the heated soy is ingested but, under such circumstances, better utilization of this as well as the other amino acids. More recently, Evans (*6*) reported no correlation between the "digestible" organic sulfur content of soy protein and its nutritional value.

The present studies, conducted with soy protein, have been extended to oats, milk, and other proteins and have yielded similar data indicating that the rate of release of individual amino acids during enzymic digestion can readily account for differences in the biological value of the proteins. However, some precautions must be exercised before rating a sample with maximal digestibility as the most acceptable. For example, excessive heat processing of soybean meal can lead to destruction of some amino acids, so that the biological value becomes the resultant of two factors: (1) the improved digestibility of the protein, leading to greater retention of the absorbed nitrogen, and (2) the imbalance in essential amino acid composition, responsible for a lowering of the biological value of the protein.

On the basis of the present findings it might seem that protein hydrolysates should be superior in biological value to the original intact proteins. In the case of the former, the amino acids would be liberated *in vivo* very rapidly and therefore be available for absorption almost as a group. However, the recent report by Woolley (*13*) indicates that there may be one or more essential peptides which the animal organism is unable to synthesize. It was found that the omission of the factor, strepogenin (presumably a peptide), from a dietary containing a hydrolysate which furnished all of the essential amino acids in satisfactory quantities was responsible for the poor biological response. Thus, in addition to a proper balance of the essential amino acids, it may be necessary to insure certain peptide linkages in the dietary for satisfactory utilization of amino acid nitrogen.

Addendum: The report by Ham and associates (*7*), which appeared while this paper was in press, suggests that the effect of heat on a proteolytic inhibitor in soybean is responsible for the differences in susceptibility of soy protein to enzymic digestion. However, the impairment in utilization of the protein derived from raw or improperly heat-processed meals must be attributed to an inhibition of the *rate* of methionine liberation for mutual amino acid supplementation and not to a reduced *degree* of methionine availability as postulated by Ham and collaborators.

References

1. Albanese, A. A., Frankston, J. E., and Irby, V. *J. biol. Chem.*, 1944, **156**, 293.
2. Almquist, H. J., Macchi, E., Kratzer, F. H., and Grau, C. R. *J. Nutrition*, 1942, **24**, 385.
3. Block, R. J., and Bolling, D. *The amino acid composition of proteins and foods.* Springfield, Ill.: Charles C. Thomas, 1945; and personal communications from Dr. R. J. Block.
4. Dunn, M. S., Camien, M. N., Shankman, S., Frankl, W., and Rockland, L. B. *J. biol. Chem.*, 1944, **156**, 715.
5. Elman, R. *Proc. Soc. exp. Biol. Med.*, 1939, **40**, 484.
6. Evans, R. J. *Arch. Biochem.*, 1945, **7**, 33.
7. Ham, W. E., Sandstedt, R. M., and Mussehl, F. E. *J. biol. Chem.*, 1945, **161**, 635.
8. Hayward, J. W., and Hafner, F. H. *Poultry Sci.*, 1941, **20**, 139.
9. Johnson, L. M., Parsons, H. T., and Steenbock, H. *J. Nutrition*, 1939, **18**, 423.
10. McCarthy, T. E., and Sullivan, M. X. *J. biol. Chem.*, 1941, **141**, 871.
11. Mitchell, H. H. *Ind. eng. Chem.* (Anal. ed.), 1944, **16**, 696.
12. Payne, D. S., and Stuart, L. S. *Soybean protein in human nutrition: advances in protein chemistry.* (M. L. Anson and J. T. Edsall, Eds.) New York: Academic Press, 1944.
13. Woolley, D. W. *J. biol. Chem.*, 1945, **159**, 753.

A "Frenching" Response of Tobacco Seedlings to Isoleucine

Robert A. Steinberg

Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland

Growth of tobacco seedlings in aseptic cultures under constant light and temperature was found to be greatly modified by the presence of isoleucine in the culture medium. The abnormalities in gross morphology resulting from the addition of this amino acid consisted in loss of dominance of the apical bud, production of numerous quite narrow leaves, and mottled chloroses. The shortened stem and excessive development of axillary buds gave a "witch's broom" type of growth. The abnormalities enumerated are characteristic of a tobacco disease called frenching, the cause of which is unknown.

Slight variations from the symptoms of frenching in the field were observable under the extreme environmental conditions employed. These abnormal conditions were very high moisture and relative humidity, low light intensity, and the use of agar instead of soil. The mottling of the leaves was not of the reticular pattern typical of frenching, nor did the narrow or strap-leaves assume the upright position to the same degree. There was little indication of wavy margins or ruffling of strap-leaves.

These abnormalities occurred regularly in every experiment with several samples of this amino acid. None of the other amino acids, sugars, vitamins, and peptones (a total of 60) tested brought about this reaction. The severity of abnormal symptoms varied with the concentration of isoleucine employed. Levels of 50 to 200 p.p.m. proved adequate under these conditions. Complete details of the experimental work will be reported later.

