rejection delayed in the treated animals, but the period of rejection was much prolonged. The grafts were less edematous and hemorrhagic than controls. A scaling or crusting of the surface epithelium was frequently noted. Some grafts showed beginning signs of rejection and then apparently recovered. The homografts on these animals would sometimes be rejected but on occasion would survive for long periods of time.

In a test on the importance of the time of treatment with promethazine, control homografted animals were compared with three groups of homografted rabbits (Table 2) which differed in the time of starting and duration of treatment.

The control grafts were rejected in the usual manner. The group receiving delayed treatment was not significantly different from the controls. Treatment 3 days before grafting and 4 days after grafting did not cause significant prolongation of homograft survival. Treatment 3 days before and 16 days after grafting did show significant prolongation of homograft survival. A striking observation was the rapid rejection process which developed soon after the treatment was stopped.

In a test of the protective action of promethazine on second-set homografts, 24 rabbits received ear-skin homografts on the ear skin, and these were allowed to be rejected. These grafts were rejected normally between the 7th and 11th day after grafting. Twelve of these 24 animals received a second graft 11 days after the primary grafting; six were treated with promethazine 3 days before grafting and until the grafts were rejected, and six served as controls. The other 12 animals received a second graft 17 days after the primary grafting. Six served as controls and six were treated with promethazine 1 day before grafting and until the graft was rejected or until 16 days after the second graft (Table 3).

The second-set grafts on both control groups were rejected promptly and significantly sooner than the primary grafts.

In the treated animals the rejection of the second-set homografts was significantly delayed.

The prolongation of survival of primary and second-set homografts by treatment with phenothiazine derivatives cannot easily be attributed to a single specific action out of the multiple actions of these drugs (*11*). Phenothiazine derivatives antagonize histamine (*12*); maintain capillary integrity (*13*); protect cell, lysosome, and mitochondrial membranes (*2, 3, 14*); and prevent cell death and necrosis (*3*). In addition to preventing some immunological phenomena (*4–6*), they also decrease leucocyte phagocytosis (*15*), protein synthesis (*16, 17*), and serum levels of complement (*15*) and γ-globulin (*16*).

We see the protective action in our experiments as the result of a series of connected events. Initially the preservation of cell, lysosome, and mitochondrial membranes diminishes the release of life-sustaining and antigenic substances from the newly grafted foreign skin. The decreased amount of antigen released provides a less intense immunologic stimulus than is present in the control animals. The immunologically competent cells exposed to the antigens may react more slowly or less vigorously in phagocytosis and degradation of the antigens or in synthesis antibody. The phenothiazines may interfere with antibody attachment to the homografted cells. Finally, after antibody reacts with the homografted cells, the phenothiazines may again protect the cells, lysosomal and mitochondrial membranes delaying or preventing the otherwise irreversible cell damage and homograft rejection. Possibly, if drug and dosage are optimum to protect the graft sufficiently during the critical periods of antigen loss from the graft and immunologic assault by the host, permanent takes of homografted skin (and organs) may be obtained without the use of traditional immunosuppressive drugs.

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Plant Growth Retardant B-995: A Possible Mode of Action

Abstract. *Inhibition of shoot elongation in dwarf and tall peas by the 1,1-dimethylhydrazide of succinic acid (B-995) was correlated with the inhibition of the oxidation of tryptamine-2-C^{14} to indoleacetaldehyde-2-C^{14} in homogenates prepared from epicotyls of young plants treated with B-995. The growth-retarding action of B-995 is attributed to the formation of 1,1-dimethylhydrazine in vivo. This hydrazine strongly inhibited tryptamine oxidation by pea epicotyl homogenates.*

In numerous species of angiosperms, shoot growth is strongly inhibited by the 1,1-dimethylhydrazides of succinic and maleic acids, designated as B-995 and C-011, respectively, and by the structurally related beta-hydroxyethylhydrazine (BOH) (*1*). These chemicals, plus a number of other struc-

Table 1. Effects of B-995 on shoot elongation in peas (*Pisum sativum* L.) of four varieties. Scarified, surface-disinfected seeds were soaked in aqueous $10^{-2}M$ B-995 solutions or in distilled water for 12 hours in darkness at 17° to 20°C before they were planted in vermiculite in a greenhouse. Shoot heights, cotyledonary node to shoot apex, were measured when the plants were 21 days old. Data are based on 20 to 44 plants per group and are expressed as the means plus or minus the standard errors of the means.

