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.

I. R. SCHNEIDER
J. F. WORLEY
Crops Research Division, Agricultural Research Service, Plant Industry Station, Beltsville, Maryland

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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-

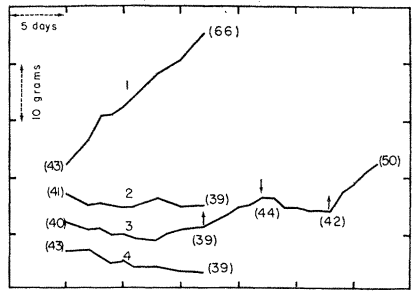

Fig. 1. Average growth response of weanling protein-depleted rats to D-leucine in the presence and absence of norleucine. The numbers in parentheses denote the average initial and final weights of four rats. Curve 1, leucine-free diet plus 0.85 percent D-leucine. Curve 2, leucine-free diet. Curve 3, 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.

stration of an antagonism between norleucine and leucine in mammals.

The mechanism of norleucine toxicity and its reversal by leucine cannot be explained at present. The growth inhibition caused by the inclusion of amino acid analogs in the diet has been frequently explained as resulting from the inhibition of protein synthesis; more recently it has been attributed to the formation of "foreign" protein, in which the particular amino acid is replaced by its analog. Indirect evidence for the latter possibility in our system is given by the recent finding that the administration, by intravenous injection, of DL-norleucine-3-C^{14} to cows resulted in the incorporation of this amino acid into casein (*6*). Norleucine does not seem to be a natural constituent of casein or of other proteins that have been investigated to date.

M. Rechcigl, Jr.
J. K. Loosli, H. H. Williams
Departments of Animal Husbandry and Biochemistry and Nutrition, Cornell University, Ithaca, New York

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Low-Angle X-ray Diffraction of Fibrous Polyethylene

The low-angle x-ray diffraction of a crystalline and highly oriented fiber of linear polyethylene has been studied (*1*). The high axial orientation possessed by these fibers is shown by the wide-angle diffraction pattern, which is similar to that of a well-developed single crystal with rotational symmetry about the fiber axis. The amorphous scatter was weak in this fiber, and the unit cell was verified to be orthorhombic with dimensions identical with those given by Bunn (*2*). Furthermore, the *c* axis—that is, the chain direction—is coincident with the fiber axis. It was also noted that, when a fiber is heated above its melting temperature and then cooled to room temperature, an axial contraction of about 40-fold occurred. This observation is a further indication of the high orientation in the sample. It is doubtful whether the properties of a synthetic macromolecule possessing this degree of axial orientation have been studied heretofore.

The low-angle camera, described elsewhere (*3*) was capable of resolving spacings up to 800 A. The specimen consisted of a fiber bundle of optimum thickness for CuK_α radiation. A well-defined, low-angle pattern, limited to meridional reflections, was observed (Fig. 1). The first-, second-, and fourth-order reflections of a long period corresponding to $d = 410 \pm 20$ A are clearly resolved. A photometry trace showing the resolution between the first two orders is shown in Fig. 2. The second-order reflection is of greater intensity than the first order, and the third-order reflection is missing.

Since equatorial and other non-meridional reflections were absent, experiments were undertaken to determine whether the fiber acted as a one-dimensional diffractor. These experiments involved the tilting of the fiber axis relative to the x-ray beam. Although diffraction persisted with angles of tilt up to 40°, the diffraction orders were poorly defined, thus making any quantitative deductions from these experiments difficult. Nevertheless, one can conclude that this highly oriented polyethylene fiber acts as a one-dimensional diffractor, its reciprocal lattice consisting of discs of large diameter which increase with the order of the diffraction.

Low-angle diffraction patterns have been observed previously in both the fibrous proteins (*4*) and in mechanically oriented synthetic polymers (*5*, *6*). The fibrous proteins usually exhibit a series of meridional low-angle reflections, as many as 30 orders having been reported for native collagen (*4*). However, the synthetic fibers previously studied displayed only a single diffuse diffraction maximum corresponding to a much smaller spacing than that reported above. Our observation that several meridional diffraction orders can be obtained from a highly oriented fiber with a principal spacing of 410 A indicates that a well-defined periodicity along the chain direction can also be developed in these substances.

In the case of collagen the low-angle diffraction pattern can be accounted for by a cylindrical band and interband model (*4*, *7*). As was suggested by Hess and Kiessig (*5*) a similar interpretation for the periodicity of the fibrous polyethylene can be made. The crystalline regions correspond to the interband and the amorphous regions to the band, with the high orientation causing these regions to approach colinearity. The amorphous regions in polyethylene occur as a consequence of the kinetic difficulties of completely crystallizing a long-chain molecule even though it may be composed of identical repeating units. On the other hand, in the fibrous proteins the chain repeating units (that is, amino acid residues) are not all identical so that a periodicity can also be developed as a consequence of chemical and structural differences along the chain. For synthetic polymers a high degree of orientation must be developed if more than one diffraction maximum is to be observed at low angles.

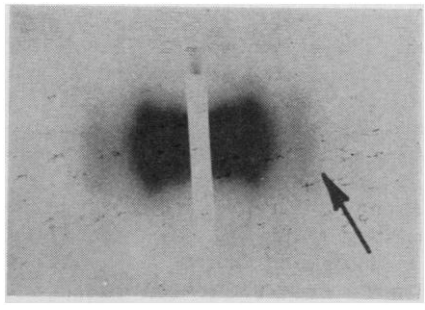

Fig. 1. Low-angle diffraction pattern of polyethylene. Copper K_α, nickel-filtered radiation; specimen-to-film distance, 22.5 cm. The arrow indicates the position of the fourth order of reflection.

Fig. 2. Photometer trace of low-angle pattern of polyethylene (less exposure time than in the example shown in Fig. 1).

Leo Mandelkern
C. R. Worthington, A. S. Posner
National Bureau of Standards, Washington, D.C.

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