The population and dry weight of ascospore suspensions were measured turbidimetrically after standardization by microscopical counting and dry-weight determination. The average dry weight of an ascospore was found to be 1.8×10^{-6} mg, of which 5 percent was RNA and 11 percent protein. Breakage of ascospores was effected by a modified French cell (*12*) at 23,000 pounds per square inch. Fractionation of extracts, RNA purification and determination of nucleotide composition were performed by methods described elsewhere (*5*). However, polyvinylsulfate was used routinely during RNA preparation to inhibit enzymatic degradation (*13*). The RNA

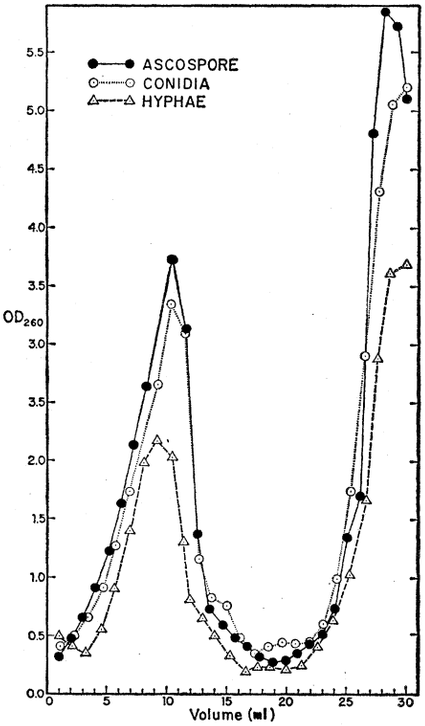

Fig. 1. Density-gradient centrifugation of extracts from ascospores, conidia and hyphae. Each extract was layered directly on a linear gradient of sucrose (20 to 3 percent) in 0.01*M* tris-HCl buffer (*p*H 7.4) containing 10 μmole/ml of $MgCl_2$. The extracts were centrifuged for 255 minutes at 25,000 rev/min in an SW25 head of a Spinco model L preparative ultracentrifuge. Fractions were collected and their optical density at 260 mμ was measured.

and protein were determined by the methods of Mejbaum (*14*) and Lowry *et al.* (*15*), respectively.

After centrifuging an extract of ascospores through a linear sucrose density gradient, two peaks are obtained: the one near the origin represents ribosomes, and the other, soluble materials (Fig. 1). The peaks of ribosomal origin from the three morphological forms are in perfect coincidence; therefore, ribosomes from conidia and ascospores, by analogy with those from hyphae (*16*), have a sedimentation coefficient at 20°C of 80 Svedberg units (80*S* or $S_{20} = 80$). Also, each of the three peaks of ribosomal origin are equal to about 50 percent of the total optical density at 260 mμ (OD_{260}).

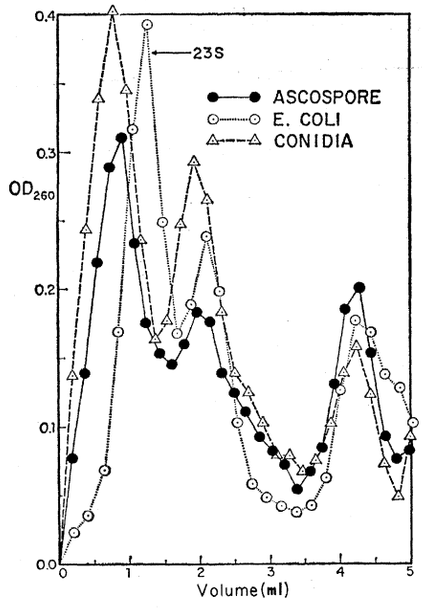

Fig. 2. Density-gradient centrifugation of phenol-purified (*19*) total RNA from ascospores, conidia, and *Escherichia coli*. The RNA was precipitated twice with absolute ethanol, dissolved in an acetate-KCl-$MgCl_2$ buffer (*20*) containing polyvinylsulfate (20 μg/ml) and dialyzed 18 hours against the same. The solutions were layered directly on a linear gradient of sucrose (15 to 2.5 percent) in a 0.01*M* tris-HCl (*p*H 7.4) containing 50μmole/ml of KCl. They were centrifuged for 5.5 hours at 36,000 rev/min in an SW39 head of a Spinco model L preparative ultracentrifuge. Fractions were collected and their optical density at 260 mμ was measured.

