was 8 to 10 times higher. Immature seeds which were leached in water for 3 hours lost over half of the *C* initially present. Compound *C* was the only fraction obtained from the perchloric acid extracts which was an effective growth inhibitor.

For inhibition studies, compound *C* was isolated from immature soybean seeds or frozen green peas (a readily available source of material) by ion-exchange chromatography, as illustrated in Fig. 1, then lyophilized to remove the formic acid. The dried residue was dissolved in water and adjusted to *p*H 5.0 with KOH. Concentrations are expressed in terms of optical density at 260 mμ. One "O.D. unit" is that amount of *C* which will give unit optical density in 1 ml of solution with 1-cm cuvettes in the Beckman DU spectrophotometer.

Table 1 gives data for the effect of compound *C* on seed germination, on root respiration and phosphate accumulation, and on oxidative phosphorylation by mitochondria isolated from soybean hypocotyls. Mature soybean seeds were soaked in the indicated concentrations of *C* for 4 hours and then planted in moist vermiculite. Germination of treated seeds was delayed 24 hours as compared with germination of controls soaked in water. After 48 hours, 60 to 90 percent of the treated seeds had germinated. The mean length of the embryonic axis of the seeds that germinated is given in Table 1. Similar responses to compound *C* were obtained with mature corn seeds.

Table 1. Effects of compound *C* on germination of soybean seeds and on the respiration and phosphate uptake of soybean root tips and isolated soybean hypocotyl mitochondria.

Germination of soybean seed			
Units of *C* per 4 ml of water	0	1	10
Length of plant (in cm) (after 48 hours)	4.5	2.5	2.0
*Activity of 1-cm soybean root tips**			
Units of *C* per 2.5 ml of buffer	0	5	25
Respiration, initial 15 minutes	780	830	1670
Respiration, after 3 hours	753	809	943
PO_4 uptake, after 3 hours	0.31	0.26	0.21
Activity of soybean mitochondria†			
Units of *C* per 2.5 ml of buffer	0	1	15
$Qo_2(N)$	917	885	702
P/O	2.69	2.27	2.08
P/N	221	179	130

* Root tips were placed in Warburg vessels in $10^{-3}M$ potassium phosphate (*p*H 5.0) labeled with P^{32}. Respiration rate is given as microliters of O_2 per hour per gram (fresh weight); phosphate uptake is given as micromoles of PO_4 absorbed per gram (fresh water).

† Mitochondria were isolated and activity was determined over a 30-minute period with α-ketoglutarate as the substrate (*8*). $Qo_2(N)$ is given in microliters of O_2 per hour per milligram of N; P/O is given in micromoles of P esterified per microatom of O_2; P/N is given in micromoles of P esterified per hour per milligram of N.

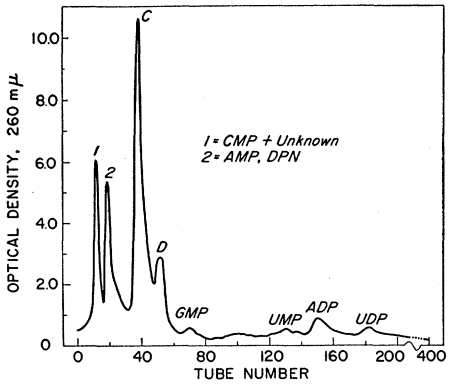

Fig. 1. Elution spectrum of soluble nucleotides from immature soybean seeds. Seeds were homogenized in 0.6*M* $HClO_4$ and centrifuged, and the perchlorate was removed from the extract as the K salt. The extract was placed on Dowex-1 (formate) and eluted with a gradient of formic acid-ammonium formate (*1*). ADP, AMP, adenosine di- and monophosphate; CMP, cytidine monophosphate; DPN, diphosphopyridine nucleotide; GMP, guanosine monophosphate; UDP, UMP, uridine di- and monophosphate.

The application of 25 O.D. units of compound *C* to root tips caused the respiration rate to increase markedly in the initial 15 minutes; this increase was followed by a gradual decline to rates comparable to control levels after 1 hour. Table 1 shows respiration rates and the amount of orthophosphate accumulated by the tissue in 3 hours. The net result in increased respiration and in decreased phosphate accumulation was similar to that produced by the uncoupling agent, 2,4-dinitrophenol.

Further evidence that compound *C* acts as an uncoupler was given in the experiments with mitochondria. Phosphate esterification per unit N was inhibited. Oxidation of α-ketoglutarate was generally depressed, as indicated in Table 1, although in occasional experiments a transitory increase in respiration was noted.

