EFFECTS OF STREPTOMYCIN ON CERTAIN GREEN PLANTS[1, 2]

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While attempting through the use of antibiotics to obtain pure cultures of various algal flagellates and their related colorless forms, Provasoli *et al.* (1948) observed that on exposure to streptomycin (SM), *Euglena gracilis* var. *bacillaris* became colorless. Provided with suitable substrates the bleached organisms could be kept alive in spite of the loss of their photosynthetic apparatus. Complete bleaching resulted from about four days exposure to 100 μg. SM/ml; bleached organisms remained colorless indefinitely and were not inhibited in growth upon subsequent serial transfer in light on SM-free media. Provasoli *et al.* (1951) reported on further experiments with green flagellates and their colorless homologues which led to the hypothesis that SM acts at two different sites in these organisms, one affecting the chloroplast (the *bleaching* site) and the other responsible for killing (the *antibiotic* site). The relative sensitivity of the two sites in a particular species determines whether it will be killed or bleached by exposure to SM. (Although the mode of action of SM is not known, absence of greenness resulting from SM treatment will, for convenience, be referred to as bleaching in this paper.)

Von Euler (1947, a, b) has reported the effect of SM on germinating seeds of barley, rye, and cress. In his experiments SM was observed to have two effects: (1) a general or non-specific growth inhibiting effect resulting in stunting and thickening of the roots and thickening of the coleoptile, and (2) a bleaching effect. Exposure to 0.2 percent SM was sufficient to cause the first and second leaves of the germinating seedlings to be green only at the tips. Higher concentrations resulted in completely white leaves. No observations are reported beyond the seedling stage. Von Euler (1947, a) states that SM does not destroy chlorophyll already formed but that further formation is inhibited.

Ziebur (personal communication) has made preliminary studies of the effect of SM on a number of higher plant species. Both the bleaching and the non-specific growth effects were obtained by a variety of treatments, including treatment of apical buds of nearly mature radish plants. Flower production by shoots treated in this manner made possible some limited observations of progeny resulting from SM-bleached plants. Female sterility and at least partial male sterility were observed. No SM effects were observed in the progeny resulting from the cross, normal female × SM-treated male. The reciprocal cross was not successful.

Other reports of SM bleaching of potentially chlorophyllous tissue include: carrot tumor tissue (De Ropp, 1948), pine seedlings (Bogorad, 1950), wheat and radish (Wright, 1951), and *Chlorella* (Dubé, 1953).

The reports reviewed above raise numerous questions concerning the genetical and physiological mechanism(s) of SM action on plants. Elucidation of these mechanisms has been hampered by the paucity of information regarding SM action on intact higher plants beyond the seedling stage, and by the apparent lack of sexuality in *Euglena*, the organism in which SM action has been most fully

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studied. Because of this situation it seemed desirable to accumulate more information concerning the effects of SM treatments on various plants, and then to examine in some detail those treatments which seemed most likely to lend themselves to clarification of the mode of action of SM. Particularly, an effort has been made to obtain in higher plants the differential effects on bleaching and growth which have been reported for the algal flagellates.

EXPERIMENTAL METHODS

Two experimental methods were employed, (1) seed treatments with species other than those employed by previous workers, and (2) leaf treatment, a modified injection technique applied to more mature plants.

Seed treatment. Attempts were made to maintain seedlings from treated seeds beyond the stage of development reported by previous workers. In an effort to circumvent, at least temporarily, the need for externally supplied metabolites, experiments were conducted with species which are known to be capable of considerable growth at the expense of food stored in the seed. Seeds of pea, bean, and corn were therefore employed.

Typical treatment was as follows: Seed of pea (Burpee's Blue Bantam) were placed in a moist chamber and allowed to begin germination. After 48 hr. they were transferred to filter paper pads in 9 cm. petri dishes. Ten ml. of 1.0 percent SM (Dihydrostreptomycin sulfate, Merck) was introduced into the dish. This volume of solution was sufficient to thoroughly soak the filter paper and to partially submerge the seeds. After 48 hr. in the dishes the seedlings were transferred to quartz sand in waxpaper cartons, four seedlings per carton. The cartons containing the seedlings were placed in the greenhouse or in a darkroom. The plants were watered daily with tap water.