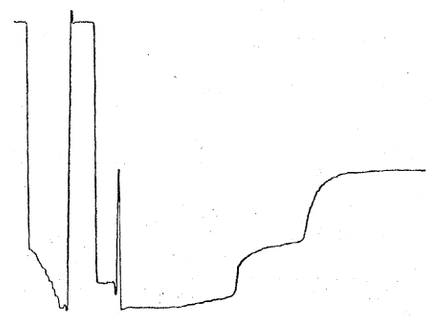

Fig. 3. Purified conidial rRNA was centrifuged at 52,640 rev/min at 20°C in the Spinco model E analytical ultracentrifuge equipped with ultraviolet optics. This figure shows a typical microdensitometric tracing with two boundaries corresponding to 28*S* and 19*S* RNA, respectively. Sedimentation boundaries are shown approximately 20 minutes after acceleration of the rotor was completed.

After centrifugation of purified ascosporal RNA through a linear sucrose density gradient, three peaks are obtained: two of ribosomal, and one of soluble origin. These three peaks have exactly the same position as those of conidial RNA. Expressed as percentages of the total OD_{260} absorbing materials, the rRNA peaks represent 74 percent and 81 percent, respectively, for ascospores and conidia. For comparison, the two characteristic 23*S* and 16*S* components of *Escherichia coli* RNA (*17*) are also shown in Fig. 2. Analyses of rRNA in the analytical ultracentrifuge confirmed the presence of two boundaries, as shown in the microdensitometer tracing presented in Fig. 3. These boundaries correspond to 18.7*S* (19*S*) and 28.5*S* (28*S*), respectively. We also found that soluble RNA has a sedimentation coefficient of 4.5*S*. Earlier determinations on rRNA from hyphae (*16*) also yielded a 12*S* component which we conclude was a degradation product, since polyvinylsulfate was not then used as an enzyme inhibitor (*18*). The results in Table 1, together with those in Fig. 2, show that rRNA constitutes the bulk of the total RNA. Table 1 also reveals that sRNA has a composition which is different from that of both total RNA and rRNA. The nucleotide composition of ascosporal RNA is thus similar to that of both conidial and hyphal RNA (*5*).

We also found that ribosomes from ascospores, conidia, and hyphae have, respectively, the following composition: 67, 55, and 58 percent RNA and 33, 45, and 42 percent protein. These ribosomes treated with trifluorotrichloroethane (a protein extractor) have compositions identical to the untreated ascosporal ribosomes. The significantly higher ratio of RNA to protein in ascospore ribosomes probably is a reflection of a very low rate of protein synthesis.

Table 1. The nucleotide composition of ribosomal, soluble, and total RNA from *N. crassa* ascospores. Results expressed as moles per 100 moles of identified nucleotides. (A, adenylic acid; G, guanylic acid; C, cytidylic acid; U, uridylic acid; Pur., purines; Pyr., pyrimidines.)

Nucleotide	Ribosomal	Soluble	Total
A	25.8±0.7*	18.1±0.7	24.9±0.4
G	27.9±0.5	26.7±0.1	28.4±0.8
C	22.5±0.9	35.0±0.9	23.4±0.6
U	24.0±0.5	20.3±0.4†	23.4±0.7
G+C	50.4±0.7	61.7±0.5	51.8±0.7
Ratios			
Pur./Pyr.	1.15	0.81	1.14
6-Am/6-K‡	0.93	1.13	0.93
A+U/G+C	0.99	0.62	0.93

* Errors are expressed as average deviation from the mean of four determinations. † Includes pseudouridylic acid. ‡ 6-Am nucleotides: adenylic and cytidylic acids; 6-K nucleotides: guanylic and uridylic acids.

We must, therefore, conclude that dormant forms retain the major parts of the equipment necessary for protein synthesis (21, 22).

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Antiserums Prepared with Acrylamide Gel Used as Adjuvant

Abstract. *Antiserums to macroglobulins were prepared by a convenient and time-saving procedure in which acrylamide gel was used as adjuvant. After electrophoresis in acrylamide gel, the macroglobulin zone from the gel pattern was homogenized and injected directly into rabbits without intermediate isolation of the purified antigen. The resulting antiserums exhibited high titers of antiserum to macroglobulin and showed only weak cross-reactions with other gamma globulins in serologic tests.*

The preliminary isolation and purification of protein antigens, which is usually required before they can be used for the preparation of specific antiserums, is laborious, time-consuming, and often wasteful of starting material (*1*). The purified antigen is usually mixed with an adjuvant to stimulate an increased antibody response. We describe here the application of acrylamide gel as the isolation medium and as the adjuvant in a technique which is simple, time-saving, and economical of material.

Acrylamide gel is a hydrophilic polymer of acrylamide, cross-linked with methylene-*bis*-acrylamide. The monomers are reported to be neurotoxic, but the gel itself has not shown evidence of toxicity. The use of acrylamide gel as an electrophoresis medium of high resolution was described previously (*2*).

