to 12 days 49 percent of the tubules are in leptotene and zygotene. After 19 days one is apt to find that nearly 50 percent of the tubular cross sections contain cells approaching the first meiotic metaphase.

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Seedling Albinism Induced by an Extract of Alternaria tenuis

Abstract. The chlorophyll deficiency in citrus seedlings known as albinism was induced by inoculating seed with certain clones of *Alternaria tenuis*, or by germination of the seed in contact with extracts from the fungus. The active material in the extracts apparently has a rather specific inhibiting effect on chlorophyll formation in seedlings of various species.

A form of chlorophyll deficiency referred to as albinism occurs sporadically in citrus seedbeds. The appearance of the affected seedlings ranges from completely white to only slight flecking of white on otherwise green leaves. Some of the less severely affected seedlings eventually develop into normal green plants, but many others die within a month or two after germination.

Perlberber and Reichert (*1*) found that the production of albinos was essentially eliminated if the seeds were treated with mercury-containing preparations or salts of mercury, copper, cobalt, nickel, or lead. Tager and Cameron (*2*) clearly showed that the seed coats are involved in the disorder, since no chlorophyll deficiency occurred when the seed coats were removed before planting. Frost (*3*) mentioned the possibility of toxic action by fungi or bacteria, and evidence suggesting that a fungus is indeed responsible has been reported (*4*). The present study was undertaken in an effort to isolate a causal organism and determine the mechanism of its action.

A number of fungi isolated from the seed coats of citrus seeds which had produced albinos were tested for activity by inoculating surface-sterilized seeds, which were then germinated on agar. In one such test all of the seedlings which grew from seed inoculated with a clone of *Alternaria tenuis* were albinos.

When this clone was grown on Czapek nutrient agar and the cultures were extracted with hot water, cholorophyll deficiency inducing activity was found in the extracts. This was determined by germinating surface-sterilized excised sweet orange (*Citrus sinensis*) embryos in contact with 1-ml samples of the extract. With few exceptions these assays gave 90 to 100 percent albinos. Autoclaving the extracts to insure freedom from the fungus did not measurably reduce activity. The active substance has subsequently been extracted with cold water and with methanol, ethanol, propanol, isobutanol, and chloroform. Butanol, chloroform, and ethyl acetate extractions of filtrates from liquid cultures have also yielded active extracts.

Mung bean (*Phaseolus aureus*) seeds germinated in an aqueous solution of the active material produce chlorophyll-deficient seedlings, and a standard assay procedure was developed for this plant. For assaying nonaqueous extracts, suitable aliquots of the extract are transferred to 2-ounce bottles and the solvent evaporated. Two milliliters of distilled water are added and five fungicide-treated mung bean seeds are placed in the solution to germinate. After 3 days at 30°C and 3 or 4 days at room temperature, the leaves have unfolded and the degree of chlorophyll deficiency can be estimated. A rating from 0 for entirely green to 5 for entirely yellow has been used. Mean values for the five seedlings in duplicate samples of an extract usually are in close agreement.

Apparently the active substance interferes with chlorophyll formation but does not destroy chlorophyll once it is formed. Repeated application of a drop of the extract to the apical bud of a mung bean plant did not appear to affect the mature tissue, but the new leaves which developed from the apical bud were partially chlorophyll deficient.

The embryos of some citrus species are green when removed from the fruit, one such species being the calamondin (*Citrus madurensis* Lour., *C. mitis* Blanco). When excised embryos of calamondin were germinated in contact with an agar culture of the active fungus, all of the shoots were without chlorophyll, while the cotyledons retained their original green color.

The chlorophyll-deficient mung bean leaves are yellow, indicating the carotenoids are present. This is in contrast to the effect of certain other chlorophyll-inhibiting chemicals. With both amino triazole and streptomycin, the affected leaves or portions of leaves are white rather than yellow.

At the threshold concentration of the extract for complete chlorophyll suppression, the initial height of the seedlings is not affected, and the size of the first pair of leaves is not noticeably reduced. These observations also are in contrast to the effect of aminotriazole and streptomycin, which severely stunt the seedlings at concentrations which suppress chlorophyll formation. The evidence suggests a rather specific effect of the active material in the fungus extract on chlorophyll formation.

After the initial stem elongation and primary leaf development, no further growth occurs in the seedlings completely lacking chlorophyll. No effort has been made to keep albino mung beans alive by supplying sugar, or by grafting as was done with citrus by Minessy (*5*), who found that albino seedlings kept alive by approach grafting to green seedlings would eventually produce green shoots when buds were forced to grow by ringing the stem of the green seedlings.

Besides citrus and mung bean, seedlings of lettuce, carrot, and cucumber have been similarly affected by the extracts. On the other hand, chlorophyll deficiency was not induced in seedlings of tomato, radish, turnip, cabbage, oats, barley, or corn germinated in contact with solutions of the active material.

A second isolate of *A. tenuis* obtained in the same way as the one discussed above has also yielded the chlorophyll-inhibiting substance, as have two isolates of *A. tenuis* (*6*) from lemon fruit and bark. On the other hand, extracts of cultures of *A. porri* and *A. zinniae*, and of *A. tenuis* from another source (*7*) have failed to induce any chlorophyll deficiency.

