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6. All burnsi and kandiyohi adults utilized as parents in previous crosses (*3–5*) were also heterozygous. No homozygous adults of these mutant types have been uncovered in breeding tests.
7. This research was supported by a grant from the National Science Foundation (NSF-G3332).
8. We assume that there is no differential mortality among the larvae. In Anderson's kandiyohi ♀ × *pipiens* ♂ and kandiyohi ♀ × burnsi ♂ crosses, 28 percent (78 of 280) and 38 percent (92 of 239), respectively, of the larvae died prior to metamorphosis. In Volpe's kandiyohi ♀ × burnsi ♂ and burnsi ♀ × kandiyohi ♂ crosses, 33 percent (24 of 74) and 48 percent (28 of 58), respectively, of the tadpoles failed to transform.
9. Professional frog dealers in the midwest estimate (a rough approximation at best) that the kandiyohi and burnsi variants each comprise 1 percent of leopard frog populations (*3–5*).
10. D. J. Merrell at the University of Minnesota is presently engaged in a study of the mutant populations in the Minnesota-Dakotas area (personal communication).

19 December 1957

Apparent Movement of Southern Bean Mosaic Virus across Steamed Areas of Bean Stems

In 1933 Grainger (*1*) reported that tobacco mosaic virus moved across steamed areas in stems and leaves. His results conflicted, however, with those of other investigators (*2*), who also had worked with strains of tobacco mosaic virus. The view based on these papers (*2*) was that mosaic viruses do not pass steam-killed sections of stems (unless they are deliberately introduced into tracheary elements) because normally these viruses are unable to pass either into or out of tracheary elements (*3*). Apparently no evidence contrary to this latter view has since been reported either for mosaic viruses or for phloem-limited viruses (*4*), but such evidence has been reported for a xylem-limited virus (*5*).

Our study of the movement of southern bean mosaic virus (SBMV) indicates that this virus, or some part of it capable of initiating infection, can pass into, through, and out of steamed portions of Pinto bean (*Phaseolus vulgaris* L.) stems. In these steamed portions, cells that possess dehydrogenase activity or the capacity of cell division, two activities associated with living cells, could not be detected. Because evidence for this pathway of movement of presumably large particles may interest biologists in various fields, the results obtained are summarized here.

The experimental method followed is based on the fact that when SBMV is introduced into a systemic host (Black Valentine bean) previously grafted to a local-lesion host (Pinto bean) a systemic necrotic reaction associated with multiplication of the virus occurs in the latter bean (*6*). This procedure minimized the possibility of spread of virus above a steamed region unless the primary infections arose from particles that passed through the steamed area, because a systemic spread of virus does not occur with superficial inoculation of Pinto in any region (*6, 7*).

In our experimental procedure each Black Valentine bean plant, which had been previously approach-grafted between the primary leaf node and the soil line region to the same region of Pinto, was mechanically inoculated on trifoliate foliage with SBMV (Fig. 1). A cellophane barrier (not shown in Fig. 1) prevented contact between Black Valentine and Pinto plants. Inoculation was made 24 to 48 hours after a portion of the internode (an inch or more in length) above the primary leaves of Pinto was steamed. Plants thus treated are hereafter designated "grafted-steamed." Each control consisted of an inoculated Black Valentine plant tied, instead of grafted, to an adjacent Pinto. (This combination is hereafter designated "nongrafted control"). In some experiments an additional type of control, Black Valentine grafted to a Pinto (designated "grafted control"), was included. This control indicated whether virus was accidentally introduced into Black Valentine and passed the section of a stem to be steamed after a graft union was established, but before the application of steam. The Black Valentine plant of the grafted control was severed above the graft union and removed about 3 hours after inoculation. An added precaution used to eliminate the possibility of insect introduction of the virus was weekly fumigation and spraying.

Assays, made by a local lesion method (*8*), of two or three samples from tissue four and more nodes above the primary leaf node from each Pinto were used to detect the presence of SBMV (Table 1). The number of lesions resulting from the assay of samples from grafted-steamed plants (38 positive cases) ranged from 0 to 450 per leaf with a mean of 60. The number of lesions resulting from the assay of samples from control plants (4 positive cases) ranged from 0 to 4 with a mean of 0.8. The purpose of the large number of controls (three times the number of grafted-steamed plants) used in the first two experiments was to increase the probability of detection of virus that might have occurred above steamed sections in Pinto without particles having moved through the graft union.

