room temperature; under such conditions, the extract has retained activity over 1-year storage periods. To test sporangium production, disks of mycelium and agar from the margin of a culture on V-8 juice agar are placed in the soil extract in petri dishes.

Formation of sporangia was reported in 1959 (*3*), and I presented further data on stimulation in 1962 (*4*).

Data reported in 1959 and from subsequent experiments with nonsterile soil extract indicated the involvement of a microbial agent in asexual sporulation. Sterilization of the extract by autoclaving [15 lb (7 kg) for 20 minutes] or filtration (Seitz or Berkefeld filters) removed the stimulatory factor. This factor was thermolabile; it was eliminated from the soil extract by incubation at 45°C for 5 days or at 50°C for 10 minutes, but not by freezing. Results with dilution and dialysis also supported the microbial theory.

The nonsterile soil extract has also stimulated formation of sporangia in the opposite (A_1) mating type of *P. cinnamomi* (*5*) and increased production of sporangia in *P. citrophthora* and *P. palmivora*.

Subsequently many soil bacteria were isolated and tested for influence on sporangium development by *P. cinnamomi* in sterile extract. Stimulation was obtained in Australia in the spring of 1964 in a number of tests with a purple-pigmented bacterium identified as *Chromobacterium violaceum* (Schroeter) Bergonzini (*6*). This bacterium was first isolated from South Australian soils by the soil-dilution plate method in 1964–65. This organism was also isolated from soil in South Australia by the rice-grain method (*7*); it produced a deeply pigmented culture, the pigment was not soluble in water, the bacterium did not grow at 37°C, and no endospores were produced.

When *C. violaceum* was added to soil extract sterilized by autoclaving, sporangium production in *Phytophthora cinnamomi* ranged from 5 to 40 percent of that in the nonsterile soil extract. Usually the bacteria were suspended in sterile distilled water; then 0.05 to 0.10 ml of this suspension was pipetted into 8 ml of sterile soil extract in a small petri dish. Stimulation of sporulation was more consistent and of higher magnitude when a culture of *Chromobacterium violaceum* which had been recently isolated from soil, or recently transferred, was used. No stimulation occurred in several tests with bacterial cultures several weeks old, including an isolate of *C. violaceum* from California soils (*8*). The *C. violaceum* freshly isolated on rice grains from California soils was effective in inducing formation of sporangia. Corpe (*9*) reported considerable variation in this group. Soils from different areas varied considerably in stimulation of sporangium production; this could reflect differences in bacterial population.

Several other bacteria and combinations of bacteria have given occasional stimulation of sporangium production in *Phytophthora cinnamomi*, in tests in California and in Australia. Washings from dilution plates containing a number of different bacteria, when added to sterile soil extract containing disks of mycelium of *P. cinnamomi*, have consistently caused sporangia to be produced. Thus, *Chromobacterium violaceum* is not the only microorganism involved in the phenomenon. This is indicated also by reports on the stimulation of sporulation in *Phytophthora cinnamomi* by bacteria or diffusates from nonsterile soil extract (*10*, *11*); in one case "aseptic" sporangia were reported on the side of Millipore membranes opposite a soil extract (*11*).

Chromobacterium violaceum, although not readily isolated from soil, is a common inhabitant of many soils throughout the world, and it also is commonly found in water. Thus this organism could well be one of the important biotic agents providing the stimulus for production of this essential spore stage by *Phytophthora cinnamomi* and other species of the genus.

That several tests of spring water and other water sources for stimulation of sporangium production have given positive results further substantiates the probable importance of an organism such as *Chromobacterium violaceum*. Another link in the involvement of this bacterium in sporulation is provided by the data on inactivation of the factor or agent at temperatures of 45° to 50°C. This correlates with the fact that *C. violaceum* does not grow at 37°C and does not produce spores.

There are indications that the substance or substances influencing production of sporangia are intimately associated with the bacterial cell, for removal of bacteria from the soil extract by filtration through a Millipore filter (0.45-micron pore size) also prevented development of sporangia. Filtration of the extract through Millipore filters with pore sizes of 3.0 or 1.2 microns did not remove bacteria; these filtrates stimulated production of sporangia. Undoubtedly *C. violaceum* and other microorganisms in the soil are producing some metabolite or metabolites that are essential for triggering sporangium production in *Phytophthora*.

