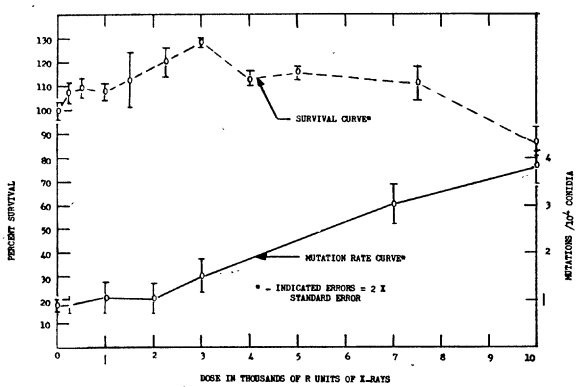

Fig. 1. Relationship of survival and mutation rate to dosage.

sures to relatively low dosages of x-rays; population sizes were determined by hemocytometer counts; and viability was determined by plating appropriately diluted aliquots of conidia onto minimal agar supplemented with glutamic acid (*8*). The number of conidia that germinated following irradiation increased with dosage up to 3000 r, at which dose between 20 and 25 percent more conidia germinated than in the untreated populations (Fig. 1). It is also evident from this figure that more conidia germinated following treatments of 250 to 7000 r than germinated without irradiation.

The second measurement of "stimulation" was that of a radiation-induced growth-rate alteration. Following irradiation, the conidia produced mycelia, in minimal medium (solution), that became perceptible to the naked eye at varying time intervals. By removing the developing mycelia (*7*), the duration of germination was measured. Unirradiated macroconidia produced mycelia in amounts sufficient to be perceptible in 15 to 20 hr at 24°C. The duration of germination was 24 to 36 hr. Conidia subjected to doses of x-rays or gamma rays between 500 and 50,000 r produced visible hyphae in 8 to 10 hr; the duration of incubation was 24 to 30 hr. Conidia treated with ultraviolet light or with thermal neutrons of varying doses were, on the contrary, retarded in growth. Perceptible growth was not detected until after 24 hr of incubation; and it continued at a rather constant rate for approximately 48 hr. There was a rather extended dose-independent range with all radiations. Since conidia one generation removed from the conidia treated with x-rays, thermal neutrons, and ultraviolet light and from untreated conidia germinated at the same rate, it was concluded that the stimulation was nongenetic.

It is evident from these data that x-rays effect changes in both genetic and nongenetic components of *Neurospora* conidia. X-rays affect the growth rate and the increased conidial germination similarly; but, in the case of the growth rate, either the biological component responsible is affected differently by the different types of radiation or different components are affected.

The relationship between mutation rate and conidial survival is more complex. The survival curve appears to be a composite of at least two independent events: (i) conidial "activation" or stimulation and (ii) inactivation. Therefore, it is difficult to determine the extent of either event, since a given survival value represents a combination of both. *A priori,* one would expect inactivation to be insignificant at low dosages, at which a rather large increment of stimulation occurs. At higher dosages, inactivation, no doubt, becomes more significant, but the increment of stimulation becomes difficult to measure. Since the survival curve is a composite, it becomes difficult to establish the correlation between either component and the mutation process. According to their relationship with x-ray dosage, stimulation is independent of genetic mutation; but, before the relationship between inactivation and mutation is established, it will be necessary to separate, experimentally, the two components that combine to produce a given survival curve.

The conidial "activation" caused by x-rays imposes certain difficulties to the interpretation of killing curves in *Neurospora.* Until the separate components are isolated, it would seem risky to attempt to separate nuclear from nonnuclear x-ray effects solely on the basis of existing killing curves.

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7 March 1954.

Liquid Sulfur Dioxide as a Solvent for Proteins and the Infrared Spectrum of Proteins in Solution

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Pure liquid sulfur dioxide (boiling point, −10.02°C) has previously been observed to have little or no solvent properties for proteins (*1*). In the case of water, it is well known that many proteins are insoluble in pure water but dissolve readily on the addition of small amounts of neutral salts (*2*). It has now been found (*3*) that a similar salting-in effect occurs with liquid sulfur dioxide, and that proteins are soluble in sulfur dioxide-neutral salt solutions.