A standard acrylamidegel electrophoresis pattern, prepared from 0.2 ml of serum from a patient with Waldenstrom's macroglobulinemia, demonstrated a macroglobulin zone adjacent to the sample slot. This zone was excised from the unstained pattern, homogenized in an iced Potter-Elvejem tissue grinder, and injected into the posterior thigh muscles of a female albino rabbit. The 7 ml of homogenate injected contained a calculated dose of 0.03 g of macroglobulin and 0.35 g of acrylamide. Seven days after injection, the antibody response was 1750 μg of antibody nitrogen per milliliter against macroglobulin eluted from another sample of the gel. Similar results were obtained in three other rabbits. Controls injected with gel alone produced no detectable macroglobulin antibodies.

Repetition of the injections of the gel-antigen combination for a 3-week period not only increased the antibody titers, but also stimulated the formation of antibodies to γ-globulin, presumably from the small amount of this component which was present in the macroglobulin zone. Six injections were given, totaling 0.09 g of macroglobulin and 1 g of acrylamide gel. Antibody titers of 3140 μg and 960 μg of antibody nitrogen per milliliter were obtained in two rabbits 10 days after the last injection. Controls receiving 1 g of acrylamide gel alone, according to the same time schedule, produced no demonstrable antibody.

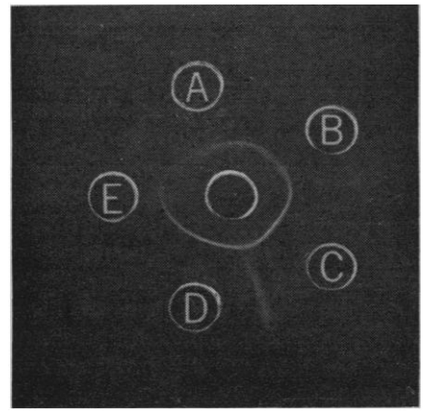
The antibodies reacted strongly in the Ouchterlony plate when the macroglobulin was used as antigen (Figs. 1 and 2). A commercial antiserum to macroglobulin (*3*) was placed in one antiserum well, and serum from each rabbit separately in other antiserum wells. The center well contained either macroglobulin prepared by diethylaminoethyl column chromatography (*4*) or material eluted from a gel electrophoresis pattern. The band formed by the commercial antiserum to macroglobulin (horse antiserum to human macroglobulin) was identical to the band formed by the rabbit antiserums. This identity was seen with both macroglobulin preparations tested, but the bands were heavier and sharper with the macroglobulin eluted from the gel. No components of the antiserums to β-globulin, α-globulin, or albumin were found on the Ouchterlony plates in which whole serum was used in the antigen well.

Table 1. Endpoints of titers obtained when rabbit antiserums were tested against coated human red blood cells.

Coating on red blood cells	Antiserum rabbit No.		Control antiserums*	
	24	E3	19S	7S
Anti-Rh (7S)	32	2		>512
Anti-Fy^a (7S)	4	0		>128
Anti-Le^a (19S)	16	16	16	Trace
Incomplete anti-HC′	4	0	2	0
Uncoated cells	0	0	0	0

* These antiserums are described by Abelson and Rawson (*7*).

Information about the nature of the antibody to γ-globulin produced by prolonged immunization was obtained from serologic data. Serums from rabbits 24 and E3 were tested with human red blood cells coated with blood-group antibodies of the 7S class (anti-Rh and anti-Fy^a) and of the 19S class (anti-Le^a) (*5*). They were also tested with human red blood cells sensitized to incomplete anti-H in the presence of complement, C′. The results (Table 1) indicated that both serums contained, in addition to antibodies to macroglobulin, small amounts of antibodies react-



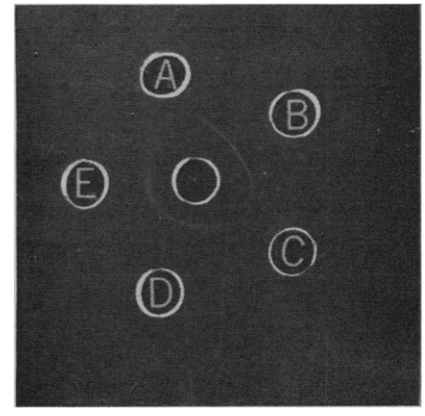


Fig. 1 (left). Photograph of Ouchterlony plate. *A*, Rabbit 21 (after 3-week course of immunization); *B*, rabbit 24 (after 3-week course of immunization); *C*, horse antiserum to human macroglobulin; *D*, rabbit 21 (after 3-week course of immunization); *E*, rabbit B20 (control—received gel alone); *center*, macroglobulin prepared by diethylaminoethyl column chromatography. Fig. 2 (right). Photograph of Ouchterlony plate. *A*, Rabbit B20 (control—received gel alone); *B*, horse antiserum to human macroglobulin; *C*, rabbit E3 (one immunization injection given 1 week previously); *D*, horse antiserum to human macroglobulin; *E*, rabbit 24 (after 3-week course of immunization); *center*, macroglobulin eluted from a gel pattern.