treated group whereupon the level of blood glucose rose sharply above the values for the treated rats which continued to work for periods up to 12 hours. The total average number of recorder revolutions for the group treated with ACE was 9482 ± 613 and the corresponding value for the untreated group was 5361 ± 261. The difference (4121 ± 1345) meets the usual requirements for statistical reliability.

Discussion. The presence of the liver and other intra-abdominal organs is not required for an effect of adrenal cortical hormones upon muscle work. This does not mean that the normal role of the liver in adrenal cortical physiology is unimportant.

The stimulation of muscle in the rat greatly accelerates the oxidation of glucose(6). On this basis it would be expected that the greater work output of the rats treated with ACE would increase glucose tolerance above that of untreated rats. Although muscle work does have a major effect upon glucose tolerance as is evidenced by the results of experiment 3 (Fig. 3), the effect of ACE in decreasing glucose tolerance can mask the effect of small differences in work output as shown in experiments 1 and 2 (Fig. 1 and 2). This effect of adrenal cortical extract in suppressing the glucose tolerance of eviscerated rats was previously observed in non-working animals(7). The basis of this extra-hepatic effect of ACE upon the utilization of glucose is not understood.

Summary. The continuous intravenous injection of adrenal cortical extract to adrenalectomized-eviscerate rats improved work output during the faradic stimulation of muscle in one hind leg. There was a concomitant elevation of blood glucose above the level of the untreated rats when the animals continued to work. When both hind legs were stimulated, muscular responsiveness was completely lost in each of the untreated rats within 6 hours and there was an immediate rise in the level of blood glucose above the values for the treated rats which continued to work.

1. Ingle, D. J., PROC. SOC. EXP. BIOL. AND MED., 1938, v39, 151.
2. ———, *Exp. Med. and Surg.*, 1949, v7, 34.
3. ———, *Endocrinol.*, 1944, v34, 191.
4. Pabst, M. L., Sheppard, R., and Kuizenga, M. H, *Endocrinol.*, 1947, v41, 55.
5. Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, 1936, v114, 583.
6. Stetten, DeWitt, Jr., Morley, E. H., and Ingle, D. J., Unpublished data.
7. Ingle, D. J., Prestrud, M. C., Nezamis, J. E., and Kuizenga, M. H., *Am. J. Physiol.*, 1947, v150, 423.

Received June 11, 1953. P.S.E.B.M., 1953, v83.

Soyin, A Toxic Protein from Soybean III. Immunochemical Properties.* (20410)

IRVIN E. LIENER AND JOSEPH E. ROSE. (Introduced by Paul D. Boyer)
From the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

Some of the biological and physical properties of soyin, a toxic protein recently isolated from defatted soybean flour, have been described previously(1-4). Electrophoretic and diffusion measurements of soyin† revealed a high degree of homogeneity, whereas sedimentation data indicated contamination with a small quantity of low molecular weight impurity(3). In the present investigation immunological technics were employed to obtain more precise information regarding the homogeneity of soyin. The data which were obtained also permitted a calculation of the soyin content of the original soybean flour from which the soyin had been prepared.

Methods. Preparation of soyin and crude

* Paper No. 3027 Scientific Journal Series, Minn. Agr. Exp. Station.

† The most highly purified fraction which has thus far been possible to prepare.

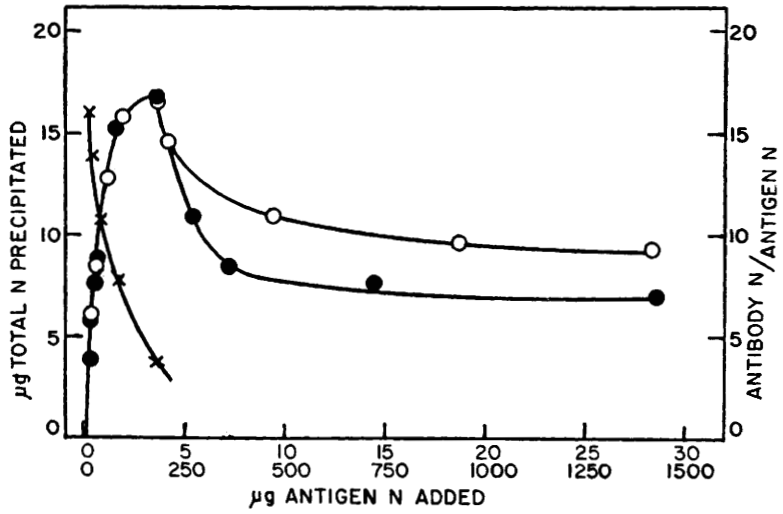

FIG. 1. Observed precipitin reaction of soyin 'Antiserum A' with soyin (-●—●-) and CSBE (-○—○-). Upper and lower abscissae refer to quantity of soyin N and CSBE N added respectively. Plot of the ratio of antibody N (total N precipitated minus soyin N) to antigen (soyin) N vs antigen (soyin) N is shown by the curve (-×—×-).