It will be noted in Fig. 1 that no triphosphate nucleotides were obtained from immature seeds. Extracts of hydrated mature seeds contained appreciable amounts of triphosphate nucleotides, particularly adenosine triphosphate. This finding and the evidence that compound *C* is an uncoupler of oxidative phosphorylation (Table 1), suggest that *C* acts as a growth inhibitor, at least in part, through inhibition of high-energy phosphate production.

The concept that endogenous inhibitors control seed germination is widely held (*3*), and Evenari (*4*) has listed over 100 species from which germination inhibitors have been obtained. Compound *C* may be an important inhibitor of germination in immature seeds. Woodford *et al.* (*5*) and van Overbeek *et al.* (*6*) have suggested that the growth inhibitions caused by high auxin concentrations may result from the accumulation of growth inhibitors. The experiments reported here (*7*) indicate that the inhibitory effects of 2,4-D applied to soybean seedlings may be the result of the accumulation of *C*.

Work on the chemical identification of compound *C* is in progress. Studies are also being made of the distribution of this compound in various species of immature seeds and of its relationship to seed germination.

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Discrimination Learning

Abstract. Eight rats were run through discrimination training sessions in which responses in the dark were not reinforced whereas the first response after the onset of a light was reinforced. The procedure generated orderly learning and latency data for the individual animal. The latency distributions are adequately described by a simple mathematical formulation.

The present study was designed, first, to determine the extent to which orderly discrimination learning curves for individual animals could be simply obtained and, second, to test the adequacy of a mathematical formulation developed by Mueller (*1*) for describing latency data.

The subjects were eight naive, adult, male albino rats which had been deprived of water for 22½ hours at the start of each experimental session. The apparatus consisted of a response chamber through one wall of which a

response lever was inserted. Depression of this lever could activate a dipper which delivered the reinforcement, a drop of water. A stimulus light was mounted directly above the lever. The response chamber was housed in a sound-shielded, light-proof room in which a random noise generator masked external sounds. The programing and recording devices were housed in a separate room.

Each rat was first given a ½-hour operant level session—that is, a session in which it had free access to the lever but in which no reinforcements were delivered. Each rat was then trained to approach and drink from the dipper on hearing it operate and, subsequently, to depress the response lever to operate the dipper. Once the animal had learned to press the lever, it was given three sessions during which each response was reinforced (a total of 375 reinforcements). The stimulus light was on throughout each of these sessions.

The procedure was then changed so that the animal, in order to receive reinforcements, was required to make a discrimination—that is, to respond when the stimulus light was on and not to respond when the stimulus light was off. Each response recycled a timer set for a 20-second interval. When this time had elapsed, the light came on in the response chamber. The first response in the light activated the dipper, which delivered a drop of water, turned out the light, and recycled the timer. A response in the dark, on the other hand, recycled the timer but did not operate the dipper. Thus, the onset of light was always 20 seconds after a response; an animal that never spaced its responses at least 20-seconds apart received neither a presentation of light nor a response reinforcement (*2*). Ten daily 1-hour sessions of discrimination training were given. The time that elapsed between each onset of light and the subsequent response was recorded in sessions 9 and 10.

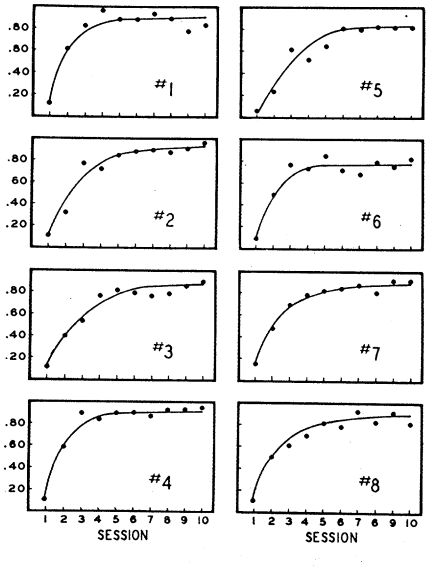

Fig. 1. Discrimination indices for each rat in each session. The animal's number appears in each graph.

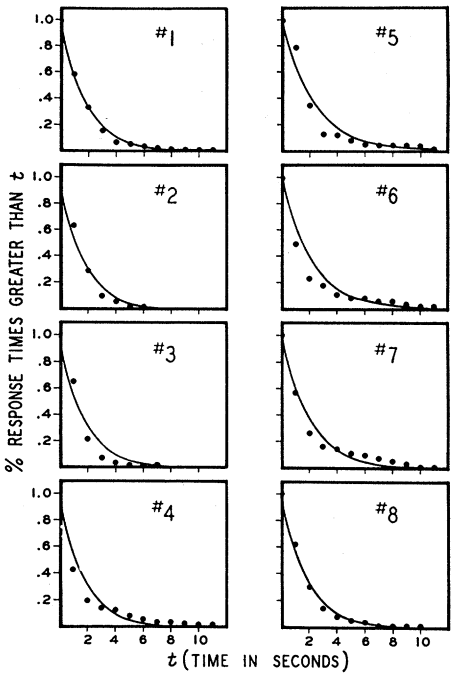

Fig. 2. The proportion of latencies (time from onset of light to time of response) greater than t plotted for each rat in session 10. The curves describe the equation e^{-rt}, where r is the reciprocal of the mean latency.