GEORGE A. ZENTMYER
Department of Plant Pathology, University of California, Riverside

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Quenching of DNA Phosphorescence

Abstract. *Paramagnetic cations quench the phosphorescence of DNA at concentrations well below one ion per DNA phosphate. The order of quenching efficiency is copper, nickel, cobalt, and manganese.*

Low concentrations of Mn^{++} quench the phosphorescence of DNA (*1*). We now report that Mn^{++}, while itself a good quencher, is inferior to other paramagnetic cations tested. The quenching efficiency from highest to lowest is in the order Cu^{++}, Ni^{++}, Co^{++}, Mn^{++}.

Studies of quenching may contribute to understanding of the interaction of DNA and small molecules and to understanding of the mechanism of radiation damage.

Salmon sperm DNA (A-grade, Calbiochem) was purified as described elsewhere (*2*). All emission spectra were run in glasses of equal volumes of glycerol and water at 77°K. The DNA concentration was $2 \times 10^{-3}M$ phosphate and contained $1.8 \times 10^{-2}M$ NaCl to prevent denaturation at room temperature. The paramagnetic ion salts were added as chlorides.

Phosphorescence was measured on an Aminco-Keirs spectrophosphorimeter with a photomultiplier photometer (Hruska Radio Co., Lutherville, Md.)

Under the conditions of our experiments, paramagnetic ions quench the phosphorescence of DNA; the quenching occurs at low ratios of cation to DNA phosphate (Fig. 1).

If S is the phosphorescence amplitude, S_0 the amplitude without added cations, and r the ratio of added cation to DNA phosphate, then, for the four ions, the initial slope $(-1/S_0)(dS/dr)$ is 20, 17, 14, and 10 for Cu^{++}, Ni^{++}, Co^{++}, and Mn^{++}. The best quencher, both with respect to the initial rate of quenching and to the final amount of quenching is Cu^{++}.

As the phosphorescence is quenched, the shape of the emission spectrum changes. The maximum emission is at 458 mμ with no added cations but shifts to 440 mμ when the phosphorescence is strongly quenched.

The curves exhibit a nonstoichiometric relation in the sense that strong quenching occurs at low values of r. This relation must be examined in light of modern theories of phosphorescence quenching. These theories (*3*) postulate either a charge transfer or spin-coupling mechanism and hence imply that there is an overlap between the wave functions of the quencher and the molecule whose emission is quenched. Since overlap of wave function decreases sharply with separation of the molecules, the quenching of emission must also diminish rapidly with such separation. It is in this connection that the nonstoichiometry exhibited in Fig. 1 must be considered. Two possibilities present themselves.

1) The phosphorescence of DNA may actually be due to certain special regions of the DNA, perhaps regions involving faults in the helix or high concentrations of a particular base pair. If paramagnetic cations were selectively bound to such regions, then the nonstoichiometry of quenching could be understood. This possibility involves two assumptions, neither of which is supported by any evidence. It must therefore be regarded as highly speculative.

2) The excitation may diffuse along the helix, being quenched when it comes to the vicinity of a paramagnetic cation. An analysis of such a diffusion process (*1*) yielded an approximate relation between the "jump time" of an excitation and the number of quenching centers. The theory included the assumption that the quenchers were randomly distributed and, when the excitation reached a quenching site, it was quenched with high efficiency. For Mn^{++}, the conclusion was reached that, if every ion added acted as a quencher, the jump time for the excitation would be about 10^{-3} second. For triplet excitation migration this time appeared much too long, times of the order of 10^{-9} second being much more reasonable (*4*). However, recent results (*5*) have indicated that a major fraction of the DNA phosphorescence originates from a negative thymine ion. Such an ion could arise by transfer, in the excited state, of the N_1 proton of thymine to the N_1 position in adenine. Nothing is known concerning the probability of migration of an excitation of this type from one thymine moiety to another. However, since a diffusion would involve proton shifts as well as electronic excitation transfer, if it could occur, it should certainly be slower than transfer between molecules in the same ionization state.

Thus a very slow transfer rate is consistent with the idea that most of the added cations act as quenchers.

