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.

protection against the intoxication ensuing once the bacteria have liberated their antigens into the bloodstream. Indeed the toxicity of *Shigella*[4] and *Salmonella*[5] vaccines employed for prophylactic immunization has been a complicating factor; and the excessive toxicity of *Shigella* vaccines has constituted a hazardous accompaniment to their successful use, even when neurotoxin-free vaccines are employed. It follows that the problem of therapeutics directed specifically at preformed endotoxin is of considerable clinical significance.

The only pharmacological (as distinguished from immunological) agents known to lessen experimentally the toxic effects of these antigens were sulfonamide drugs, whose effectiveness, however, in this respect was low, protecting mice against only a few lethal doses of the endotoxin.

Accordingly, over several years we have undertaken a search for pharmacological agents which might be more effective than sulfanilamide in counteracting the effects of the endotoxin. We have tested the compounds and combinations of compounds listed below* for protective effects against the endotoxin in mice. The rationale governing their selection for trial need not be given here. It may be noted, however, that nearly

[4] Goebel, W. F., Perlman, E., and Binkley, F., *Science*, 1944, **99**, 412.

[5] Wilson, G. S., and Miles, A. A., *Topley and Wilson's Principles of Bacteriology and Immunity*, Williams & Wilkins, Baltimore, 3rd Ed., 1946, p. 1553.

* (1) N^1-acetylsulfanilamide; (2) N^4-acetylsulfanilamide; (3) *p*-aminoazobenzene; (4) *o*-aminobenzene sulfonic acid; (5) *p*-aminobenzoic acid; (6) anthranilic acid; (7) arginine; (8) arginine, cysteine, methionine; (9) ascorbic acid; (10) ascorbic acid, cortical adrenal extract; (11) ascorbic acid, guanine, thiamine, methionine; (12) ascorbic acid, cysteine, glycine, methionine, *p*-aminobenzoic acid, calcium gluconate, sodium acetate; (13) ascorbic acid, glutathione, (14) *p*-aminophenol; (15) atropine; (16) atropine, ergotoxin; (17) benzedrine; (18) benzenesulfonylamide; (19) bulbocapnine; (20) butanediol; (21) calcium gluconate; (22) carrot diet; (23) camphor; (24) catechol; (25) catechol (acetylated); (26) casein hydrolysate; (27) casein hydrolysate, ascorbic acid, adrenal cortical extract, sodium chloride; (28) choline; (29) coramine; (30) coramine, sulfanilamide, calcium gluconate; (31) coramine, atropine; (32) coramine, metrazol; (33) concanavalin; (34) cortical adrenal extract; (35) cortical adrenal extract, amino acid mixture; (36) cysteine; (37) cysteine, methionine; (38) cysteine, methionine, sodium acetate; (39) cysteine, glycine, yeast, nucleic acid, arginine, sodium acetate; (40) 4,4-diaminodiphenyl sulfone; (41) diaminophenol; (42) diacetyl; (43) diphenylsulfone; (44) ergotoxin; (45) glycolic acid; (46) glycine; (47) glutathione; (48) glutathione, monoethalolamine salt of ascorbic acid; (49) heparin; (50) hesperidine; (51) hesperidine, ascorbic acid; (52) histamine; (53) histamine, tryptophane, ascorbic acid, methionine; (54) histamine, tryptophane, methionine; (55) histidine; (56) hydroquinone; (57) *p*-hydroxybenzoic acid, hydroquinone triacetate, hydroquinone; (58) insulin; (59) insulin, dextrose; (60) liver (Difco); (61) liver (Lederle, 6E, 6C, 9C); (62) liver, sulfadiazine; (63) methionine; (64) metanilamide; (65) metrazol; (66) nicotinic acid; (67) *o*-nitrophenol; (68) *p*-nitrosophenol; (69) *p*-nitrobenzoic acid; (70) yeast nucleic acid; (71) nupercaine; (72) orcinol; (73) *o*-phenetidine; (74) *p*-phenetidine; (75) *o*-phenylenediamine; (76) picrotoxin; (77) quinone; (78) resorcinol; (79) sodium acetate; (80) sodium chloride; (81) sodium formaldehyde sulfoxylate; (82) sulfanilic acid; (83) thiamine; (84) thiouracil; (85) tryptophane; (86) tyrosine; (87) yeast, liver diet; (88) yeast extract (Difco).