Alkali metal (and ammonium) iodides and thio-

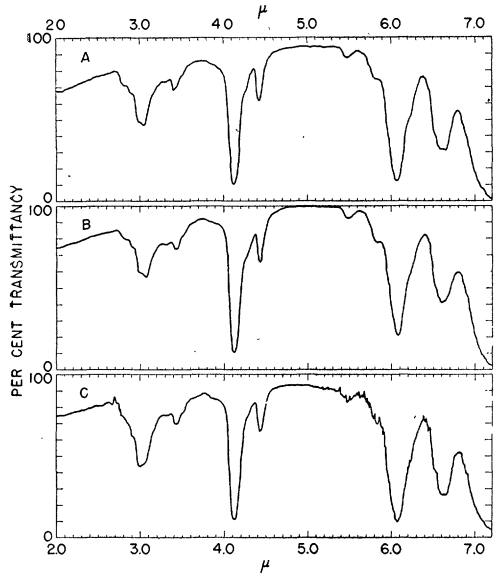

Fig. 1. Infrared spectra of globular proteins in 2.5*M* KI-SO_2 solution. Protein concentration, 10 mg/ml; cell thickness, 0.25 mm. (*A*) Insulin; (*B*) cytochrome *c*; (*C*) bovine gamma globulins.

cyanates are among the most soluble of the inorganic salts in liquid sulfur dioxide (*4*), and these have been successfully used as salting-in agents. The concentration of salt required to effect salting-in appears considerably greater in sulfur dioxide than in water. Nearly saturated solutions (at room temperature) of ammonium thiocyanate (6*M* NH_4CNS) and potassium iodide (2.5*M* KI) have been the most extensively used. Thus, bovine plasma albumin, bovine gamma globulins, insulin (*5*), crystallized egg albumin, trypsin, cytochrome *c*, urease, catalase, peanut protein globulin, zein, hide collagen, silk fibroin, and rattail tendon all dissolve readily in 6*M* NH_4 CNS-SO_2 solution. Nylon and poly-L-aspartic acid (*6*) are also soluble. Solutions with a protein concentration of 10 mg/ml have been prepared of all these proteins; the solubility limit is probably substantially higher for most of these proteins, and a solution of bovine plasma albumin of 100 mg/ml in 6*M* NH_4 CNS has been prepared.

These experiments were carried out on a metal vacuum line fitted with polychlorotrifluoroethylene reaction tubes; this made it possible to prepare and observe these solutions at room temperature. Sulfur dioxide-potassium iodide solutions are not as powerful solvents as are the ammonium thiocyanate-sulfur dioxide mixtures. Although many of the proteins listed in the foregoing paragraph dissolve in 2.5*M* KI-SO_2, silk fibroin, collagen, nylon, and gelatin swell but do not seem to dissolve. As ordinarily prepared, protein solutions in SO_2-KI are red, and those in SO_2-NH_4 CNS are yellow; no visible changes in these solutions are noted after standing at room temperature for several days. Proteins dissolved in sulfur dioxide-salt solutions can be recovered by dilution; the addition of sulfur dioxide to reduce the concentration of the salt leads to the precipitation of the protein. The properties of the proteins recovered from such solutions still remain to be examined in detail.

It has been pointed out that there are certain advantages to the study of the infrared spectrum of proteins in solution (*7*) rather than as films or fibers. This has been, at best, a difficult task, since the usual protein solvents obscure at least a major portion of the infrared spectrum of the protein. With liquid sulfur dioxide as a solvent, the situation is greatly improved, since both the 3-μ and 6-μ regions are transparent (*8*); because potassium iodide is transparent in the infrared, the infrared spectrum of proteins dissolved in SO_2-KI solution can be readily studied.

Figure 1 shows the infrared spectra of dissolved insulin, cytochrome *c*, and bovine gamma globulins. The 3-μ and 6-μ regions are both clearly visible; the useful region extends to a little above 7 μ before the intense sulfur dioxide absorption sets in. These spectra were obtained at room temperature with a Model 21 double-beam Perkin-Elmer infrared spectrograph equipped with calcium fluoride optics and using a 0.25-mm cell (*9*). (The cell was not compensated, and two SO_2 peaks appear at 4 μ.)

The infrared spectrum of a bovine plasma albumin solution in the 6- to 7-μ region is shown in greater detail in Fig. 2. The infrared spectra so far obtained appear to resemble quite closely those of extended chain polypeptides described by Ambrose and Elliott (*10*). Particularly noteworthy is the shift of the 6.45-μ band to the vicinity of 6.60 μ. This shift, which may be interpreted as the result of denaturation and unfolding, is not surprising in view of the known denaturing effect of iodide and the thiocyanate ions (*11*).

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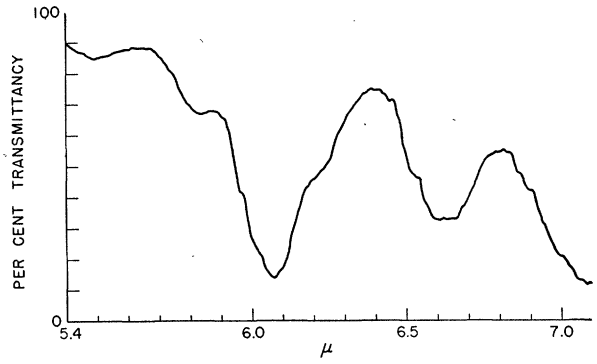

Fig. 2. Infrared spectrum of bovine plasma albumin in 2.5*M* KI-SO_2 solution in the 6-μ region. Protein concentration, 10 mg/ml; cell thickness, 0.25 mm.

through the courtesy of Sharp and Dohme and Burroughs, Welcome Co.
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6 January 1955.