Additional work is now under way in both greenhouse and field to determine the degree in identity of symptoms under normal conditions of growth for tobacco. Data are also being sought to aid in determining whether the response of the tobacco plant to isoleucine is of a primary or secondary nature. Secondary causatives of this type have already been reported by McMurtrey (*1*) as thallium and Spencer (*2*) as sulfanilamide.

References

1. McMurtrey, J. E., Jr. *Science*, 1932, **76**, 86.
2. Spencer, E. L. *Amer. J. Bot.*, 1939, **26**, 14 S.

On the Specificity of Epidemic and Murine Typhus

Vincent Groupé and Richard Donovick[1]

The Biological and Chemical Laboratories
E. R. Squibb and Sons, New Brunswick, New Jersey

That the rickettsiae of epidemic and murine typhus are closely related antigenically is shown by the fact that infection of guinea pigs with epidemic typhus confers complete immunity to infection with murine typhus and vice versa. That they are, however, not antigenically identical has been shown by the following observations: (a) Immunity induced with one inoculation of killed murine rickettsiae protected guinea pigs only against infection with the homologous type, although repeated inoculations of the same vaccine also immunized against the heterologous type (*7*). (b) Serum obtained from a horse after one series of inoculations with killed murine rickettsiae protected guinea pigs only against infection with the homologous type (*8*) although serum from the same horse after a second series of inoculations with the murine vaccine protected guinea pigs against both homologous and heterologous types (*9*). (c) Purified rickettsial suspensions reacted in complement-fixation tests only with homologous convalescent human and guinea pig sera, although the corresponding "soluble antigens" reacted with both homologous and heterologous sera (*5*). (d) Absorption of sera from cases of Brill's disease with murine antigen removed murine but not epidemic antibody, although epidemic antigen removed both murine and epidemic antibody (*4*). (e) Sera obtained from guinea pigs immunized with murine and epidemic typhus vaccine, respectively, neutralized only the homologous toxic substance (*6*).

The specificity of epidemic and murine typhus has been further demonstrated by the active immunization of mice in the experiments described below. Murine (Wilmington strain) and epidemic (Breinl strain) typhus vaccines were prepared from heavily infected yolk sacs[2] and two groups of 50 mice[3] each were immunized with one intraperitoneal inoculation of 0.5 cc. of epidemic and murine vaccine, respectively. Fourteen days after inoculation half of each group of immunized mice was challenged with homologous and the remainder with heterologous toxic substance (*1, 3*). The challenge dose, consisting in each case of 0.5 cc. of toxic substance, representing 3 to 4 LD_{50}, was injected intravenously in the tail vein. The results, recorded 18 hours after challenge, are presented in Table 1. It

TABLE 1

Specificity Shown by Active Immunization of Mice

Challenged I.V. with 0.5 cc. of:	Vaccinated I.P. with 0.5 cc. of:		Controls	
	Epidemic typhus vaccine	Murine typhus vaccine	Toxic substance diluted: 1–20	1–40
Epidemic toxic substance diluted 1–10 (3 to 4 LD_{50})	20/23*	0/23	0/10	8/10
Murine toxic substance diluted 1–10 (3 to 4 LD_{50})	1/23	22/23	0/11	7/10

* Number of mice surviving/total number of mice.
I.V.—Intravenously.
I.P.—Intraperitoneally.

will be seen that 20 of 23 mice immunized with epidemic typhus vaccine and 22 of 23 mice immunized with murine typhus vaccine were protected against 3 to 4 LD_{50} of the homologous toxic substance. Furthermore, none of 23 mice immunized with murine typhus vaccine and only one of 23 mice immunized with epidemic typhus vaccine was protected against the heterologous toxic substance. It is evident from these data that one inoculation of epidemic or murine typhus vaccine protected mice only against the homologous toxic substance.

Summary: The specificity of epidemic and murine typhus has been shown by active immunization of mice with killed rickettsial suspensions and subsequent challenge with heterologous and homologous toxic substance.

During the preparation of this manuscript, Fitzpatrick published findings showing that 3 of 8 mice immunized with murine typhus vaccine were protected against 3 MLD of epidemic toxic substance administered intravenously and that 8 of 16 mice immunized with epidemic vaccine were protected against <1 MLD of murine typhus rickettsiae administered intraperitoneally (*2*). These results suggested to Fitz-

[1] On temporary leave from the Division of Microbiology, Squibb Institute for Medical Research.

[2] National Institute of Health, Washington, D. C. Communication on the preparation of epidemic typhus vaccine, 10 August 1942 (unpublished).

[3] Young albino Swiss mice (Webster strain) weighing 12–13 grams were used throughout these experiments.