Leaf Treatment. Another means of circumventing the need for exogenous metabolities was to induce bleaching in a part of an otherwise green plant. The bleached portion presumably would be supplied with carbohydrates by the unaffected chlorophyllous tissue of the same plant. The bud treatment experiments of Ziebur, mentioned earlier, are illustrative of this approach.

Another variation of this method was a modified "injection" technique in which bean seedlings were treated by rolling one of the primary leaves slightly and inserting it into a tube containing the SM solution. In most cases treatment was begun at the time of almost complete expansion of the primary leaves, before the bud of the first trifoliate leaf had begun to expand. All leaf treatments were conducted in the greenhouse. Plants were maintained in individual pots and were watered daily with tap water.

RESULTS

Table 1 presents results of a typical experiment in which pea seeds were treated in the manner described in the preceding section. In this experiment control plants, which remained in the moist chamber for 96 hours, were grown in dark and in light under the same conditions as the treated plants. Data reported in the table are from measurements taken after 4 weeks growth, at which time the plants were discarded.

This experiment demonstrated a toxic effect of SM apparently not attributable to its effect on the photosynthetic mechanism. If the effect of SM were only on chlorophyll formation, it would be expected that SM-treated plants which grew in the dark would resemble dark-grown controls. However, treated plants, both in the dark and in the light, were extremely stunted. Roots were short and thick and no lateral roots developed. Stems were extremely short. Although not noted in the table above, the leaves of SM-treated plants, both in the dark and in the light, were less expanded than those of dark-grown controls. Leaves of the dark-grown controls were yellow. Leaves of treated plants, both in the dark and in the light, were white, and hard and waxy to the touch. At the time that the measurements were made it was noted that the cotyledons of treated plants were still fairly large and firm, indicating that although increase in size of the treated plants had apparently ceased, there was still considerable stored food in the cotyledons. A number of treated seedlings were dying at the conclusion of the experiment, and the cotyledons of a number of them were infected with bacterial colonies. The controls in the light were in flower at the termination of the experiment.

Similar treatment of bean seeds (Burpee's Stringless Green) with SM resulted in bleaching and in the general toxic effects noted above for pea. However, treated plants which grew in the light varied in their appearance. The hypocotyls of all treated plants were extremely stunted. Primary leaves varied in color from pale green to white, and although they unfolded on some plants, they consistently failed to expand. Treated plants failed to develop further than this before dying.

With corn, seed treatments have shown more promise because bleached seedlings seemed to be more resistant to the toxic effect of SM than pea or bean seedlings, although results of seed treatment were extremely irregular. Of the seedlings resulting from the same experiment some were green and apparently normal, others were uniformly pale green, and still others were completely white. A large number had white or partially white primary leaves, but green second leaves. This last group suggests that differences in degree of bleaching result from differences in the permeability to SM of the seed coat (pericarp) of different seeds.

TABLE 1

Effects of streptomycin on growth of pea plants in light and dark

	1.0% SM light	1.0% SM dark	Control light	Control dark
Stem length (av.)	1.34 cm.	1.82 cm.	15.94 cm.	30.85 cm.
Root length (av.)	1.50 cm.	1.88 cm.	16.21 cm.	7.60 cm.
Stem color	white	white	green	white
Leaf color	white	white	green	yellow

Bleached corn seedlings, while smaller and slower growing than untreated plants, were relatively less stunted than were the treated pea and bean seedlings. Even completely bleached corn seedlings had fair root development, with considerable branching of the roots. Stunting was roughly proportional to the degree of bleaching, and pale green seedlings grew more than completely white ones, although both died early. The pale green seedlings reached a height of about 8–10 cm. before dying, and the white ones about 3–6 cm. A few of the completely white seedlings were kept alive longer than the rest by moving them from the greenhouse into low-intensity light in the laboratory where they were supplied with carbohydrate by immersion of the tip of one of the leaves in 0.4 M sucrose.

Results of Leaf Treatment Experiments. In an early experiment bean plants were treated in the manner described above with 1.0, 0.5, 0.25, and 0.12 percent SM. Bleaching effects were observable after 9 days. The effects were most striking in the first trifoliate leaf as it expanded, and the intensity of SM effects increased with increase in concentration of SM. (Note: The first trifoliate leaf will hereafter be referred to as TFL-1, the second as TFL-2, etc.)