The response data are treated in terms of a "discrimination index" computed for each rat in each session. This index of learning is the ratio of the number of responses made in the light to the total number of responses (those made in the light plus those made in the dark). As the animal learns, an increasingly large proportion of the total number of responses occurs in the light; the index can accordingly shift from 0 to 1 as learning progresses (*3*). Such discrimination indices, plotted for each rat, are shown in Fig. 1.

With each occurrence of an interresponse time of at least 20 seconds, the stimulus light appeared and, within a brief period, the animal depressed the lever. The procedure provided, then, latencies measured as time from onset of light to time of response. The latency distributions obtained in session 10 are plotted in Fig. 2.

The values plotted are the proportions of responses made after various times t on the abscissa. If an animal emitted ten responses with a latency of 1 second, ten with a latency of 2 seconds, and ten with a latency of 3 seconds, for example, the corresponding proportions would be 1.00, 0.67, 0.33, and 0, plotted against t values of 0, 1, 2, and 3 seconds, respectively. The curves drawn through the points describe the equation $Y=e^{-rt}$, where Y is the proportion of response times greater than any time t, r is the reciprocal of the mean latency, and e is the natural base of logarithms.

This equation is one developed by Mueller (*1*) on the assumption that responses are randomly distributed in time. In his treatment, r corresponds to the response rate developed in a "free responding," operant situation; that is, the value of r is given by the ratio of the number of responses made to the total time available for responding. In the present context, then, r is equal to the reciprocal of the mean latency (*4*). The curves plotted in Fig. 2 were drawn by computing the reciprocal of the mean latency, substituting this value in the e^{-rt} equation for the various t values, and plotting the resultant Y values (*5*). These data and the details of procedure have been more completely described elsewhere (*6*).

Figures 1 and 2 indicate that the procedure generated rather orderly learning and latency data for the individual animal. It may therefore be of some value in assessing the effects of various independent variables on these kinds of behavior in the single animal, as opposed to behavior in some arbitrary, "average" organism. Further, the general adequacy of the e^{-rt} equation in describing the data of Fig. 2 (*7*) suggests that this equation could be useful as a starting point for the development of mathematical descriptions of certain more complex forms of behavior.

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2. This general procedure was originated by D. M. Page [cited in M. P. Wilson and F. S. Keller, *J. Comp. and Physiol. Psychol.* **46**, 190 (1953)].
3. These indices are a rather gross composite of at least three variations in the animal's behavior. (i) With training, the animals presumably come to respond more rapidly after the onset of the light and thus "gain" further opportunities for presentation of light. (ii) As the discrimination is learned, fewer responses are made in the dark, and thus more occasions are provided for presentation of light and subsequent responses. (iii) The indices are affected by the distribution, in time, of those responses that are made in the dark; for example, responses made after 19 seconds of darkness decrease the total number of opportunities for presentation of light more than do those made after 5 seconds.

4. In an operant situation, r is equal to N/T, where N is the number of responses and T is the time available for responding. Here, N equals the number of responses in the light and T the sum of all latencies. The mean latency is thus T/N, and its reciprocal is r.
5. A more sensitive, and therefore preferable, way of treating latency (L) data is in terms of the ratios of the frequency of any L to the number of opportunities for that L to occur. The limitations of the present experiment, however, were such that the difficulties inherent in the use of this ratio could not be overcome. [See D. Anger, *J. Exptl. Psychol.* **52**, 145 (1956)]. The e^{-rt} function is here taken as a fairly acceptable first approximation to the data.
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7. In the study discussed here the latencies between successive responses made during the preliminary sessions, when each lever depression was reinforced, were not recorded. Data from another, unpublished, study suggest, however, that the e^{-rt} function, when allowance is made for the time the animal spends consuming the water (reinforcement), provides an adequate description of the distributions obtained under these conditions.

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Role of Trehalose in Ascospores of Neurospora Tetrasperma

Abstract. The anthrone-positive material extractable in 80 percent alcohol, whose disappearance is correlated with the breaking of dormancy, has been found to be a non-reducing sugar which yields only glucose upon hydrolysis. On the basis of its crystal structure, infrared spectrum, melting point, specific rotation, and chromatographic properties, this material has been identified as trehalose.

