tains glycine.

Methods. Seventeen male, adult albino rats, approximately 6 months of age and previously subsisting on Purina Dog Chow were used for this study. Creatinine and hippurate clearances were done to ascertain the control rates of glomerular filtration and renal plasma flow respectively. Approximately one week later, each rat received 50 mg of glycine per 100 g of body weight by stomach tube and the clearance studies were repeated immediately afterwards. The methods and calculations used in determining the clearances have been reported in a previous study.[7] All clearances have been expressed in cc per hour per 100 g of body weight.

TABLE I.
The Effect of Glycine on Creatinine and Hippurate Clearance of the Rat.

	Before Glycine			After Glycine		
Rat	U.V.*	C.C.†	H.C.‡	U.V.	C.C.	H.C.
27	1.75	30.4	180	2.9	41.5	101
25	3.60	40.8	212	4.4	36.2	149
93	4.95	39.0	90	5.2	41.5	144
72	3.70	39.4	139	2.0	28.0	182
92	5.0	48.8	175	3.5	39.2	157
57	4.0	39.5	202	4.7	40.0	146
10	0.60	18.6	91	4.75	44.0	185
12	2.4	34.8	146	3.55	38.6	89
98	2.3	34.6	108	2.80	28.6	119
35	3.6	34.4	158	2.40	40.8	142
29	4.9	28.5	115	0.90	20.8	64
88	3.9	43.7	160	2.10	24.7	108
21	3.6	38.7	134	3.30	19.8	122
09	3.5	38.8	128	2.75	39.7	128
20	4.6	32.5	73	2.25	45.7	81
77	4.6	37.0	138	2.85	36.8	191
93	4.55	50.5	152	3.05	42.8	138
Av.	3.62	37.1	141	3.14	35.8	132

* U.V. equals urine volume in cc per hour.
† C.C. equals creatinine clearance in cc per 100 g per hr.
‡ H.C. equals hippurate clearance in cc per 100 g per hour.

Results. Inspection of Table I indicates that no significant change occurred in either the creatinine or hippurate clearance of the rat following the ingestion of glycine. Thus the average creatinine clearance was 37.1 cc per hour before and 35.8 cc per hour after the ingestion of glycine. Likewise, the average hippurate clearance was 141 cc per hour before and 132 cc per hour after glycine had been given. The average urine volume per hour was 3.62 cc before and 3.14 cc after ingestion of glycine. This average decrease of 13% is perhaps of questionable significance.

It should be noted that Pitts[5] also found no increase in the creatinine clearance of dogs after heavy glycine feeding, nor could Beyer *et al.*[6] observe an increase in the dog's creatinine clearance after feeding of such essential amino acids as tryptophane, leucine, isoleucine or valine. Finally it should be emphasized that the results of this present study only indicate that *excess* feeding of glycine appears to have no effect on renal hemodynamics. It is conceivable that were glycine to be reduced abnormally in the blood, changes in renal hemodynamics might occur.

Conclusion. The administration of glycine to the rat had no demonstrable effect on either its rate of glomerular filtration or its renal blood flow.

The author wishes to express his thanks to Barbara Trousdale and Frances Greenberg for their technical assistance.

[7] Friedman, M., *Am. J. Physiol.*, 1947, to be published.

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Differentiation of Soy Bean Antitryptic Factors.

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In a preliminary report, the author called attention to a water-soluble antitryptic fraction of navy beans which can be readily precipitated with alcohol. Although a large

part of the trypsin-inhibiting material of soy beans is not precipitated with alcohol, it is precipitated with acetone.[1] It was further pointed out that soy and other beans as well as navy beans do, however, contain a trypsin-inhibiting fraction which can be repeatedly reprecipitated with alcohol.[2]

Kunitz soon crystallized this latter factor from soy beans and described some of its properties.[3,4] He found it to be a crystalline globulin which can be further recrystallized from 20% alcohol. Further comment regarding the occurrence of this factor is hardly necessary; however, the trypsin-retarding fraction of soy beans, which is quite soluble in 60% alcohol but insoluble in acetone, would appear to deserve further attention. Following the publication of our description of this fraction,[1] it was found that a note by Ham and Sandstedt[5] had appeared shortly before referring to what appears to be this material, and we wish to acknowledge their paper. They indicated that this antitryptic substance is destroyed upon treating an aqueous extract of the beans with 60% alcohol, and they found no inhibiting action in aqueous extracts of soy beans previously treated with 45% alcohol. Other properties given also differentiate it from the antitryptic substance which is found in alcohol precipitates of aqueous extracts of soy beans. These investigators may have discarded the latter substance with the kaolin used to remove proteins since it will adsorb on kaolin.

