

corticosterone would be superior to cortisone (6).

Summary. Under the conditions of these experiments 17-hydroxycorticosterone was found to have about twice the biologic potency of cortisone in the work performance test on

adrenalectomized-nephrectomized male rats.

We wish to express our appreciation to Dr. Augustus Gibson, The Medical Division of Merck and Co., who supplied the cortisone and to Dr. W. J. Haines, Endocrinology Department, The Upjohn Research Laboratories, who supplied the 17-hydroxycorticosterone for these studies.

6. Sprague, R. G., personal communication.

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Nutritive Value of Legume Seeds. XI. Counteracting the Growth Inhibitor of Raw Soybeans.* (18981)

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The existence of a distinct growth-inhibiting factor for the chick in raw soybeans was demonstrated by Ham, Sandstedt, and Mussehl (1). That the soybean growth inhibitor similarly affects the rat has been established subsequently in several reports. One of the objectives of the present writers' investigations of the soybean growth inhibitor (SGI[†]) has been to answer this question: Is there a factor which will counteract the growth inhibitor of raw soybeans? Results of a search for an anti-SGI[†] are here described.

Experimental. The substances to be tested for anti-SGI activity replaced an equal weight of starch in the following ration: 25 g autoclaved or raw soybean oil meal, 0.6 g DL-methionine, 2 g salt mixture(2), 0.5 g choline

chloride, 0.5 mg thiamine HCl, 0.5 mg Ca pantothenate, 2.5 mg niacin, 0.125 mg pyridoxine HCl, 0.5 mg riboflavin, 5 g cod liver oil, 15 g lard, and corn starch sufficient to make 100 g. The raw soybean oil meal was prepared with a minimum of heat treatment by the solvent process according to the manufacturer's statement. The autoclaved soybean oil meal was prepared from this by autoclaving at 15 lb pressure for 30 minutes. The rations were fed to weanling rats of the Sprague-Dawley strain. Animals of each litter were paired as to sex and initial weight between the 2 experimental rations, autoclaved soybean oil meal plus the material being tested for anti-SGI activity versus raw meal plus the same material. The rats were housed in individual screen-bottom cages with food and water available *ad libitum*. The number of rats fed each ration ranged from 8 to 20, the feeding period from 20 to 42 days. Some of the materials tested for anti-SGI activity are listed in Table I with the average gain per day at the end of 20 days feeding. Longer feeding periods did not affect the results other than to increase statistical significance. Of some 25 materials tested only trypsin powder has been found to be effective in counteracting the growth inhibitor in raw soybeans. Because of the interest in the trypsin inhibitor of raw soybeans, the apparent trypsin inhibitor activity of a mixture of 25 g raw soybean oil meal and 5 g trypsin

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1. Ham, W. E., Sandstedt, R. M., and Mussehl, F. E., *J. Biol. Chem.*, 1945, v161, 635.

† It is proposed that the abbreviation "SGI" be used for the soybean growth inhibitor until identification has been accomplished. A factor which counteracts the growth inhibitor will be termed "anti-SGI."

2. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, v14, 273.

TABLE I. Anti-SGI Activity of Several Materials.

Material added	Rat growth g/day $\pm$ SE g gain/g food consumed		Raw auto-claved, %
	Autoclaved	Raw	
10% cow manure	3.32 $\pm$.20 (.39)	1.79 $\pm$.14 (.28)	54
5% fish solubles	3.47 $\pm$.10 (.46)	2.55 $\pm$.15 (.33)	73.5
Whey, 75 ml/100 g ration	3.94 $\pm$.14 (.48)	2.90 $\pm$.21 (.40)	73.6
0.1% Xanthine, 2 mg α -tocopherol in oil/day	2.83 $\pm$.14 (.43)	2.10 $\pm$.13 (.31)	74.3
5% yeast	3.63 $\pm$.17 (.49)	2.70 $\pm$.20 (.36)	74.4
None	3.27 $\pm$.14 (.45)	2.51 $\pm$.19 (.32)	76.7
0.5% L-arginine, HCl	3.91 $\pm$.20 (.49)	3.08 $\pm$.15 (.43)	78.8
5% whole liver powder	3.54 $\pm$.19 (.50)	2.83 $\pm$.10 (.42)	80
0.2% MK-42*	3.09 $\pm$.19 (.47)	2.53 $\pm$.13 (.42)	81.8
5% casein, tech.	3.69 $\pm$.15 (.48)	3.08 $\pm$.07 (.41)	83.5
5% trypsin powder†	3.44 $\pm$.24 (.50)	3.57 $\pm$.12 (.47)	103.9

* MK-42 stated to contain 12.5 mg vit B₁₂ activity and 30 g streptomycin base per lb., courtesy of Dr. M. A. Schooley, Merck & Co., Rahway, N. J.

† Trypsin powder (1-110), Pfanstiehl Chemical Co., Waukegan, Ill.

powder, the proportions as fed, was determined by the method of Borchers(3). The trypsin inhibitor activity of the extract from a mixture of raw meal and trypsin was 32.4×10^{-3} units per ml, the activity of the extract from raw meal alone was 40.8×10^{-3} units per ml.