Variety	Shoot height (cm)		Inhibition (%)
	Control	B-995	
Tall pea varieties			
Tall Telephone	20.9±0.72	7.3±0.46	65
Alaska	18.3±0.71	7.7±0.34	58
Dwarf pea varieties			
Dwarf Telephone	7.8±0.35	5.8±0.28	26
Little Marvel	6.4±0.23	5.1±0.19	20

turally unrelated growth retardants, have become effective tools in investigations of the biosynthesis and modes of action of auxin and gibberellins.

The inhibition of shoot elongation in many different plant species has led to speculation that B-995, as well as other growth retardants, may interfere with the metabolism or regulatory action of auxin or gibberellin. However, B-995 failed to inhibit gibberellin biosynthesis in *Fusarium moniliforme* (*2*), and also did not block the gibberellin-induced release of reducing sugar from barley endosperm (*3*). Dahlgren and Simmerman (*4*) suggested that C-011 may retard plant growth because of the formation of 1,1-dimethylhydrazine (UDMH) and 1,1-dimethylhydrazinium hydrogen maleate. Both compounds are reported to be produced by the intramolecularly catalyzed decomposition of the growth retardant in aqueous solutions. 1,1-Dimethylhydrazine inhibits diamine oxidase in animals (*5*). One of us (D.J.R.) showed previously that β-hydroxyethylhydrazine inhibits tryptamine oxidation in pea-seedling homogenates, and suggested that B-995 may affect auxin concentrations in vivo by inhibiting the oxidation of tryptamine by diamine oxidase (*6*).

Pea plants of four varieties were grown from scarified seeds which had been soaked in aqueous solutions of B-995 for 12 hours before they were planted. Alternatively, in certain experiments, B-995 in 0.05 percent Tween 20 (polyoxyethylene sorbitan monolaurate) was applied to the shoot tips of 9-day-old Alaska and Little Marvel peas at doses of 400 μg per plant. Shoot elongation was markedly inhibited in the plants grown from seeds soaked in $10^{-2}M$ B-995 (Table 1); only slight inhibition was obtained with $10^{-3}M$ solutions, and $10^{-1}M$ solutions were lethal to the majority of treated seeds. In each of the four different varieties, the degree of inhibition of shoot elongation was directly proportional to the relative rate of shoot elongation in that variety. The Tall Telephone and Alaska varieties were inhibited by two or three times as much as the dwarf varieties Little Marvel and Dwarf Telephone. Shoot elongation was also markedly inhibited by applications of B-995 to the shoot tips of seedlings. Dry weight of shoots was not affected greatly by any method of B-995 treatment.

To test the possibility that inhibition of shoot elongation by B-995 might result from inhibition of diamine oxidase, the activity of this enzyme in homogenates of normal and treated pea plants was measured. Scarified, surface-disinfected seeds of Alaska and Little Marvel peas were soaked either in $10^{-2}M$ B-995 or in distilled water for 12 hours in darkness. The seeds were then planted in sterilized vermiculite in a growth chamber programmed to provide 16 hours of light at 20° ± 1°C (light intensity, 7800 lux) and 8 hours of darkness at 17° ± 1°C. These environmental conditions closely approximated those for the greenhouse experiments (Table 1). Groups of 20 epicotyls each of control and treated seedlings of both varieties were excised at the cotyledonary node at intervals of 54, 88, and 163 hours after the beginning of the 12-hour treatment period. Ten epicotyls of each group were dried at 80°C for 24 hours; the remaining 10 epicotyls were weighed and assayed immediately for diamine oxidase activity as follows. Epicotyls (3 to 8 mg fresh weight per milliliter of buffer) were homogenized in borate-phosphate buffer (0.033*M* each) at *p*H 8.1 (*6*) and centrifuged to remove cellular debris. The supernatant was used immediately in the assay. Diamine oxidase activity, represented by the oxidation of tryptamine-2-C^{14} (New England Nuclear Corp.) to indoleacetaldehyde-2-C^{14}, was assayed essentially by the method described by Wurtman and Axelrod (*7*) for animal monoamine oxidase. The oxidation of tryptamine-2-C^{14} to indoleacetaldehyde-2-C^{14} was allowed to proceed for 30 minutes, after which time the reaction was stopped by the addition of 2*N* HCl and the indoleacetaldehyde-2-C^{14} formed was extracted in toluene. After centrifugation, the radioactivity in a sample of the toluene extract was counted in a liquid scintillation spectrometer. Less than 0.05 percent of the remaining tryptamine-2-C^{14} was extracted by toluene.