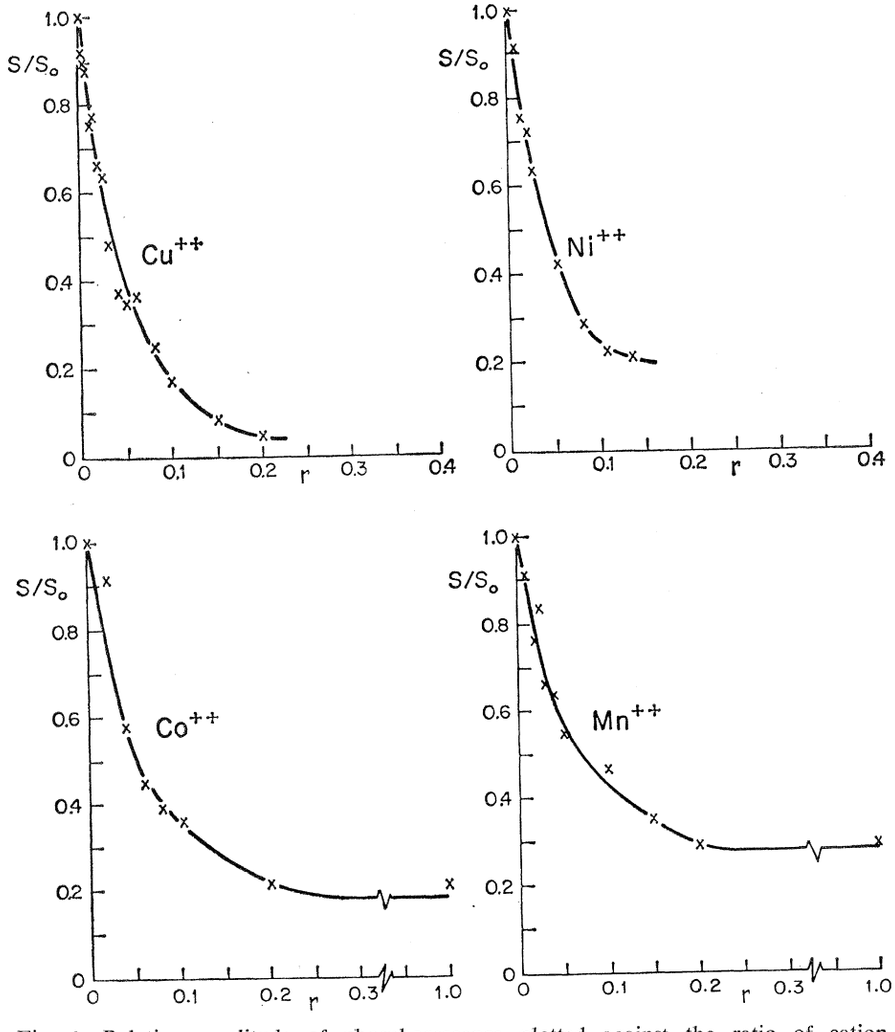

Fig. 1. Relative amplitude of phosphorescence plotted against the ratio of cation added to DNA phosphate. All points are averages of four to six measurements.

Since, from studies of nuclear magnetic resonance, it now appears firmly established (*6*), that most, if not all, of the added Mn^{++} and Co^{++} becomes attached to the nucleic acid phosphates, the possibility of a quenching mechanism that operates by way of several saturated bonds must be seriously considered.

The quenching data presented here should aid in establishing mechanisms of radiation damage in DNA since such states may occur by way of the excited triplet.

I. ISENBERG*
S. L. BAIRD, JR.*
R. ROSENBLUTH*

Institute for Muscle Research, Marine Biological Laboratory, Woods Hole, Massachusetts

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* Present address: Science Research Institute, Oregon State University, Corvallis.

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Allograft Survival: Effect of Antiserums to Thymus Glands and Lymphocytes

Abstract. *Although antiserums to lymphocytes and thymus cells have similar effects on lymphocytes and thymocytes in vitro, the antiserum to thymus has more persistent lymphopenic effect in vivo and prolongs allograft survival time more markedly. Since only thymus glands of animals treated with antiserum to thymus showed depletion of small lymphocytes, antibody to thymic humoral factor may be operational.*

Antiserums to lymphocytes and thymocytes produce lymphopenia and suppress delayed hypersensitivity reaction (*1, 2*). Antiserum to lymphocytes, however, has lymphopenic effect of relatively short duration. On the other hand, antiserum to thymocytes, given daily from the day of birth, has long-lasting lymphopenic effect in mice (*2*). We now report a significant difference between antiserum to rat lymphocytes and antiserum to rat thymus in the effect of each on prolongation of allograft survival in young adult rats.