soybean extract (CSBE). The soyin preparation used in this work was the same as that used for the physical studies previously reported(3). The crude soybean extract was prepared by suspending 1 part of defatted soybean flour‡ in 10 parts of 0.9% NaCl. The pH was adjusted to 6.7, and the solution was stirred vigorously for 1 hr at room temperature. The clear, yellow supernatant that remained after centrifuging will be hereafter referred to as CSBE. Hemagglutinating tests (described below) on extracts of the insoluble residue showed that all of the hemagglutinating activity had been removed by the first extraction. *Preparation of soyin antiserum.* A rabbit was immunized by injecting soyin intravenously at levels of 1.5 mg, 3.0 mg, and 6.0 mg 3 times per week during the first, second, and third weeks respectively. Five days after the last injection blood was withdrawn by cardiac puncture and allowed to clot. The serum, centrifuged free of the retracted clot, was preserved by adding 0.1 mg merthiolate per ml, and stored in the refrigerator. No attempt was made to remove the complement from the serum. Serum obtained from this rabbit will be referred to as 'Antiserum A'. *Precipitin technic.* Various dilutions of soyin or CSBE (0.9% NaCl as the diluent) in a volume of 1 ml were added to 1 ml of diluted soyin antiserum (1 part serum + 2 parts of 0.9% NaCl). Appropriate controls of diluent plus antiserum and diluent plus antigen were likewise prepared. The tubes were held at 37°C for 1 hr, and kept in the refrigerator for at least 48 hrs before centrifuging. The precipitates were washed according to the procedure of Kabat and Mayer(5) and analyzed for nitrogen by the method of Lanni *et al.*(6). The latter procedure was also found to be very satisfactory for determining the nitrogen content of CSBE and solutions of soyin. *Toxicity and hemagglutinating activity.* The methods used for determining toxicity and hemagglutinating activity have been described previously(1). The effect of soyin antiserum on the hemagglutinating activity of soyin and CSBE was assessed in a quantitative manner by the following modification of the procedure described by Kabat and Mayer(5). To various dilutions of the antiserum (with 0.9% NaCl as the diluent) in a volume of 0.5 ml was added a constant amount of soyin or CSBE in a volume of 0.2 ml. After the tubes were kept at 37°C for 1 hr, 0.2 ml of a 4% washed suspension of rabbit erythrocytes in 0.9%

‡ Nutrisoy XXX, Archer-Daniels-Midland Co., Minneapolis, Minn.

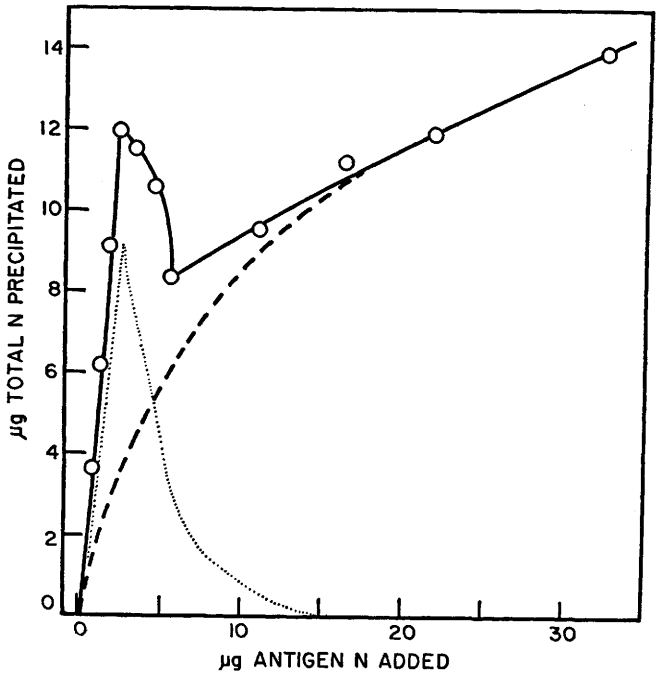

FIG. 2. Observed precipitin reaction of soyin antiserum B with soyin (-○——○-). Extrapolation to 0 of that portion of the observed curve extending beyond 15 μg antigen N added is shown by the curve (-----). Subtraction of the extrapolated curve from the observed curve gives the true soyin-antisoyin curve (.....).