Auxin Gradient Theory of Abscission Regulation

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Abscission of leaves, flowers, and fruits is known to be accelerated or retarded by many factors, including light intensity, photoperiod, temperature, water, mineral nutrients, carbohydrates, auxins, oxygen, carbon dioxide, anesthetics, mechanical injury, disease, insects, and senescence.

Several hypotheses and theories concerning the regulation of abscission have been advanced. Each has emphasized some important factor in abscission, such as turgor (*1*), nutrient balance (*2*), leaf-fruit ratio in fruit abscission (*3*), acidity (*4*), and hormone-ethylene balance (*5*). Few consider all known factors; none are adequately comprehensive. These hypotheses and theories will be analyzed in a forthcoming article (*6*). This paper reviews some aspects of the physiology of abscission and describes an auxin gradient theory of the regulation of abscission.

In an investigation of auxin in beans, Shoji *et al.* (*7*) found the concentration of auxin in the leaflets (distal to the leaflet abscission zone) approximately three times the concentration in the leaf stalks (proximal to the abscission zone). Shortly before the leaflets abscised, the auxin concentration in the leaflets fell, but in the leaf stalk it remained unchanged. This suggested that the auxin gradient across the abscission zone is a factor in the regulation of abscission. A similar but more extensive investigation in cotton has confirmed these results (*8*).

In excised leaflet abscission zones of beans, abscission was accelerated by the application of auxin to the proximal side of the abscission zone (*9*). This was confirmed in excised abscission zones of cotton (*10*) and in greenhouse beans (*11*). In *Coleus*, auxin transported from young leaves accelerated abscission of debladed petioles below the leaves (*12*). These observations further support the idea that the auxin gradient is a regulator of abscission.

Additional support is given by the auxin relationships of other factors affecting abscission: oxygen,

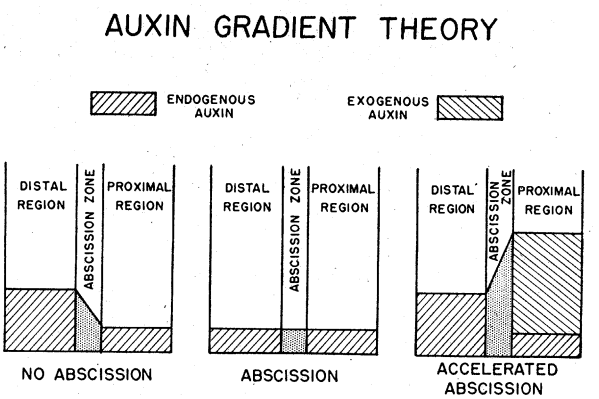

Fig. 1. Relationships between the auxin gradient across the abscission zone and abscission. Based on Shoji *et al.* (*7*) and Addicott and Lynch (*9*).

which accelerates abscission, is required for auxin inactivation (*13*); under oxygen deficiency, abscission is retarded and auxin increased (*14*). Chemical defoliants lead to a rapid decrease in leaf auxin (*8*, *11*). Ethylene, which also accelerates abscission, decreases auxin in some species (although not in others) (*15*). Injury by disease or insects may reduce auxin; for example, the fungus *Omphalia* defoliates coffee, apparently through the production of an auxin-inactivating enzyme (*16*). Zinc deficiency, which often accelerates abscission, decreases auxin (*17*).

On the basis of this and other evidence (*6*), the following theory is proposed: Auxin is the principal endogenous regulator of abscission; its gradient across the abscission zone regulates onset and rate of abscission. Abscission does not occur with auxin gradients characteristic of healthy, mature tissue: with high auxin distal to the abscission zone and low auxin proximal to the abscission zone. Abscission occurs after a fall in the ratio of distal to proximal auxin. Abscission is accelerated when the gradient is reversed. Figure 1 shows these relationships. There is evidence of positive correlation between the auxin gradient and the rate of abscission (*10*).

Application of auxin to an intact plant frequently does not retard abscission (*18*), and may even accelerate it (*19*). On the basis of this theory, such results would be expected if applied auxin were translocated or inactivated as rapidly as it is absorbed, not enough remaining distal to the abscission zone to maintain a retarding gradient. If auxin accumulated on the proximal side of the abscission zone, the reversed gradient would accelerate abscission.

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