Ascospores of *Neurospora* remain dormant unless provided a heat-shock (*1*), furfural or furfuryl alcohol (*2*), other furans and heterocyclic compounds (*3*), or certain organic solvents (*4*). Activation of these cells is accompanied by a 20-fold increase in respiratory rate (*5*), the origin of which is still uncertain. Our recent data have revealed that whereas the dormant ascospore utilizes endogenously contained lipids as the respiratory substrate, activated ones consume an endogenous carbohydrate fraction which is extractable in 80 percent ethanol (*6*). Thus, within a few minutes after exposure to temperatures which break dormancy, this material begins to disappear, and it is almost completely exhausted by the time the germ tube is protruded. The present report concerns the analysis of the carbohydrates in the 80-percent-ethanol-soluble fraction and the identification of the principal component as the nonreducing disaccharide trehalose.

Weighed aliquots of ascospores were killed by boiling in 80 percent ethanol for 5 minutes and then were centrifuged free of the supernatant fluid. The spores were broken in a Nossal disintegrator (*7*) and defatted by extraction in ethyl ether for 24 hours. The defatted cells were extracted in 80 percent ethanol at 60°C until no more anthrone-positive material could be removed, after which the supernatant was clarified by centrifugation. The extract was decolorized by mixing with Norit (1 percent by weight) at 60°C; this process was followed by boiling for 15 minutes. After removal of the Norit, the extract was concentrated under a vacuum until a thin syrup was obtained, which was added to a mixed-bed resin containing Dowex 50 (H^+) and Dowex 1 (CO_3^-). Approximately 95 percent of the anthrone-positive materials were recovered by washing the resin with deionized water, and the clear solution was concentrated into a thick syrup, in a vacuum. The syrup was put in a beaker, and an equal volume of hot 80 percent ethanol was added; the beaker was placed in a desiccator at 4°C, and crystals formed within 24 hours. When left undisturbed in the cold for 3 weeks, most of the liquid evaporated and large numbers of clear orthorhombic crystals formed, some as large as 4 by 2 mm. These crystals were redissolved in hot 80 percent ethanol, recrystallized, washed again in cold ethanol, and dried under a vacuum. The yield, on the basis of dry weight of ascospores, was 10.2 percent; this represented approximately 78 percent of the anthrone-positive materials in the 80-percent-alcohol extract.

Various analyses of the crystalline material were carried out, including chromatography with N-butanol, acetic acid, and water (4:1:5) as a solvent system, and a single spot was found which corresponded to trehalose. The periodate-permanganate reagent of Lemieux and Bauer (*8*) was used, and the length of time required for the development of the spot was found to be identical for the material obtained from the ascospores and for an authentic sample of trehalose. Therefore, the characteristics of the crystals obtained from the ascospores were compared with those of authentic trehalose; the data are summarized in Table 1. In addition, the infrared spectra of these two samples were found to be identical when Nujol mulls were observed in a Perkin-Elmer model 21 spectrograph (*9*). Finally, the crystalline material was found to be nonreducing and liberated only glucose upon hydrolysis in 1*M* H_2SO_4, as revealed by analysis with the glucose oxidase system (*10*).

Extracts that had not been passed through the resins yielded another spot upon being chromatographed. Such material, when concentrated, formed not only the rhomboidal crystals of trehalose but a small number of needle-shaped crystals as well. This second substance was found also to be nonreducing, but it did not give the blue-green color with anthrone. For these reasons, and because its R_f in the solvent system described above was identical with that of mannitol, it was tentatively identified as that sugar-alcohol.

Table 1. Comparison of properties of extract from ascospores of *Neurospora tetrasperma* and trehalose.

Property	Ascospore extract	Trehalose
Melting point	97°–99° and 205°–210°	96.5°–97.5° and 203°*
Mixed melting point	97°–99° and 203°–206°	
$[\alpha]_D^{20}$	+176	+176
R_f in butanol, acetic acid, and H_2O	0.06	0.06

* Data from (*12*).

These data suggest that trehalose is probably the substrate, soluble in 80 percent ethanol, whose utilization is correlated with the activation of ascospores of *Neurospora tetrasperma.* Investigations are now under way to determine the locus of the metabolic block which prevents the consumption of trehalose, thereby restraining the development of the dormant ascospores (*11*).

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9. We are indebted to Venkoba Rao, of the Randall Laboratory, University of Michigan, for having obtained the infrared spectra.
10. The assay for glucose was based upon the "Glucostat" reagent, which was obtained from the Worthington Biochemical Corp., Freehold, N.J.
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