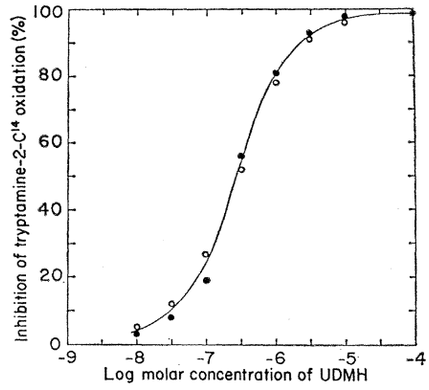

Fig. 1. The effect of UDMH on the rate of tryptamine-2-C^{14} oxidation by pea epicotyl homogenates. Each assay mixture consisted of 50 mμmole of tryptamine-2-C^{14} (233,000 dpm) in 0.1 ml borate-phosphate buffer, UDMH in 0.1 ml buffer, 0.1 ml enzyme preparation and buffer to make a total volume of 0.4 ml. The assay mixtures were incubated immediately after addition of the enzyme preparation for 30 minutes at 25.5° ± 0.5°C. Data were corrected for blank values obtained by incubating tryptamine-2-C^{14} with boiled enzyme. Open and solid circles denote average values for two replicate experiments.

Table 2. Comparative rates of tryptamine-2-C^{14} oxidation by homogenates of pea epicotyls excised from control and B-995–treated seedlings of two varieties. Each value represents the average of four replicate assays. Maximum deviation from the mean values was ± 0.3 mμmole. Each assay mixture consisted of 50 mμmole of tryptamine-2-C^{14} [233,000 disintegrations per minute (dpm)] in 0.1 ml borate-phosphate buffer, 0.05 or 0.10 ml enzyme preparation, and buffer to make a total volume of 0.4 ml; the assay mixtures were incubated immediately after addition of the enzyme preparation for 30 minutes at 25.5° ± 0.5°C. Data were corrected for blank values obtained by incubating tryptamine-2-C^{14} with boiled enzyme.

Age of seedlings (hr)	Tryptamine-2-C^{14} converted (mμmole/30 minutes)			
	Per epicotyl		Per mg dry wt	
	Control	B-995	Control	B-995
Alaska				
88	15.6	7.7	18.2	9.6
163	60.8	34.6	18.4	13.5
Little Marvel				
88	6.9	2.4	7.5	3.1
163	39.9	21.2	13.2	8.5

The amine oxidase reaction was linear with enzyme concentration over a range of 0 to 0.20 ml of enzyme preparation.

The rate of tryptamine-2-C^{14} oxidation by homogenates prepared from epicotyls of Alaska (tall) and Little Marvel (dwarf) peas was reduced 40 to 65 percent (Table 2). The inhibition for treated Alaska pea epicotyls was 51 percent at 88 hours and 43 percent at 163 hours. A similar comparison of Little Marvel control and treated pea epicotyls showed 65 percent and 47 percent inhibition of the rate of tryptamine-2-C^{14} oxidation at 88 and 163 hours, respectively. Practically no tryptamine-2-C^{14} oxidation (less than 3 percent of the 88-hour-old epicotyls) was detectable in epicotyl homogenates of control or treated peas at the 54-hour stage of development. The rate of tryptamine-2-C^{14} oxidation was substantially higher in homogenates from tall pea epicotyls than in similar enzyme preparations of dwarf epicotyls at both the 88- and 163-hour stages. Similar experiments were performed with Alaska and Little Marvel peas which had been treated when 9 days old by applications of 400 μg of B-995 per plant to the shoot apices. A marked effect of B-995 on the capacity of shoot tips to oxidize tryptamine-2-C^{14} was revealed when the shoot tips were assayed 8 days after application of B-995.