NaCl was added to each tube. After holding at 37°C for an additional hr, the highest dilution of the serum causing complete inhibition of agglutination was recorded.

Results and discussion. Precipitin tests. In Fig. 1 are presented the data obtained when 'Antiserum A' was tested against soyin and CSBE. Plotting the total amount of N precipitated as a function of soyin N added gave a curve with the characteristic zones of antibody excess, equivalence, and antigen excess. The curve obtained with CSBE could be superimposed on the soyin curve in the region of antibody excess by reducing the units along the antigen axis by 1/50. If one assumes that these curves represent the reaction of a single antigen and a single antibody, the soyin content of CSBE would be 2%.§ Actually, however, the following lines of evidence suggested that the system involved here was not a simple reaction between 2 single components: a) as shown in Fig. 1 a plot of the ratio of antibody N (Ab) to antigen N (An) vs An failed to give a straight line such as would be expected from the Heidelberger-Kendall equation(7) Ab/An = a-bAn for a homogeneous antigen-antibody system, b) tests on the supernatants revealed an excess of both antigen and antibody at the point of maximum precipitation, and c) complete solubility of the specific precipitates in the region of antigen excess was not obtained. According to Cohen *et al.*(8) these facts provide strong evidence that the antibody-excess portion of the curves shown in Fig. 1 actually represents the sum of the reactions of soyin with its antibody and of a minor impurity with its antibody. This would, of course, invalidate any attempt to determine the soyin content of CSBE without first applying suitable corrections for the impurity.

To resolve the problem imposed by the presence of an impurity, advantage was taken of the fact that prolonged immunization favors antibody production against minor impurities (9). A rabbit injected with soyin according

§ The phrase "soyin content of CSBE" as employed in this paper refers to the ratio of soyin N to CSBE N (x 100 if expressed percentagewise).

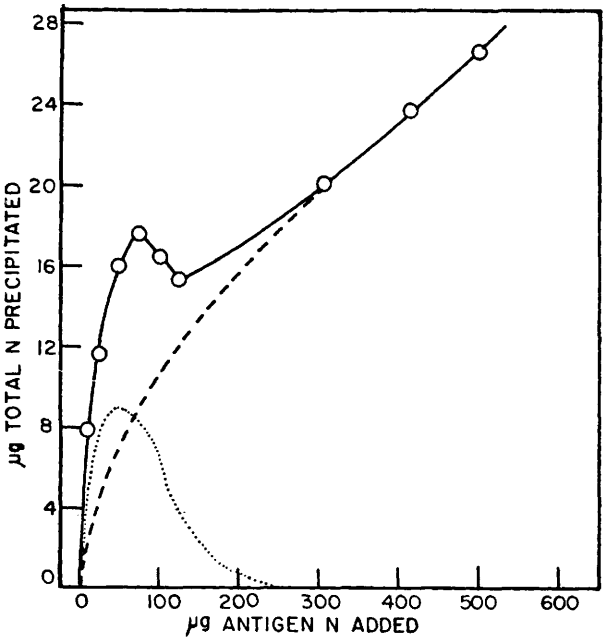

FIG. 3. Observed precipitin reaction of soyin antiserum B with CSBE (-○—○-). Extrapolation to 0 of that portion of the observed curve extending beyond 250 μg antigen N added is shown by the curve (- - - - -). Subtraction of the extrapolated curve from the observed curve gives the true soyin-antisoyin curve (.....).

to the previously described schedule was given additional injections of 2 mg at intervals of 1 week over a period of two months. As shown in Fig. 2 and 3 the serum from such an animal ('Antiserum B') when tested against soyin and CSBE now gave unequivocal evidence for the presence of at least 2 components.‖ In fact the rabbit produced more antibody against the impurity than against soyin itself. Following the procedure suggested by Cohen *et al.*(8), the equations describing the reaction of the impurity with its antibody (portions of the curves exceeding 15 and 250 μg antigen N in Fig. 2 and 3 respectively) were calculated, and the curves were extrapolated to 0. The true soyin-antisoyin curve is then given by the difference between the observed and extrapolated portion of the curve. It can be readily shown that the true soyin-antisoyin curves obtained with soyin and CSBE (Fig. 2 and 3 respectively) are superimposable in the region of antibody excess when the ratio of soyin N to CSBE N along the antigen axis is 1:10. This would indicate therefore that the soyin content of CSBE is closer to 10% than the 2% value which had been obtained when the presence of the impurity had not been taken into account:

