by supplementation with excess pyridoxal phosphate. However, if incubation was carried out with 3×10^{-2}M inhibitor, the inhibitor effect can no longer be reversed by supplementation with cofactor. The inhibition of phenylalanine and its derivatives, phenylpyruvic, phenyllactic, and phenylacetic acid, is competitive and substrate-dependent at pH 8. The K_m for 5 HTP decarboxylase is estimated to be 5.12×10^{-4}.

The authors wish to express their appreciation to Marilyn Pease for technical assistance.

1. Clark, C. T., Weissbach, H., Udenfriend, S., *J. Biol. Chem.*, 1954, v210, 139.
2. Buzard, J. A., Nytch, P. D., *ibid.*, 1957, v227, 225.
3. ———, *ibid.*, 1957, v229, 409.
4. Davison, A. N., Sandler, M., *Nature,* 1958, v181, 186.
5. Wang, H. L., Waisman, H. A., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 533.
6. McCaman, M. W., Robins, E., *J. Lab. Clin. Med.*, 1962, v59, 885.
7. Berry, J. P., Woolf, L. I., *Nature,* 1952, v169, 202.
8. Huang, I., Tannenbaum, S., Hsia, D. Y.-Y., *ibid.*, 1960, v186, 717.
9. Lineweaver, H., Burk, D., *J. Am. Chem. Soc.*, 1934, v56, 658.
10. Pare, C. M. B., Sandler, M., Stacey, R. S., *Lancet,* 1958, v2, 1099.
11. Kirman, B. H., Pare, C. M. B., *ibid.*, 1961, v1, 117.
12. Knox, W. E., *Pediatrics,* 1960, v26, 1.
13. Lovenberg, W., Weissbach, H., Udenfriend, S., *J. Biol. Chem.*, 1962, v237, 89.
14. Tashian, R. E., *Metabolism,* 1961, v10, 393.
15. Nachmias, V. T., *Neurochem.*, 1960, v5, 202.
16. Yulwiler, A., Louttit, R. T., *Science,* 1961, v134, 831.

Received September 10, 1962. P.S.E.B.M., 1963, v112.

"Bound" Growth Inhibitor in Raw Soybean Meal.* (27957)

RAYMOND BORCHERS (Introduced by John L. Adams)
Department of Biochemistry and Nutrition, University of Nebraska, Lincoln

The poor growth of experimental animals fed raw soybean(1) as a source of protein, when compared with cooked soybean, would suggest the existence of specific growth inhibitor(s) in the raw soybean. Repeated attempts in this laboratory to prepare such a factor have yielded erratic and inconclusive results. Working with various soybean fractions, Rackis *et al.*(2) have recently concluded, "All raw fractions contained widely different levels of trypsin inhibitor activity as measured chemically; and, there appears to be no direct relationship between trypsin inhibitor activity, growth inhibition and pancreatic hypertrophy." Generally, the results of Birk and Gertler(3), Rackis *et al.*(2), and Saxena *et al.*(4) suggested that a water extracted soybean residue was more growth inhibitory than the soluble fraction. These reports and the results obtained in our laboratory suggested that the soybean growth inhibitor may exist in a "bound" or insoluble form. This hypothesis was examined by digesting raw soybean oil meal with papain, later by merely soaking in water. In this connection, McGinnis and Menzies(5) reported that papain digestion improved growth over raw soybean while Desikachar and De (6) reported no improvement from papain digestion.

Methods. The raw soybean meal used in this study was prepared by the solvent extraction process. Heated meal was prepared by autoclaving the raw meal at 15 lb pressure for 30 minutes. The raw or heated soybean meal was suspended in 10 ml water per g of meal and maintained at 37° with stirring for the indicated period. Commercial crude papain, when used, was added at a level of 50 mg per g of meal. The "digests,"

* Published with approval of the Director as paper no. 1276, Journal Series, Nebraska Agri. Exp. Station. The investigation was supported in part by grant from Nat. Inst. Health, Bethesda, Md.

TABLE I. Growth Rate of Rats Fed "Digested" Soybean Meal Compared with Autoclaved Soybean Meal.

Treatment*	Digestion, hr 0	16	40
	Growth rate as % of control†		
Raw soybean meal			
Untreated	77		
Papain digested	74	59	10
Water "	75	64	45
Papain digested, then autoclaved	100	99	36
Autoclaved soybean meal			
Papain digested	98	94	84

* See text for conditions of digestion treatment. Autoclaved indicates subjecting to heating in a steam autoclave at 15 lb pressure for 30 min.

† With the feeding of each experimental preparation to a group of 4 female and 4 male weanling rats, a control group, paired as to litter, sex, and initial wt, was fed an autoclaved soybean meal ration (12% of protein) for a 20-day period. Avg daily gains of control groups were from 3.92 g to 4.56 g.

after drying, were incorporated in rations to supply 12% of protein (N × 6.25) and fed to weanling rats of the Holtzman strain weighing 40 to 45 g. Otherwise, ration composition and feeding procedures were as previously described(7).

Results. Digesting raw soybean meal with papain resulted in increased growth inhibition over that of the untreated raw soybean meal. After 40 hours of digestion, the preparation barely supported growth. Merely suspending in water and maintaining at 37° resulted in similar increased growth inhibition but to a lesser extent. Autoclaving the "digested" preparation after 16 hours incubation resulted in the usual growth rate obtained with autoclaved soybean meal; after 40 hours, autoclaving improved the growth promoting value but not to normal. Digesting previously autoclaved soybean meal resulted in no effect on growth rate with 16 hours' digestion but some effect was noted at 40 hours. Growth rates on the experimental preparations are presented in Table I as per cent of growth rate on a control autoclaved soybean meal ration.

Discussion. The increased growth-inhibiting or decreased growth-supporting properties of raw soybean meal incubated with papain, or in water alone, suggest that the soybean growth inhibitor exists in raw soybean in a "bound" form. The "liberation" of such a bound form would appear to be accomplished by enzymes or microorganisms associated with the raw soybean meal. An additional proteolytic enzyme, papain, resulted in some increased liberation as judged by poorer growth.

An alternate explanation to that offered above must, however, be strongly stressed. Raw soybean meal "digestions," as carried out in these *in vitro* procedures, may result in liberation or formation of factors not normally released or formed in the usual process of *in vivo* digestion of raw soybean meal.

Summary. Incubating raw soybean meal at 37° in water resulted in further reduction, over that of raw soybean meal, in the growth rate of rats fed such meal; papain accentuated this effect. The results are interpreted as supporting the hypothesis that the raw soybean growth inhibitor(s) exists in a "bound" form in raw soybean.

1. Osborne, T. B., Mendel, L. B., *J. Biol. Chem.*, 1917, v32, 369.

2. Rackis, J. J., Smith, A. K., Nash, A. M., Robbins, D. J., Booth, A. N., *141st meeting, Am. Chem. Soc.*, 1962, Washington, D.C.

3. Birk, Y., Gertler, A., *J. Nutrition*, 1961, v75, 379.

4. Saxena, H. C., Jensen, L. S., McGinnis, J., *Poultry Sci.*, 1962, v41, 1680.

5. McGinnis, J., Menzies, V. H., *Poultry Sci.*, 1946, v25, 538.

6. Desikachar, H. S. R., De, S. S., *Science*, 1947, v106, 421.

7. Borchers, R., Mohammad-Abadi, D., Weaver, J. M., *J. Agri. Food Chem.*, 1957, v5, 371.

Received September 13, 1962. P.S.E.B.M., 1963, v112.