The tests in most instances consisted of injecting groups of 6 mice each with intraperitoneal doses of 2-LD_{50} of the endotoxin. Previously to or simultaneously with the injection of the endotoxin, the mice received by repeated intraperitoneal injections or by stomach tube, the test chemicals in sub-toxic doses. Survival of the mice at the end of 24 hours after injection of the endotoxin served as the index of reduction in toxicity brought about by the test chemicals.

The mice used in these tests and in those experiments presented below were all young males of a Swiss line in the weight range of 18-21 g, obtained from the Rockland Farms. The animals were maintained during the experimental period, unless otherwise specified on a commercial mouse diet and tap water *ad libitum*.

The methods used in preparing the endotoxin material from *Salmonella typhimurium* employed in the above tests have been previously described.[6]

[6] Zahl, Paul A., Hutner, S. H., and Cooper, F. S., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 187.

TABLE I.

Dose of endotoxin (intraper.) mg	Dose of thiouracil (intraper.) mg	No. of animals	% survival
—	3.0 (×6)	10	100
0.50	3.0 "	27	96
0.50	C*	31	78
0.75	3.0 "	20	95
0.75	C	20	35
2.00	3.0 "	24	60
2.00	C	25	12

* C = Control.

all these tests were conducted before the recognition of sulfonamides as goitrogenic agents. It suffices to say that the only non-sulfonamides counteracting to any observable degree the toxicity of the endotoxin were thiouracil† and *p*-aminobenzoic acid. The further experimental basis for these findings and certain collateral experiments are described here.

Procedure and Results. Protection by Thiouracil Against Endotoxin-induced Death. In a series of experiments involving a considerable number of mice, the animals were pretreated with thiouracil, administered intraperitoneally, for a period of 72 hours, by means of a 3-mg dose given about every 12 hours. The total dosage over 72 hours was thus 18 mg. Following the last thiouracil dose, lethal amounts of the endotoxin material[6] of *Salmonella typhimurium* were injected. Survival of the mice at the end of 24 hours was taken as a measure of the effectiveness of the thiouracil in lessening the toxicity of the endotoxin. Because of the general consistency of the results, the experiments are summated in Table I, rather than presented individually.

It is evident from the data listed in Table I that thiouracil affords a degree of protection against the toxic effects of the endotoxin. In other exploratory experiments in which only a few hours elapsed between the time of the first dose of thiouracil and the injection of the endotoxin, very little protective effect could be observed, due presumably to the slowness of interference with thyroid function by thiouracil. Also, when the pretreatment with thiouracil was extended to 7 days before the injection of the endotoxin, little if any protective effect was observed, due possibly to the general debilitating effects of sustained thiouracil treatment, as shown by the fact that mice so treated and with such relatively high dosages failed to gain weight at the normal rate.

Protection by Thiouracil Against Endotoxin-induced Tumor Hemorrhage. The toxicity of the endotoxins of Gram-negative bacteria is generally considered to result primarily from injury to the vascular endothelium. This action is readily studied experimentally by the induction of hemorrhage in transplanted tumors, as well as by the over-all lethality. We had previously found that the induction of tumor hemorrhage provides a sensitive, almost quantitative method for assaying small fractions of a lethal dose of endotoxin.[2] This belief that the hemorrhage-inducing property and the death-inducing property of the endotoxin are in fact low- and high-dosage manifestations of the same action, could undergo additional test by determining, using methods described elsewhere,[2] whether thiouracil could protect mice against endotoxin-induced tumor hemorrhage. Table II gives data indicating a positive tumor hemorrhage protective effect on the part of thiouracil.

Protective Effects of Thiouracil Against Other Poisons. A series of experiments was performed to determine whether thiouracil had any protective effects against liver poisons of the class typified by 1,2-dichloroethane. The effects of poisons of this class are physiologically quite different from those typified by the endotoxin. It seemed of interest, therefore, to ascertain whether a goitrogen such as thiouracil is effective against dichloroethane. The results of these experiments are summarized in Table III. It is concluded that thiouracil has some protective action against the toxicity of dichloroethane.

A similar type of experiment was performed with colchicine. This poison is presumed to

† Kindly supplied by Dr. R. O. Roblin, Jr., of the American Cyanamide Company, Stamford, Conn.

TABLE II.

Dose of endotoxin (intraper.) mg	Dose of thiouracil (intraper.) mg	No. of animals	Degree of tumor hemorrhage*
0.10	3.0 (×6)	18	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ++
0.10	(control)	14	+++ +++ +++ +++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ 0 0

* Each designation below indicates the degree of tumor hemorrhage induced in each mouse. 0 = no hem.; + = slight hem.; ++ = considerable hem.; +++ = marked hem.; ++++ = maximal hem.