The following experiments were carried out in an effort to define some of the characteristics of the anti-SGI factor in the trypsin powder. The anti-SGI activity remained after the dry trypsin powder was autoclaved with live steam at 15 lb for 30 minutes. Autoclaving in this manner destroyed the trypsin activity as determined by the method of Anson(4). The anti-SGI activity was stable during autolysis when suspended in 10 ml water per g and incubated at 37°C and pH 8.5 for 48 hours, the activity being present in the soluble portion. Suspending the powder in 10 ml water per g, adding HCl to pH 3, adding ethanol to 60% concentration, and centrifuging yielded the active product in the centrifugate. The following tests were made on the 60% alcohol centrifugate after concentration *in vacuo* to remove the alcohol and diluting so that one ml was equivalent to 0.1 g original trypsin powder. Continuous extraction with n-butanol failed to remove any of the activity. Addition of acetone to 80% concentration yielded a relatively small precipi-

tate which was inactive. Saturation with ammonium sulfate yielded a small precipitate without activity. Dialysis of the water solution of the 60% alcohol centrifugate against running tap water for 20 hours resulted in loss of activity of material remaining within the membrane. Results are detailed in Table II.

Discussion. The results presented establish a positive answer to the initial objective, that is, there is a factor which will counteract the growth inhibitor of raw soybeans. Although the results presented indicate that the factor occurs only in crude trypsin powder, preliminary data seem to indicate that the anti-SGI factor may have a more widespread occurrence but at insignificant levels when assayed against 25% raw soybean oil meal.

The feeding of crude trypsin powder was initially undertaken in an effort to remove the trypsin inhibitor from soybean extracts as the trypsin-trypsin inhibitor complex of Kunitz (5). As shown by the results, such addition of crude trypsin powder to the ration did counteract the growth inhibiting effect of raw soybeans. However, data presented indicate that addition of 5 parts crude trypsin powder to the ration containing 25 parts raw soybean oil meal reduced the trypsin inhibitor activity to approximately 80% of that of raw soybean alone. Also, since autoclaving destroyed trypsin activity but not the anti-SGI activity of the trypsin powder, it is evident that trypsin

3. Borchers, R., Ackerson, C. W., and Sandstedt, R. M., *Arch. Biochem.*, 1947, v12, 367.

4. Anson, M. L., *J. Gen. Physiol.*, 1938, v22, 79.

5. Kunitz, M., *J. Gen. Physiol.*, 1947, v30, 311.

TABLE II. Some Properties of the Anti-SGI Factor in Trypsin Powder.

Trypsin powder $\approx$ 5% (see text for details)	Rat growth g/day $\pm$ SE g gain/g food consumed		Raw auto- claved, %
	Soybean oil meal		
	Autoclaved	Raw	
Autoclaved	3.84 $\pm$.24 (.50)	3.88 $\pm$.18 (.44)	101
Autolysate soluble	3.37 $\pm$.29 (.49)	3.44 $\pm$.14 (.51)	102.1
pH 3, 60% ethanol soluble	3.40 $\pm$.14 (.46)	3.46 $\pm$.11 (.45)	101.7
n-butanol insoluble	3.39 $\pm$.19 (.46)	3.43 $\pm$.18 (.43)	101.2
acetone ppt.	3.83 $\pm$.15 (.45)	2.31 $\pm$.08 (.28)	60.3
ammonium sulfate ppt.	2.99 $\pm$.08 (.40)	2.16 $\pm$.14 (.26)	72.3
dialysis residue	3.94 $\pm$.13 (.49)	3.43 $\pm$.15 (.42)	87.1

activity is not essential to the anti-SGI activity.

The importance of the anti-SGI factor of crude trypsin powder in animal nutrition is difficult to outline at present. Nutritional effects have been ascribed to preparations from pancreas, particularly lipocaic. Also, no untoward effects, that is deficiency symptoms other than depressed growth, have been reported as a result of feeding raw soybean oil meal. The relation of the anti-SGI factor of crude trypsin powder to other nutritional factors as well as to the normal nutrition picture requires further investigation.

Summary. Growth of rats fed a ration containing 25% raw soybean oil meal was improved to the same rate of gain as rats fed autoclaved meal when 5% crude trypsin powder was added to each ration. The ability of the crude trypsin powder to counteract the soybean growth inhibitor (SGI) has established the existence of an anti-SGI. That the anti-SGI activity of crude trypsin powder was not dependent upon trypsin was established by heat stability.

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Differences in Cellular Pathogenicity of Two Immunologically Related Poliomyelitis Viruses as Revealed in Tissue Culture.* (18982)

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With the exception of antigenicity, biological differences within the group of poliomyelitis viruses have not been intensively investigated. This may be due in part to the fact that convenient technics are not available for such a study, as is the case with the influenza viruses. However, recent results from this laboratory (1) have shown that 2 immunologically related poliomyelitis strains, Lansing and Yale SK, differ in their ability to elicit antibodies after feeding to cynomolgus monkeys. The work of Enders, Weller, and Robbins(2,3) on the

growth of poliomyelitis viruses in tissue culture has opened new avenues for exploration in this field. These workers have recently reported the capacity of Lansing and Brunhilde strains to cause cell injury and death in human embryonic tissues(4). We had found that the poliomyelitis viruses will grow in monkey testes *in vitro* (but not *in vivo*),

2. Enders, J. F., Weller, T. H., and Robbins, F. C., *Science*, 1949, v109, 85.

3. Weller, T. H., Robbins, F. C., and Enders, J. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1949, v72, 153.

4. Robbins, F. C., Enders, J. F., and Weller, T. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v75, 370.

5. Syverton, J. T., Scherer, W. F., and Butorac, G., *PROC. SOC. EXP. BIOL. AND MED.*, 1951, v77, 23.

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1. Melnick, J. L., *J. Immunol.*, 1951, v67, 219.