TFL-1 arises from the main axis at an angle of 90 degrees from the primary leaves. From 0.5 to 0.12 percent SM inclusive, the SM effect was greatest in the portion of TFL-1 which was nearest (*i.e.*, made the smallest angle with) the treated primary leaf. The leaflet nearest the treated primary leaf was most bleached, the middle leaflet less bleached, and then mostly on the side nearest the treated primary leaf, and the leaflet furthest from the site of treatment was least bleached. The leaflets showed a reduction in amount of expansion which was roughly proportional to the degree of bleaching.

The SM tubes were removed from the leaves after 25 days, at which time the treated leaves were all dead, whereas the primary leaves of controls (immersed in distilled water) were alive.

In this experiment treatment with 1.0 percent SM was lethal. For the lower concentrations, total amount of bleaching in TFL-1 varied with the concentration of SM employed. In the plants treated with 0.5 percent SM the primary leaf opposite the treated leaf developed a mottled necrosis and died prior to the rest of the plant. The plants themselves were dead 29 days after the start of treatment, TFL-2 failing to open. Treatment with 0.25 and 0.12 percent SM permitted limited development of TFL-2. Both bleaching and toxic effects were very slight at the latter concentration. Plants treated with 0.25 percent SM were observed to develop a necrosis in the most severely bleached portion of TFL-1.

By varying SM concentrations and duration of treatments an attempt was made to examine the report of Hutner, *et al.* (1949) that *Euglena* bleaching is a function of total exposure to SM (*i.e.*, duration × concentration = k). However, it was found that bean plants treated in this manner under greenhouse conditions were, as with seed treatments, not sufficiently sensitive in their reaction to SM treatment to demonstrate an effect from small variations in exposure times and/or SM concentrations. At least 48 hr. treatment was necessary to demonstrate an SM effect at concentrations of 1.0–0.25 percent. Treatment of sufficient duration to cause bleaching generally resulted in the death of the treated leaf.

The use of a wetting agent (0.1 percent Tween-20) in combination with the SM solution appeared to enhance the toxic effect of SM to such an extent that the plants were killed before bleaching could be detected. 1.0 percent SM, even in the absence of the detergent, resulted in the death of the plant without expansion of TFL-1, or if the exposure time was reduced, in no effect at all. Therefore, all subsequent experiments were conducted with SM at concentrations of 0.5 and 0.25 percent.

At these concentrations the minimal exposure necessary to produce bleaching also resulted in toxic effects which severely injured the plants. TFL-2 usually expanded only very slightly. If the plant survived at all, subsequent growth resulted from development of buds in the axils of the cotyledons, primary leaves, and TFL-1 and -2. This would indicate SM injury to the apical growing region resulting in reduction of apical dominance. Even these axillary buds developed only slightly, and the plants usually died without flowering. The axillary shoots, when they developed, had pale green to yellow leaves.

An attempt was made to counteract the toxic effects of SM leaf treatments with 0.2 M sucrose dissolved in the 0.5 percent SM leaf treatment solution. When treated in this manner it was noted that TFL-2 was evenly bleached a pale yellow to white. The characteristic pattern of bleaching (*i.e.*, bleaching inversely proportional to the distance of the tissue from the site of treatment) which developed in the plants treated with SM alone, was absent. In the presence of added sucrose the compound leaves apparently were completely lacking in chlorophyll, and since both primary leaves were usually lost after treatment the plants were virtually devoid of chlorophyll and thus prevented from further growth. The lives of a few such plants were prolonged by immersing the center leaflet of TFL-1 in 0.4 M sucrose after the SM treatment. This treatment resulted in prolonging the lives of some of these plants by about a week beyond that of SM treated controls. TFL-1 of plants treated with SM and sucrose expanded uniformly to about one-fourth to one-third the width of TFL-1 on untreated plants.

A few attempts were made to induce bleaching in more mature plants in the hope that they would be able to reach the flowering stage in spite of toxic effects. Although such efforts were not successful it was noted that when TFL-1 of a plant having three TFL's was treated, TFL-4 was white when it expanded, expansion being about one-third of normal. No appreciable effects were discernible in the intervening leaves.

In these experiments the bleaching effect was manifested only in immature leaves. Leaves which were already expanded and green could not be made to bleach by SM treatment. Treated primary leaves died without bleaching, and the primary leaves opposite the treated ones, although often exhibiting a toxic effect in the form of a spotty brown necrosis, did not develop the characteristic SM-induced bleaching.