Osborne-Mendel strain (not inbred) rats kept in a closed colony for 35 to 36 generations were used. Antiserums were produced in albino rabbits (3 to 4 kg) by immunization with a mixture of 20-percent saline suspension of homogenized thymus or mesenteric lymph nodes (from rats, aged 6 to 8 weeks) and Freund's complete adjuvant, in equal volume. The mixture (5 ml) was injected into the rabbits intramuscularly and subcutaneously in multiple locations once a week for 5 weeks, and the animals were bled 1 week after the last injection for the subsequent 4 weeks. Some animals were given two booster injections at weekly intervals and bled weekly for another 4 weeks. All the serums were heated at 56°C for 30 minutes and pooled.

The effect of the antiserums was studied by agglutination tests with rat red blood cells, lymph node cells, and thymocytes. Agglutinin titers expressed by reciprocals of the maximum dilutions of serum giving positive agglutination are shown in Table 1. Thymocyte agglutinin titers were equal in both antiserums and lymphocyte agglutinin titers differed only by one dilution.

Two groups of seven animals each were injected daily intraperitoneally with 1 ml of either antiserum for 4 weeks or more, and the lymphocyte count was made daily on each animal. The mean lymphocyte count, prior to injection, for the group receiving antiserum to lymphocytes was 4774 ± 458, and it was 5225 ± 480 for the group receiving antiserum to thymus. The injections of antiserum to lymphocytes produced lymphopenia lasting up to 3 to 4 weeks, but the white counts gradually returned toward normal thereafter. The mean value of the lowest lymphocyte counts observed in the seven animals after treatment with antiserum to lymphocyte for at least 3 weeks was 2645 ± 520. In contrast, injection with antiserum to thymus resulted in more persistent lymphopenia of intense degree, and the mean of the lowest lymphocyte counts in this group after the animals had been injected with this antiserum for at least 3 weeks was 1388 ± 510. There was no significant change in the number of polymorphonuclear leukocytes, and the hematocrit remained essentially unchanged in most of the animals receiving either antiserum.

Table 1. Agglutinin titers of antiserums to rat thymus (ARTS) and lymphocytes (ARLS) for red blood cells (RBC), lymphocytes, and thymocytes.

Rabbit serum	Agglutinin titers (maximum dilution)		
	RBC	Lympho-cyte	Thymo-cyte
ARTS	1:40	1:320	1:160
ARLS	1:80	1:160	1:160
Normal	< 1:5	< 1:5	< 1:5

A full-thickness graft of circular skin (18 mm in diameter) was exchanged between 6- to 8-weeks-old male rats (120 g). The graft was sutured with silk to a bed prepared on the lateral thoracic wall. No dressing was applied, and the graft was observed daily. Complete disappearance of macroscopically intact grafted skin, also confirmed microscopically, was scored as the time of rejection. Mean survival time in nontreated animals was 10.9 days (Table 2). In animals receiving daily intraperitoneal injection of 1 ml of antiserum to lymphocytes beginning 1 day before grafting, the mean survival time was prolonged to 18.8 days. In the group of animals treated with daily injection of 1 ml or less of antiserum to thymus intraperitoneally, however, survival was more than three times longer than that of the nontreated group. Daily injection of 1 ml of antiserum to thymus not infrequently resulted in death of animals with intact graft. Reduction of the dose to as low as 0.5 ml every 5 days has been tolerated well without ill effect on the graft.

Table 2. Survival time of allografts, in groups treated with antiserum either to lymphocyte or to thymus.

Dose (ml/day)	Animals (No.)	Graft survival (days)	Mean
		No treatment	
0	15	10, 10, 10, 10, 10, 10, 11, 11, 11, 11, 11, 12, 12, 12, 12	10.9
		Antiserum to lymphocytes	
1	12	11, 13, 14, 14, 14*, 15, 15*, 17, 18, 25, 34, 36	18.8
		Antiserum to thymus	
1 or < 1	12	19*, 20*, 20*, 27*, 27*, 35†, 36*, 37†, 56*, 56†, 71†, 71†	39.8

* Animals died with intact graft. † Animals still living with intact graft.