'Antiserum B' gave a negative capillary precipitin test with the crystalline soybean trypsin inhibitor described by Kunitz(11).¶ This fact coupled with the observation that soyin has no antitryptic activity(1) makes it extremely unlikely that the impurity revealed by the quantitative precipitin test is the soybean trypsin inhibitor of Kunitz.

Toxicity and hemagglutinating data. A comparison of the toxicity and hemagglutinating activity of soyin and CSBE is made in Table I. The inhibitory effect of 'Antiserum A' on the hemagglutinating activity of soyin and CSBE is shown in Table II. The results obtained with 'Antiserum B' were essentially the same as those given for 'Antiserum A'. The soyin content of CSBE calculated from

‖ The agar diffusion technic of Becker and Munoz (10) revealed presence of two distinct bands when 'Antiserum B' was reacted with soyin.

¶ Purchased from Worthington Biochemical Laboratory, Freehold, N. J.

TABLE I. Toxicity and Hemagglutinating Activity of Soyin and a Crude Soybean Extract (CSBE).

	Soyin	CSBE
Toxicity*		
LD_{50}, mg N/kg body wt	7.0	77.0
% toxic N	100.0†	9.1
Hemagglutinating activity		
N content of original sol., μg N/ml	57.5	300.0
Highest dilution showing agglutination	1:16	1:8
Hemagglutinating titer, μg N‡	3.6	37.5
% hemagglutinin N	100.0†	9.6

* Administered intraper. into young weanling rats as described in(1).

† Assumed as point of reference.

‡ N content of highest dilution showing positive evidence of agglutination under conditions specified in(1).

TABLE II. Inhibition of Hemagglutinating Activity of Soyin and a Crude Soybean Extract (CSBE) by Soyin 'Antiserum A.'

	Soyin	CSBE
μg N added to each tube	11.5	60.0
Dilution of antiserum causing complete inhibition of agglutination	1:4	1:8
μg N neutralized by 1 ml antiserum	46	480
% hemagglutinin N	100.0*	9.6

* Assumed as point of reference.

TABLE III. Calculation of Soyin Content of Defatted Soybean Flour.

(a) mg CSBE N/g flour*	49.5
(b) mg soyin N/mg CSBE N†	.095
(c) Total mg soyin N/g flour (a × b)	4.7
(d) mg soyin N/g soyin‡	146.0
(e) % soyin in flour (c/d × 100)	3.2

* This represents total amount of N extracted from 1 g soybean flour by 10 ml 0.9% NaCl at pH 6.7 and 25°C.

† Avg value for soyin content of CSBE based on quantitative precipitin, toxicity, and hemagglutinating data. See Tables I and II in conjunction with text.

‡ N content of soyin on dry wt basis = 14.6%.

toxicity data, hemagglutinating activity and inhibition of hemagglutinating activity by soyin antiserum was 9.1%, 9.6%, and 9.6% respectively. These values are in substantial agreement with the amount of immunologically active soyin present in CSBE (10.0%).

Soyin content of soybean flour. Using an average value of 9.5% for the soyin content of CSBE, it is possible to calculate the soyin content of the soybean flour from which soyin was prepared. As shown in Table III this value is approximately 3%. This information proved to be particularly useful in rat growth studies designed to determine to what extent soyin was responsible for the poor nutritive value of raw soybean protein(2). In these experiments it was necessary to incorporate soyin into a basal ration containing heated soybean flour at a level which approximated the soyin content of a similar ration containing the unheated flour. These studies showed that soyin accounted for one-half of the growth inhibition that is obtained when unheated soy flour replaces the properly heated flour in the diet.

Miscellaneous observations. Anaphylaxis. Guinea pigs were sensitized with an intramuscular injection of 0.1 mg soyin, and, three weeks later, were challenged with an intracardial dose of 20 mg. Death which ensued within 20 minutes was preceded by symptoms characteristic of anaphylactic shock (rubbing of the nose, dyspnea, and convulsions). Nonsensitized controls receiving the same intracardial dose of soyin also succumbed, but death in this instance was delayed several hours with no evidence of anaphylaxis.