Durbin (*8*) reported chlorophyll deficiency in citrus seedlings after seed inoculation with *Aspergillus flavus*, which had previously been shown to induce chlorophyll deficiency in corn

(*9*). Repeated efforts were made in our laboratory to produce chlorophyll deficiency in citrus by inoculation with *Aspergillus flavus, Aspergillus niger*, and other *Aspergillus* species isolated from the coats of albino-producing seeds (*10*). Also included were trials with *Aspergillus flavus* sent to us by Durbin. The results were completely negative.

The evidence indicates that the albinism occuring sporadically in citrus seedbeds is probably due to the action of *Alternaria tenuis* growing on the seed coats and producing a substance which interferes with chlorophyll formation in the developing seedling. The chemical nature of the active substance is being investigated.

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Transmissible Agent Associated with Some Mouse Neoplasms

Abstract. The association between some forms of murine cancer and an agent that increases the lactic dehydrogenase activity in the plasma of normal mice is confirmed. This agent, however, is not associated with all mouse neoplasms.

All 26 murine tumors studied by Riley *et al.* had a transmissible agent associated with them. This was demonstrated by injecting normal mice intraperitoneally with 0.1 ml of plasma from mice bearing any of these 26 tumors, whether spontaneously or as transplants. The lactic dehydrogenase activity in the plasma of the recipients increased several fold, and apparently the capacity to increase this enzyme activity can be passaged serially through normal mice indefinitely (*1*).

A similar agent is associated with some of the mouse tumors available to us, but not with all, so that the association is more probably coincidental than significant.

Our methods are exactly the same as those used by Riley *et al.*, but we express our results in units 10 times larger than theirs. (We define our unit as the activity reducing the optical density by 0.01 per minute per milliliter of plasma, and all results are expressed as average activity plus or minus the standard error of the mean, followed by the number of animals in parentheses.)

The plasma of normal mice of the four strains we have used (C57/BL, BR6, C3H, and A) has a lactic dehydrogenase activity of 50 ± 3(30); the activity is not affected by the strain or sex of the mouse. The lactic dehydrogenase activity in the plasma of mice bearing any sort of tumor tends to be high. (This is not unexpected since this enzyme activity is increased in a variety of diseased conditions.) In the case of a mammary adenocarcinoma that is transplantable into only a proportion of recipients, those in which the transplants are growing have an elevated enzyme activity in their plasma [138 ± 5(4)], and those in which it fails to grow have a normal activity [35 ± 6(4)]. Similarly, a mouse with a successfully transplanted lymphoma had an enormous lactic dehydrogenase activity in its plasma (1600) while mice in which transplantation failed had normal values [51 ± 11(3)].

Table 1. Lactic dehydrogenase activity in plasma of tumor-bearing mice and of mice injected with 0.1 ml of such plasma.

Donor mice		Enzyme activity in plasma	
No. and strain	Tumor	Donor mouse	Recipient mice
	Spontaneous tumors		
1 (BR6)	Mammary carcinoma	74	61 ± 5(8)
2 (C3H)	Mammary carcinoma	38	44 ± 9(5)
3 (AKR)	Leukemia	540	43 ± 2(3)
4 (AKR)	Leukemia	750	43 ± 7(2)
	*Transplanted tumors**		
5 (C57/BL)	Sarcoma B/BP	47	56 ± 21(2)
6 (A)	Mammary carcinoma (i/36)	78	80 ± 24(5)
7 (C57/BL)	Sarcoma MC48	220	220 ± 12(6)
8 (A)	Fibrosarcoma (SA/γ)	790	235 ± 15(2)
9 (C57/BL)	Sarcoma 37	350	305 ± 35(2)

* Originally spontaneous (i/36, Sa/γ, and 37) or chemically induced (B/BP and MC48).

Plasma (0.1 ml) from nine mice bearing various tumors (*2*) was injected intraperitoneally into normal mice. The lactic dehydrogenase activity in the plasma of the recipients was measured 2 days later. The results (Table 1) showed that the agent was clearly present in three mice (Nos. 7–9) bearing three sorts of transplanted sarcoma. The agent from one of these mice (No. 7) has been transmitted serially through seven passages; 2 days after injection of 0.1 ml of plasma, the lactic dehydrogenase activity in the plasma of each recipient was increased to 223–470. The activity in the plasma of the first recipient remained elevated for at least 1 month.

No agent was demonstrable in the plasma of the other six mice, and this was still so when higher volumes of plasma or cell-free extracts of the tumors were tested. These negative tumors were spontaneous mammary carcinomas (mice 1 and 2) or leukemia (mice 3 and 4) and transplanted sarcoma or carcinoma (mice 5 and 6).

If the difference between the positive and negative results is merely a quantitative one, then it must be enormous because active plasma is still fully effective after a 1000-fold reduction of

Table 2. Response to injection of diluted plasma from mice carrying agent derived from mouse 7 (Table 1).

Dose of plasma (ml)	Lactic dehydrogenase activity in plasma of recipient mice	
	Expt. 1	Expt. 2
0	34 ± 4(2)	
0.2	228 ± 24(5)	
0.1	222 ± 5(5)	293 ± 17(5)
0.05	288 ± 8(4)	
0.025	229 ± 10(5)	
0.0125	209 ± 23(4)	
0.01		280 ± 6(5)
0.001		298 ± 8(5)
0.0001		317 ± 20(5)