TABLE III.

Dose of dichloroethane (in corn oil, by stomach tube) mg	Dose of colchicine (intraper.) mg	Dose of thiouracil (intraper.)	No. of mice	% survival
16	—	3.0 mg (×6)	81	53
16	—	(control)	66	18
—	0.50	3.0 mg (×6)	41	44
—	0.50	(control)	42	50

act primarily on the vascular endothelium, as does the endotoxin, and also produces a set of symptoms overtly similar to those produced by the endotoxin.[7] Therefore, testing colchicine against a goitrogen such as thiouracil seemed in order. The results are tabulated in Table III, and it is concluded that thiouracil has no protective effect against the toxicity of colchicine.

Effect of Temperature on the Toxicity of the Endotoxin. From other studies it has been concluded that the effects of thiouracil (on anoxia, etc.) are attributable to a general lowering of metabolic rate via interference with thyroid gland activity.[8] If such an action accounts for the thiouracil effect on the toxicity of the endotoxin and dichloroethane, then a similar effect on these poisons should be observed by altering general body metabolism in other ways. Accordingly, an experiment was set up in which groups of mice were kept at various temperatures for the 24-hour period following injection of the endotoxin. The results of this experiment are summarized in Table IV. Clearly, mice maintained at high environmental temperatures are more susceptible to the toxicity of the endotoxin than those kept at lower temperatures.

TABLE IV.

Air temperature of incubator, °C	Dose of endotoxin (intraper.)	No. of mice	% survival
14	0.75 mg	19	74
14	(control)	13	100
22	0.75 mg	20	35
22	(control)	20	100
30	0.75 mg	20	10
30	(control)	14	100

Effect of p-Aminobenzoic Acid on the Toxicity of the Endotoxin and of Colchicine. It has been reported that large doses of *p*-aminobenzoic acid have an action on the thyroid gland similar to that of thiouracil.[8] Experiments were performed to determine if this action would parallel that of thiouracil, when tested against the toxicity of the endotoxin and that of colchicine. The *p*-aminobenzoic acid was administered by stomach tube in 3 doses of 33 mg each; the first, one hour before injection of the toxic material, the other 2, at one-hour intervals after the injection. The results of these experiments are summarized in Table V. It is evident that *p*-aminobenzoic acid offers some protection

[7] Ludford, R. J., *J. Nat. Cancer Inst.*, 1945, **6**, 89.

[8] Gordon, A. S., Goldsmith, E. D., and Charipper, H. A., *Endocrinology*, 1945, **37**, 223.

TABLE V.

Dose of endotoxin (intraper.) mg	Dose of colchicine (intraper.) mg	Dose of *p*-aminobenzoic acid (stomach tube)	No. of mice	% survival
—	—	33 mg (×3)	15	100
0.75	—	33 mg (×3)	20	65
0.75	—	(control)	20	25
—	0.50	33 mg (×3)	15	0
—	0.50	(control)	15	14

against the effects of the endotoxin, but that, like thiouracil, it is ineffective against colchicine.

Discussion. The sulfonamides are now recognized as possessing a goitrogenic action.[9] Likewise the most evident physiological effects of thiouracil and of *p*-aminobenzoic acid are ascribable to interference with thyroid function.[8]

It seems reasonable to suppose, then, in view of the additional data presented in this paper, that the general protective effects of these 3 classes of compounds may be ascribed to a common mechanism, *viz.*, interference with thyroid function. This conclusion receives further support from some exploratory experiments in which mice for several days before endotoxin injection were fed on a diet containing 1% of thyroid extract. These animals appeared to be more susceptible to the toxic action of the endotoxin than were the untreated controls.

As to how thyroid interference and the presumed lowered metabolism renders the endotoxin less toxic, very little may be said. If, as has been postulated for colchicine,[10] the endotoxin is first oxidized to products which themselves are the effective poisons, then it is reasonable to suppose that the production of these secondary poisons would be retarded by a lowered metabolism, possibly allowing the normal excretory and detoxication systems of the body to cope with the antigen in its nontoxic phase.

