DOPA decarboxylase. It seems likely, therefore, that phenylalanine loading leads to alterations of a number of metabolic processes and that the decrease in central serotonin is an auxiliary phenomenon (*12*).

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References and Notes

1. C. M. B. Pare, M. Sandler, R. S. Stacey, *Lancet* **I**, 551 (1957).
2. ———, *ibid.* **II**, 1099 (1958).
3. A. N. Davison and M. Sandler, *Nature* **181**, 186 (1958).
4. H. Weil-Malherbe, *J. Mental Sci.* **101**, 735 (1955).
5. J. H. Fellman, *Proc. Soc. Exptl. Biol. Med.* **93**, 413 (1956).
6. R. T. Louttit, unpublished doctoral dissertation, department of psychology, University of Michigan.
7. Phenylalanine was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.
8. A detailed report on the behavioral studies is in preparation.
9. D. F. Bogdanski, A. Pletscher, B. B. Brodie, S. Undenfriend, *J. Pharmacol. Exptl. Therap.* **117**, 82 (1956).
10. A similar effect of phenylalanine diet on brain serotonin has recently been reported by H. L. Wang, V. R. Harwalker, H. A. Waisman, *Federation Proc.* **20**, 6 (1961).
11. S. M. Schanberg, C. A. McIlroy, N. J. Giarman, *Federation Proc.* **20**, 144 (1961).
12. This study was supported by a National Science Foundation grant (G-15600). We thank Linda Larsen for technical assistance.

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Calendar of Gametogenic Development in the Prepuberal Male Mouse

Abstract. The timing of the first meiotic cycle in the young male mouse was established by serial sacrifices and subsequent paraffin sectioning of testis and epididymis. Meiosis commences in mice 8 to 10 days old. The highest frequency of any one phase of meiosis was found at days 17 to 19, when 47 percent of the tubules were found to contain late pachytene.

In the course of an electron microscope study of meiosis in the mouse (*1*), it appeared desirable to obtain certain stages of meiosis in higher frequencies than those obtainable in the adult mouse (60 to 100 days). The tubular stages of the adult mouse show a random distribution, depending on the time required for their respective completion (*2*).

Hermann (*3*) and Clermont and Perey (*4*) have studied the development of the seminiferous epithelium of the mouse and rat, respectively. However, it appeared desirable to observe and record gametogenesis in the prepuberal mouse in order to establish a timetable of progressive development of the germinal cells similar to that established by Clermont and Perey in their studies of the rat. An experiment (*5*) was therefore undertaken for the specific purpose of determining the optimal prepuberal ages for obtaining the desired meiotic stages in high frequencies.

Table 1. Age of latest cell types observed in testicular tubules expressed as percent of the tubules counted. The average number of tubules counted for each age group is 108. Meiosis does not begin until day 8. Abbreviations: Lept., Leptotene; Zyg., zygotene; M-I, first meiotic division; M-II, second meiotic division.

Age of mice (days)	Gonia	Resting cytes	Lept.	Zyg.	Pachytene			M-I and M-II	Spermatids (1–8)*
					Early	Mid	Late		
8–10	80	7	13						
10–12	11.3	24.5	11.3	37.7	9.4	5.6	0		
15–17			3.9	13.7	22.9	28.5	30.8		
17–19		1	0	7	14.5	22.0	47.0	3.5	
22–24						2	30.0	12.0	36.6

* These numbers refer to the spermiogenic stage (*2, 4*).

Approximately 30 newborn male CF No. 1 mice were randomly selected from different litters. These were then placed in groups of five, with a lactating female, and allowed to grow until the time of sacrifice. Mice were killed at birth and at 3, 5, 8 to 10, 10 to 12, 15 to 17, 17 to 19, 22 to 24, 29 to 31, 36 to 38, and 43 to 45 days. The testes and epididymides were immediately excised and fixed in Carnoy or in Zenker-formol. After embedding in paraffin, the tissues were sectioned at 7 μ and stained with Feulgen-fast-green or periodic acid Schiff.

We are in agreement with the results stated by Clermont and Perey (*4*) that in the prepuberal state, the supporting cells give rise to Sertoli cells and the gonocytes constitute the germinal stem line.

In newborn (0 to 1 day) mice cross sections of the testis measure about 1 mm. The tubules are widely spaced; they are nonconvoluted and do not possess lumina. The tubular cross sections had an average diameter of 40 to 50 μ. The testis cross sections contained from 20 to 30 tubules. The tubular content corresponds to that described for the newborn rat by Clermont and Perey (*4*). Throughout the development of meiotic stages the mouse is approximately 2 days ahead of the rat.

At 3 days some gonocytes are positioned on the basement membrane and about 10 percent of them are in some phase of mitosis. As the number of supporting cells seems to be diminishing, we expected to find occasional ones in the epididymis. We did. The epididymis also contained those gonocytes that did not become aligned on the basement membrane.

At 5 days there is an increase in the number of tubular cross sections, approaching 200. Within each tubule the spermatogenic descendants of the gonocytes number up to a dozen; 50 percent of these are in active mitosis, and the remainder appear to be resting.

At 8 to 10 days the beginning of meiosis is apparent for the first time. Thirteen percent of the tubules counted contained spermatocytes in leptotene. The spermatogonia are approaching the adult pattern of the A, Intermediate, and B types. It appears that the earliest transition from spermatogonia to spermatocytes is abrupt, thus shortening those stages known in the adult as type B spermatogonia and resting spermatocytes. Thus the intermediate spermatogonial cells appear to progress directly into leptotene soon after telophase.

Tables 1 and 2 show the observations on the mice from 10 to 12 days through 6 weeks. The tables show that at 10

Table 2. Percentage of tubular sections in the various stages of the cycle. The number of tubules in each case is 100.

Age of mice (days)	Percentage in					
	I*	II–III	IV–VI	VII–VIII	IX	X–XII
29–31	12	18	36	9	8	13
36–38	6	19	21	26	10	18
43–45	14	15	10	29	16	16
100	18	7	4	23	23	25

* These numbers refer to the spermatogenic stage (*2, 4*).

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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References and Notes

1. B. R. Nebel and E. M. Hackett, *Nature* **190**, 467 (1961).
2. E. F. Oakberg, *Am. J. Anat.* **99**, 507 (1956); *Nature* **180**, 1137 (1957).
3. F. Hermann, *Arch. mikroskop. Anat. u. Entwicklungsmech.* **34**, 429 (1889).
4. Y. Clermont and B. Perey, *Am. J. Anat.* **100**, 241 (1957).
5. This work was performed under the auspices of the U.S. Atomic Energy Commission.
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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