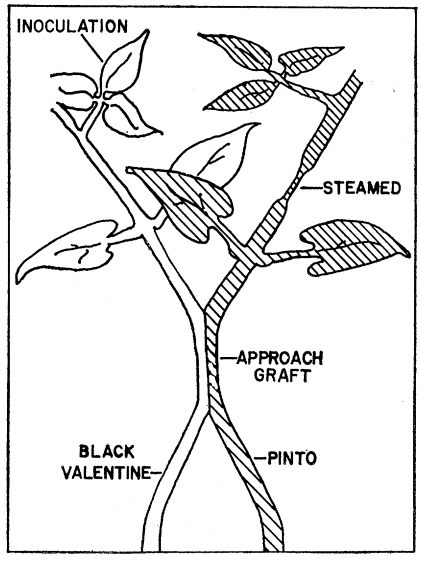

Fig. 1. The relation between the region of inoculation of southern bean mosaic virus and the steamed section in grafted bean plants.

Table 1. Detection of southern bean mosaic virus in Pinto bean plants. The numerator represents total number of Pinto plants in which virus was detected; the denominator represents the total number of plants assayed.

Expt. No.	Grafted-steamed	Non-grafted control	Grafted control
1	12/12	0/36	
2	6/12	1/36	
3	9/11	0/8	0/9
4	11/14	1/10	2/13

In addition to the detection of virus by assay of random samples, necrotic areas were noted above steamed sections in Pinto; these areas contained virus. Such necrotic areas occurred in 18 of the 38 plants in which virus was detected above the steamed portions of stems. The necrotic areas indicate that particles, after passing through steamed sections of stems, reached a cellular environment that supported multiplication. Necrotic symptoms never occurred in the control plants (112 plants).

The source of contamination (Table 1) that would explain the detection of virus in 4 out of 112 control plants is not known. The low virus level and low frequency of this contamination, however, do not account for the frequent high level of virus and the characteristic systemic necrotic symptoms that resulted only on the Pintos of grafted-steamed plants above steamed portions of stems. These symptoms occurred 12 to 36 inches above steamed portions in most cases. As a previous statement in this paper indicates, such a spread of SBMV would not occur from an accidental contamination since this is equivalent to a superficial inoculation of Pinto (*6, 7*).

The steamed sections were studied in

the following ways to determine whether they contained living cells that might support multiplication of SBMV: (i) by determining whether any cells in steamed areas retained their ability to proliferate after steam treatment; (ii) by testing for dehydrogenase activity in the steamed areas.

Cell division occurred in control Pinto stems when tissues external to the xylem were removed. Extensive proliferation was apparent within 8 days after the tissues had been removed. Cell proliferation disturbed the regular alignment of vascular bundles and forced some bundles outward. The hollow core of the pith became filled with cells as a result of this proliferation. In contrast, microscopic examination of the steamed sections of stems 2 weeks after the steam was applied showed no evidence of cell proliferation, and the central core of the pith remained unfilled.

2,3,5-Triphenyl tetrazolium chloride (TTC) was used to detect dehydrogenase activity (*9*) in steamed regions. Ten pieces of stems from an equal number of plants were cut from areas immediately below the steamed regions and within the same internode. These control samples showed a definite reaction to TTC (development of red color) in less than 1 hour at 32°C in the dark. The color was apparent in 0.2-mm sections of these stem pieces in cells of the epidermis, cortex, phloem, xylem, and pith. In contrast, no color was detected macroscopically in pieces of the steamed regions even after 24 hours in TTC (at 32°C in the dark). In addition, no color was detected microscopically in sections 1 mm or more thick taken from the ten different steamed stems.

From these results it appears that SBMV, or some part of the virus capable of initiating infection, was able to pass from living cells through steamed portions of stems of Pinto bean plants. Subsequently, at least some of these particles moved out of the steamed areas into an environment above the steamed sections that supported virus multiplication. The particles passed through a section of stem in which living cells were not detected.