The effect of UDMH on the rate of tryptamine-2-C^{14} oxidation by homogenates of control Alaska pea epicotyls 88 hours old was investigated by using a range of concentrations from 1×10^{-8} to $1 \times 10^{-4}M$ UDMH. A concentration of approximately $3.3 \times 10^{-7}M$ of UDMH resulted in 50-percent inhibition of the rate of tryptamine-2-C^{14} oxidation (Fig. 1). Thus, hydrolysis of less than 0.1 percent of the administered B-995 at the hydrazide group could produce a level of UDMH adequate to cause a 50-percent inhibition of tryptamine oxidation. Work by Martin *et al.* (*8*) with C^{14}-labeled B-995 indicated that the growth retardant underwent a slow decomposition in apple seedlings. Clarke and Mann (*9*) established the oxidation of tryptamine to indoleacetaldehyde by diamine oxidase purified from pea seedlings. Therefore, we assume that tryptamine oxidation in our experiments occurred via diamine oxidase.

We conclude that the inhibition of shoot elongation in peas by B-995 can be correlated with the inhibition of tryptamine oxidation by way of diamine oxidase.

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Hereditary Absence of Sebaceous Glands in the Mouse

Abstract. *An autosomal recessive mutation, characterized by an absence of sebaceous glands, and by hyperkeratosis, alopecia, and single (rather than the usual multiple) hair-follicle units, has occurred spontaneously in the BALB/c strain of mouse. Studies in which reciprocal transplantations of skin were made between normal and mutant mice suggest that some diffusible substance(s) synthesized by normal skin can stimulate hair growth and alleviate the hyperkeratosis characteristic of the skin syndrome.*

Disturbances of the keratinization process constitute an important group of skin diseases of man, and in many cases the disorders are known to be genetically controlled (*1*). Mutations that affect the skin, hair, or both, also occur in laboratory animals. However, such mutations, particularly those affecting the keratinization process, are not common, and descriptions of the disorders have been limited to morphology (*2*). The role of skin appendages, such as the sebaceous gland, in maintaining or promoting keratinization is not known. We were therefore interested in the spontaneous appearance, in an inbred strain of mice, of a previously undescribed mutation in which the animals affected were characterized by the complete absence of sebaceous glands. In this report we describe the physiology and dermatopathology of the condition during the life of an affected animal and present evidence for the mode of inheritance.

The mutant mice originated from a sibmated colony of strain BALB/cCrglGa (albino) maintained by one of us (A.H.G.). The spontaneous mutation initially appeared at F_{74} in litters from two different pairs of parents which were two generations removed from a common ancestor. Test matings provided unequivocal evidence (Table 1) that the condition is controlled by a single (autosomal) recessive gene with complete penetrance. The gene has been named *asebia* (without sebum) and the letters *ab* have been adopted provisionally to symbolize the mutation (*3*). Brother-to-sister matings, with forced heterozygosity of the gene, have been continued since initial discovery of the mutation. The subline has now been maintained for six generations and will be designated BALB/cGa-+*ab*.

Normal (heterozygous) and mutant mice in infancy and adulthood are illustrated in Fig. 1, *A* and *B*. The condition may be recognized as early as

Table 1. Number of mutants lacking sebaceous glands and total offspring from various matings. Proportion of mutants observed and expected (based on the hypothesis that a single recessive gene is responsible for the condition).

Type of mating	No. of offspring		Proportion of mutants		χ^2	p
	Mutant	Total	Obs.	Exp.		
Mutant × mutant	45	45	1.00	1.00		
Mutant × normal (F_1)	0	87	0.00	0.00		
Carrier × carrier (F_2)	37	122	.30	.25	1.8	>.1
Carrier × mutant (backcross)	88	176	.50	.50		