Toxic effects resulting from leaf treatments were at least as profound as from treatments of germinating seeds. In many bean plants which were leaf-treated with 0.12 and 0.25 percent SM the toxic effect persisted much longer than did the bleaching. Bleaching was most evident in TFL-1 and -2. The most marked effects on leaves which developed later, whether they were derived directly from the apical meristem or from axillary buds, were toxic effects such as reduced size, puckering, and abnormalities in the shape of the leaflet blades.

DISCUSSION

The experiments described above extend the number of species of green plants known to be "bleached" by exposure to SM. In addition, a new method for treatment of plants with SM (*i.e.*, leaf treatment) is described.

The present investigations have failed to reveal the marked difference in sensitivity to the bleaching and toxic actions of SM reported by Provasoli and coworkers for *Euglena* (and suggested for other algal flagellates). Rather, in the present experiments bleaching was always accompanied by toxic effects, and both bleaching and toxicity were intensified as the SM concentration was increased within a certain range (0.12–0.50 percent). In pea and bean the toxic effect of SM is evidenced by suppression of lateral root formation, stunting and thickening of roots and stem, and inhibition of leaf expansion.

Neither the seed treatments nor the leaf treatments described here have yielded highly uniform and reproducible results. Under greenhouse conditions it was found that fairly uniform results were often obtainable with plants within a single experimental series, but that series run at different times often differed widely in reaction to the same treatment. For this reason and also because of the apparently lower sensitivity to SM of higher plants as compared to *Euglena*, it has not been possible to confirm the claim made for the latter organism that bleaching is a function of total exposure.

Attempts to reduce toxicity without reducing bleaching, by shortening the exposure time or by reducing the concentration of SM have not been successful. However, corn appears to be less susceptible to the toxic action than the other species tested, and provision of carbohydrate by immersion of leaf tips in sucrose may provide a means of prolonging the life of bleached plants of corn and other species.

The wide variety of plants which are profoundly affected by exposure to low concentrations of SM is of considerable interest, particularly with regard to the mechanism(s) of action of this naturally occurring compound. The marked general toxic effects of low concentrations of SM are worthy of further attention in their own right. These effects range from the well known bactericidal action on numerous pathogens to the stunting effects reported here and elsewhere for higher plants. The fundamental nature of these effects is indicated by the report of Tanaka and Satô (1952). They conclude from observations of the effect of SM on mitosis in onion root tips that SM is a mutagenic agent.

The bleaching effect as reported from studies with *Euglena*, which suggests that SM induces a "heritable" somatic variation, is particularly interesting. It may be assumed that studies on the transmission of the bleaching effect to sexual progeny, both in protists and in flowering plants, will shed light on the mechanism of SM action and on plastid inheritance. But in order to examine the effect of SM on sexual progeny of bleached plants it will first be necessary to reduce toxicity without reducing bleaching, or else to find a plant which is insensitive to the toxic action of SM while susceptible to bleaching.

SUMMARY

Germinating seeds and portions of plants at later stages of development have been treated by exposure to stretomycin. Within a certain range of low concentrations of streptomycin (0.12–1.0 percent) this treatment results in absence of greenness in potentially chlorophyllous tissue, and in toxic effects such as stunting of stems, roots, and leaves.

The plants investigated were pea, bean, and corn. Seed treatment and leaf treatment were employed as means for exposing plants to streptomycin. Seed treatment consisted of placing germinating seeds in streptomycin for varying lengths of time, followed by non-aseptic cultivation in quartz sand. Leaf treatment, attempted only with bean, consisted of immersing one of the primary leaves of an intact plant in streptomycin solution prior to expansion of the first trifoliate leaf.

The toxic effects of streptomycin on these plants may be independent of the bleaching effect. However, contrary to reports regarding streptomycin treatment of some algal flagellates, it was not possible to obtain bleaching without toxic effects, although less marked toxic effects were noted in corn than in the other species tested.

In leaf treatments of immature bean plants streptomycin effects are manifested primarily in the first and second trifoliate leaves when these expand. Both bleaching and toxic effects are greatest in the portions of the trifoliate leaves which are nearest to the treated primary leaf. The use of sucrose in combination with streptomycin in these experiments results in a more even distribution of the bleaching effect in the trifoliate leaves.

In a few experiments the lives of plants subjected to lethal treatments with streptomycin were prolonged by supplying sucrose through the leaves.

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