A number of soy bean preparations having antitryptic properties have been used in various investigations. Employing an acetone precipitate of an aqueous extract, Ham, Sandstedt, and Mussehl pointed out that the proteolytic-inhibiting material has a retarding effect on the growth of chicks.[6] Klose, Hill and Fevold observed similar growth effects and indicated that the substance which they employed is nondialyzable, can be precipitated by salts, is inactivated by heat, and appears to be a protein which is concentrated in the acid-soluble (pH 4.2) fraction of soy bean protein.[7] Tagnon and Soulier, employing the inhibitor obtained by acetone precipitation of aqueous extracts as well as the crystalline material of Kunitz, observed marked anticoagulant activity on human whole blood and plasma.[8] Employing the acetone-insoluble and alcohol-insoluble fractions, the author has been unable to show a consistent influence on trypsin shock.[2] Thompson has indicated that the alcohol-insoluble factor of navy beans does not modify anaphylactic shock produced by egg albumin.[9]

Unless definite precautions are taken, crude soy bean antitryptic preparations may consist of varying proportions of at least 2 factors. This occurs especially when acetone is used to precipitate the active material from an aqueous extract. The object of the present report is to call attention to the difference between the soy bean antitryptic substance which can be extracted with water and precipitated with alcohol, called the alcohol-insoluble factor, and the active material which can be extracted with 60% alcohol and precipitated with acetone, called the acetone-insoluble factor. Both are soluble in water.

Methods. Relative inhibition of tryptic activity was estimated by adding the experimental material to triplicate casein digestion preparations, and the degree of digestion was compared with that of the same number of control uninhibited preparations of equal volume, run simultaneously. With final concentrations of 0.01% of crude trypsin and 3.1% casein, approximately half of the casein was digested at 38°C in one hour in the absence of inhibiting agents. The actual degree of digestion was determined by the increase in

1 Bowman, D. E., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 139.

2 Bowman, D. E., *Fed. Proc.*, 1945, **4**, 84.

3 Kunitz, M., *Science*, 1945, **101**, 668.

4 Kunitz, M., *J. Gen. Physiol.*, 1946, **29**, 149.

5 Ham, W. E., and Sandstedt, R. M., *J. Biol. Chem.*, 1944, **154**, 505.

6 Ham, W. E., Sandstedt, R. M., and Mussehl, F. E., *J. Biol. Chem.*, 1945, **161**, 635.

7 Klose, A. A., Hill, B., and Fevold, H. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 10.

8 Tagnon, H. J., and Soulier, J. P., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 440.

9 Thompson, R. L., personal communication.

TABLE I.
Comparison of Antitryptic Preparations.

Treatment and portion used	% of normal uninhibited digestion in the presence of inhibiting substances		
	Acetone-insoluble fraction, 0.25%*	Alcohol-insoluble fraction, 0.04%*	Crystalline material, 0.04%*
Untreated	49.9	52.5	46.9
Material insoluble in 60% alcohol	103.0	54.6	47.3
Untreated	45.5	54.2	46.8
Material insoluble in a solution 40% saturated with ammonium sulfate	102.0	56.6	49.5
Untreated	44.8	51.0	47.0
Material soluble in 2.5% trichloracetic acid	46.0	97.0	99.4

* Concentration of untreated trypsin inhibiting fraction in the casein digestion mixtures. The concentration of the treated material would have been the same except for the influence of the treatment.

the refractive index[10] of the filtrate following the isoelectric precipitation of undigested casein with a constant amount of acetic acid. In all cases triplicate unincubated negative controls were run in which the casein was precipitated before the trypsin was added and the filtrate was employed immediately. Triplicate values ordinarily agreed quite well throughout.

The material referred to as the acetone-insoluble factor was prepared by first extracting 100 g of finely ground untreated soy beans with one liter of 60% ethyl alcohol. The active material was then precipitated from the centrifuged and filtered alcohol extract by adding 2 volumes of acetone. The precipitate was centrifuged down and immediately dried in a vacuum desiccator. The majority of such preparations were further redissolved and heated in 2.5% trichloracetic acid at 98°C for 5 minutes. After centrifuging down and discarding the precipitate the active substance was reprecipitated from the neutralized solution with 90% acetone.