The failure of colchicine to be affected by thiouracil treatment is provocative. There appears to be a general parallelism between the toxic effects of the endotoxin and the stereotyped response of the body to a considerable series of toxic agents. This body response has been termed "the general adaptation syndrome" by Selye,[11] and includes the diverse phenomena usually designated as "shock." It seemed appropriate to us to determine whether the action of goitrogens was specific for the endotoxin, or whether their effect might in an over-all way be directed at the harmful phases of "the general adaptation syndrome." Considerable interest was attached to colchicine in this connection, for colchicine poisoning induces a syndrome overtly resembling that of poisoning by the endotoxin, even to a parallelism in tumor hemorrhage induction.[7] Selye has stated that colchicine is an especially good reagent for eliciting the "alarm reaction"—the first stage of "the general adaptation syndrome." If one agrees that colchicine is indeed a typical elicitor of "the general adaptation syndrome," then goitrogen therapy in the later damaging phases of this syndrome is not likely to prove very useful, in view of the above reported failure of goitrogens to protect against colchicine. Some doubt, on the other hand, may be cast on the legitimacy of regarding colchicine as a reliable reagent for producing "the general adaptation syndrome" of Selye, especially in view of the protection obtained by goitrogens against dichloroethane poisoning, which bears little immediate resemblance to either colchicine or endotoxin poisoning. It may be remarked that protection against dichloroethane has also been reported for sulfanilamide and *p*-aminobenzoic acid, both of whose actions, like that of thiouracil, are goitro-

9 Astwood, E. B., *J. Pharmacol.*, 1943, **78**, 79.

10 Fuhrman, F. A., *Physiol. Rev.*, 1946, **25**, 247.

11 Selye, H., *J. Allergy*, 1946, **17**, 231; *ibid.*, 1946, **17**, 289.

genic.[12] Hence, it must be concluded that the influence of goitrogens on "the general adaptation syndrome" is an open issue.

Penicillin has lately been reported to afford protection against the toxic action of the gonococcal endotoxin.[13] It will be of interest to determine whether this protection is based on goitrogenesis.

Another question arising from the data presented in this paper concerns the role and value of fever in resistance within the body to the toxemia of infections caused by Gram-negative bacteria. From our data it would seem that the heightened metabolism of the febrile state would enhance the toxicity of any antigen released during the course of an infection; and that low temperature or lowered metabolism would have an opposite and helpful effect. On the other hand, it is supposed that fever promotes within limits both antibody formation and phagocytic activity.[14] Whether in therapy one of these actions should be supported at the expense of the other, cannot be said in the absence of adequate quantitative information.

Summary. (1) An extensive list of pharmacological agents is presented, whose protective effects against the *Salmonella* endotoxin is reported as negative. The endotoxin of *S. typhimurium* was chosen for such tests because it is considered to be typical of the endotoxins generally found in Gram-negative bacteria. (2) Thiouracil and *p*-aminobenzoic acid have a limited protective effect against the lethal action of the endotoxin. Thiouracil protects against endotoxin-induced tumor hemorrhage. It is concluded that these effects, as well as the similar effects of the sulfonamides, is via a common mechanism, *viz.*, interference with thyroid activity. (3) Thiouracil has some protective effect against 1,2-dichloroethane, but is ineffective against colchicine. *p*-Aminobenzoic acid is likewise ineffective against colchicine. (4) Mice maintained at high environmental temperatures are more vulnerable to the endotoxin than those kept at lower temperatures. (5) The application of Selye's concept of "the general adaptation syndrome" to the action of certain poisons is briefly discussed.

[12] Heppel, L. A., Neal, P. A., Perrin, T. L., Endicott, K. M., and Porterfield, V. T., *J. Pharm. and Exp. Therap.*, 1945, **84**, 53.

[13] Miller, C. P., and Boor, A. K., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 18.

[14] Perla, D., and Marmorston, J., *Natural Resistance and Clinical Medicine,* Little, Brown and Co., Boston, 1941, p. 1253.

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Growth Inhibition of Tubercle Bacilli by Fusarium, Sp.

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Growth of tubercle bacilli on media-containing egg or similar complex substances is inhibited by contamination of the medium with either fungi or bacteria. This inhibition is apparently not specific but results from the production of protein split product, deprivation of oxygen, etc.

On simpler mediums (*e.g.* Long's) the picture is very different. While some fungi are unable to grow on those mediums, others grow freely. As a rule they do not interfere with the growth of the tubercle bacillus and may even favor it.

A search was made for fungi capable of producing antibiotic agents. The following technic was used.

An alundun capsule (20 × 2 cm, dense) was introduced into a 250 cc Erlenmeyer flask containing 100 cc of Long's medium.

* Died October, 1946.