An attempt to produce active immunization. Sera obtained from rats which had been given intramuscular injections of soyin (0.1 mg 3 times per week for 3 weeks) gave a positive capillary precipitin test with soyin. Such animals, however, were just as susceptible to the toxic effects of soyin as normal controls.

Effects of oral ingestion. Experiments were conducted to ascertain if soyin is absorbed from the gastrointestinal tract of animals consuming the unheated flour. Sera taken from rats fed diets containing unheated soybean flour gave no precipitin reaction with soyin. Guinea pigs fed diets containing the raw flour did not become sensitized to a subsequent shocking dose of soyin, nor did sensitized guinea pigs display any evidence of anaphylactic shock when they were subsequently fed diets containing raw soy flour. Soyin, administered to rats by stomach tube up to a level of 500 mg/kg, did not produce death. This is about 10 times the dose which is toxic

intraperitoneally. This evidence strongly suggests that soyin may not be absorbed, or it may be inactivated as the result of digestive processes *in vivo*. This view is not necessarily inconsistent with the previous observation that the growth inhibition of rats fed soyin is simply the result of lowered food intake(2).

Summary. The quantitative precipitin technic revealed that the most highly purified preparation of soyin available was not strictly homogeneous as had been previously indicated by electrophoretic and diffusion measurements. By applying suitable corrections for the minor impurity present in soyin, the soyin content of a crude saline extract of defatted soybean flour was found to be approximately 10%. Toxicity and hemagglutinating data gave results which were in substantial agreement with this value. The soyin content of defatted soybean flour is about 3%.

1. Liener, I. E., and Pallansch, M. J., *J. Biol. Chem.*, 1952, v197, 29.
2. ———, *J. Nutrition*, 1953, v49, 527.
3. Pallansch, M. J., and Liener, I. E., *Arch. Biochem. and Biophys.*, in press.
4. Liener, I. E., Pallansch, M. J., and Rose, J. E., *Fed. Proc.*, 1953, v12, 790.
5. Kabat, E. A., and Mayer, M., *Experimental Immunochemistry*, Charles C. Thomas, Springfield, 1948.
6. Lanni, F., Dillon, M. L., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 4.
7. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, v62, 697.
8. Cohen, M., Wetter, L. R., and Deutsch, H. F., *J. Immunol.*, 1949, v61, 283.
9. Cohen, M., *Methods in Medical Research*, The Year Book Publishers, Inc., Chicago, 1952, v5, 280.
10. Becker, E., and Munoz, J., *J. Immunol.*, 1950, v65, 47.
11. Kunitz, M., *Science*, 1945, v101, 668.

Received June 15, 1953. P.S.E.B.M., 1953, v83.

Excretion of 3-(3-Amino-2,4,6-Triiodophenyl)-2-Ethyl-Propanoic Acid (Telepaque) by Man.* (20411)

J. SPALDING SCHRODER AND DONALD ROONEY. (Introduced by Arthur P. Richardson.)
From the Department of Medicine, Emory University School of Medicine, Emory University, Ga.

Recent reports(1-8) conclude that 3-(3-amino-2,4,6-triiodophenyl)-2-ethyl-propanoic acid (Telepaque) is superior to any of the cholecystographic media now available because of a significant reduction in the incidence of undesirable side effects and better gallbladder visualization. Studies on the excretion of this material by man have not been reported. McChesney and Wyzan(9) have studied the excretion of Telepaque by rats and recovered from 77.5% to 84.5% of ingested dye in the feces and from 2.1% to 4.8% in the urine, after the animals had been on the Telepaque-containing diet for 7 days. 11.3% to 11.8% was recovered from the digestive tract, and most of this material would have appeared in the feces in time. These findings are in sharp contrast to the excretion of beta-(4-hydroxy-3,5-diiodophenyl)-alpha-phenyl-propionic acid (Priodax) by man as reported by Crismer(10), who was able to recover 19% of the total ingested Priodax from the feces and 74% from the urine. The purpose of this communication is to report our findings on the excretion of Telepaque by man.

Method. Two grams of Telepaque (containing 1333.6 mg iodine) was given to five adults with normal gastrointestinal tracts. The 4 tablets were taken with supper at 6 P.M. when collection of 24-hour urine and fecal specimens was started. All the urine and feces were collected, thoroughly homogenized, and analyzed for total iodine present during the five 24-hour periods following the administration of the Telepaque. The iodine

* This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.