The alcohol-insoluble factor was obtained by extracting 100 g of finely ground untreated soy beans with one liter of water overnight, after adjusting to pH 4. After centrifuging, the supernatant aqueous extract was precipitated by adding alcohol to 60% concentration. The precipitate was centrifuged and dried in a vacuum desiccator.

Results. Numerous preparations of the 2 factors were employed and compared with a crystalline preparation provided by Dr. Kunitz. Typical results are given in Table I. As may be expected from the methods of preparation, the solubilities of the 2 factors in 60% alcohol are quite different. This can be shown more objectively by comparing the trypsin-inhibiting capacity of a suitable weighed amount of the untreated substance with the inhibiting capacity of an equal amount after extraction with 100 parts of 60% alcohol by weight. The acetone-insoluble factor appears to completely dissolve, and no activity is apparent after centrifuging and drying any possible insoluble material and adding it to casein digestion mixtures. Under the same conditions the alcohol-insoluble factor and the crystalline material appeared to be insoluble and, as judged by trypsin-inhibiting capacity, essentially entirely recovered in the undissolved material.

The acetone-insoluble factor does not precipitate when present in 1% concentration in a solution 40% saturated with ammonium sulfate. After centrifuging down any slight amount of insoluble material present, the latter was redissolved and the pH adjusted to that of the digestion mixture. No antitryptic activity was observed. In contrast, under similar conditions the alcohol-insolu-

[10] Robertson, T. B., *J. Biol. Chem.*, 1912, **12**, 23.

ble factor and the crystalline material give a definite precipitate and, in each case, the trypsin-inhibiting capacity of the precipitate indicates essentially complete recovery.

Although crystalline soy bean antitrypsin is precipitated when heated in 2.5% trichloracetic acid as pointed out by Kunitz,[3] the acetone-insoluble factor can be almost quantitatively recovered from the filtrate of such a solution. This is indicated by the final antitryptic capacity retained after so treating a weighed amount of the material. After heating this inhibitor, in 2% concentration, in 2.5% trichloracetic acid at 98° C for 5 minutes only a very slight precipitate forms. After centrifuging and discarding this precipitate and neutralizing the supernatant solution, the active material can be almost completely recovered by precipitating with 90% acetone.

Upon treating the alcohol-insoluble factor or the crystalline material in a similar manner, an appreciable precipitate is seen in the trichloracetic acid solution and the amount of antitryptic substance obtained from the neutralized filtrate by precipitating with 90% alcohol is insignificant.

A typical preparation of the acetone-insoluble fraction was found to contain approximately 1% nitrogen and gave only faintly positive biuret and Millon tests. Further observations dealing with the nature of the active material are in progress. Its solubility characteristics and a phosphorus content of about 5% in the present preparations suggests that some attention be given to phosphorus-containing substances.

Summary. In a number of respects, the trypsin-inhibiting factor of soy beans which may be extracted with 60% alcohol and precipitated with acetone differs from the antitrypsin which can be extracted with water and precipitated with 60% alcohol. These substances may be differentiated on the basis of their solubilities in a solution 40% saturated with ammonium sulfate, in 2.5% trichloracetic acid as well as in alcohol.

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Pharmacological Protection Against *Salmonella* Endotoxin and Certain Other Poisons.

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In previous work sulfonamides were found effective to a limited degree in counteracting the systemic toxicity of certain typical endotoxins of Gram-negative bacteria.[1] Analysis of this action of sulfonamides has led to the conclusion, reported here, that the efficacy of sulfonamides in this respect depends on their goitrogenic activity, and posed again the problem of devising an efficient therapy directed against this class of poisons.

The endotoxin-containing somatic (O) antigens of Gram-negative bacteria are frequently the principal source of damage in infections by these organisms.[2] In contrast to the classical bacterial exotoxins, antibodies for these somatic antigens have little antitoxic potency.[3] The action of these endotoxins is usually aborted by checking the multiplication of the bacteria either by means of previous immunization with appropriate vaccines or, when the infection has gained a foothold, by administration of antibiotics such as streptomycin. Although these measures are often dramatically effective when directed against the primary infection, they afford little or no

[1] Hutner, S. H., and Zahl, Paul A., *Science*, 1942, **96**, 563; Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 285.

[2] Zahl, Paul A., Starr, M. P., and Hutner, S. H., *Am. J. Hyg.*, 1945, **41**, 41.

[3] Zahl, Paul A., and Hutner, S. H., *Am. J. Hyg.*, 1944, **39**, 189.