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3 December 1957

Effect of Norleucine on the Utilization of D-Leucine by the Rat

Utilization of D-leucine for growth by the rat was recently demonstrated in this laboratory (*1*). This finding is contrary to the experiments of Fierke (*2*) and Rose (*3*). The study described in this report (*4*) was undertaken in an attempt to clarify the apparent discrepancy.

In reading over the experimental procedure used by Fierke (*2*), we noted that a basal diet was used which contained 2 percent of DL-norleucine. There were also described experiments in which it was shown that norleucine is dispensable for the growth of rats, and, moreover, it was found that either of its isomers as well as the racemic form is toxic to them.

In view of these facts it occurred to us that the failure of Fierke and Rose to observe any beneficial effect of D-leucine when it was added to a leucine-free diet was due to the presence of norleucine. The structure of this compound closely resembles that of leucine, and it seems possible that it might act as an antagonist of leucine. If this were true, one would expect a lower utilization of leucine in the presence of norleucine.

This hypothesis was tested in the following way. Sixteen weanling albino rats of the Yale strain were placed on a nitrogen-free diet for a period of 2 weeks. At the end of this protein-depletion period, the animals were divided into four groups of four animals each, which were then fed appropriate diets for 12 days. The composition of the basal diet and the amino acid mixture has been described elsewhere (*1*). The experimental rations included nitrogen-free and leucine-free diets, and the latter diet supplemented with D-leucine alone or with leucine and DL-norleucine.

The results of these experiments are shown in Fig. 1. Protein-depleted rats fed the nitrogen-free and leucine-free diets lost an average of 4 g and 2 g of weight, respectively, during a 12-day experimental period. Diets supplemented with 0.85 percent of D-leucine resulted in an average growth response of 23 g, while the inclusion of 2 percent of DL-norleucine in the same diet resulted in a loss of weight of 1 g. These rats started to gain in weight when the content of D-leucine in the diet was increased.

Growth inhibition, caused by the inclusion of norleucine in the diet, was always accompanied by anorexia and low water consumption. The rats on such a regimen consumed an average of 36 g of food and drank 63 ml of water, whereas those on a similar diet which was devoid of norleucine ate 75 g of food and drank 130 ml of water. Food efficiency (grams of gain per gram of food) and nitrogen efficiency (grams of gain per gram of food nitrogen) were also markedly affected by the addition of norleucine to the diet, as was evidenced from the average figures of −0.03 and −1.0, respectively. The comparable figures for the control group were 0.31 and 16.0. Feeding of higher levels of D-leucine, which brought about the reversal of growth inhibition observed in animals on the former diet, also resulted in an increase in the rats' desire for food and water and a rise in food and nitrogen efficiency ratios.

These experiments confirm our hypothesis—that the inclusion of norleucine in the diet will greatly reduce the utilization of D-leucine by rats. This appears to explain why Fierke and Rose failed to observe any stimulatory effect of D-leucine.

Prior to our study, an antagonism between norleucine and leucine as well as between norleucine and other amino acids had been observed in bacteria (*5*). But the study discussed in the present report is believed to be the first demon-

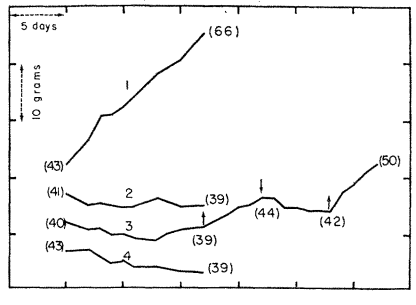

Fig. 1. Average growth response of weanling protein-depleted rats to D-leucine in the presence and absence of norleucine. The numbers in parentheses denote the average initial and final weights of four rats. Curve 1, leucine-free diet plus 0.85 percent D-leucine. Curve 2, leucine-free diet. Curve 3, leucine-free diet plus 0.85 percent D-leucine plus 2 percent DL-norleucine. The two solid arrows pointing to the top of the graph indicate points at which the percentage of D-leucine was increased from 0.85 to 1.60 and 2.20, respectively, while the solid arrow pointing to the bottom of the graph indicates the point at which the dietary level of D-leucine was decreased to that at the beginning of the experiment (0.85 percent). Curve 4, nitrogen-free basal diet.