proline, whose isoelectric point is pH 6.3, *i.e.*, quite close to that of DAP-pH 5.8(6). In fact, Work(2) has isolated it with the neutral amino acids by electrodialysis.

Summary. The new amino acid, discovered by Work, α-ε-diaminopimelic acid, is absorbed on and eluted from starch similarly to lysine by the solvents 1:2:1 n-butyl-n-propyl-0.1 N-HCl and 2:1 n-propyl-0.5 N-HCl. It migrates in electrophoresis in barbiturate buffer pH 8.6 between glutamic acid and lysine but close to proline, whose isoelectric point is near to that of the diaminopimelic acid.

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Effect of Adrenal Cortical Extract upon Work Output and Glucose Tolerance of Adrenalectomized-Eviscerate Rat. (20409)

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The data of this study confirm an earlier report(1) that adrenal cortical extract (ACE) has a positive effect upon the work output of adrenalectomized-eviscerate rats. There is an accompanying decrease in the tolerance for intravenously administered glucose.

Methods. Male rats of the Sprague-Dawley strain from the Upjohn breeding colony were maintained on a diet of Archer Dog Pellets. The technic of evisceration, which is done in 2 stages, has been described(2). When the animals reached 250 ± 2 g they were anesthetized (intraperitoneal injection of 18 mg of cyclopal sodium) and were subjected to the second stage of the operation in which the adrenal glands were removed with the other intra-abdominal organs. The stimulation of muscle was started immediately following evisceration. The procedure was according to Ingle(3) with the following modifications. A Nerve Stimulator, Model B, Upjohn, was used to stimulate muscle 5 times per second by a D.C. pulse of 20 ma having a duration of 20 msec. The gastrocnemius muscle of the left hind leg was weighted with 100 g. In Exp. 1 and 2 this leg only was stimulated. In Exp. 3, both back legs were stimulated. The distance that the weight was lifted was registered on automatic work recorders. Each recorder revolution represented approximately 400 g-cm of work. A solution containing glucose, regular insulin (Lilly), penicillin and streptomycin with and without ACE [1 glycogen unit(4) per ml] was infused into the jugular vein of each rat. The animals were enclosed in a cabinet with temperature constant at 28 ± 0.5 C. The infusions were started simultaneously with the stimulation of muscle immediately following the operation. The analyses of glucose were made on tail blood by the method of Miller and Van Slyke (5).

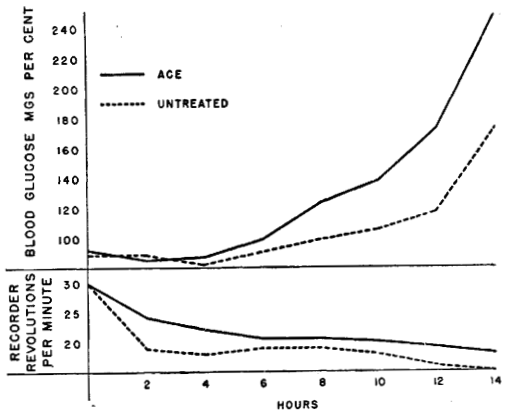

FIG. 1. Effect of ACE upon work output and glucose tolerance of adrenalectomized-eviscerate rats given a fluid load of 40 ml per rat per 24 hr. Stimulate one back leg. Averages.

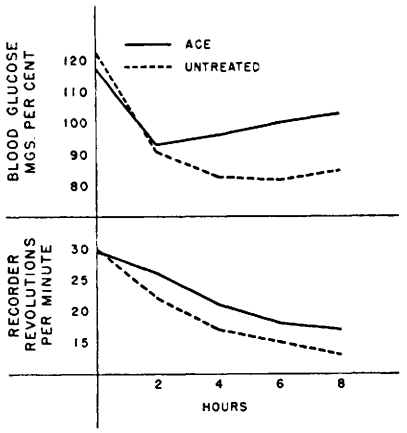

FIG. 2. Effect of ACE upon work output and glucose tolerance of adrenalectomized-eviscerate rats given a fluid load of 20 ml per rat per 24 hr. Stimulate one back leg. Averages.

Experiments and results. In experiment one, 31 adrenalectomized-eviscerate rats were each given 40 ml of solution per 24 hours containing 4 units of insulin, 10,000 units of penicillin, 20 mg of streptomycin and a glucose load of 125 mg per 100 g of rat per hour. Fifteen of the rats each received 20 glycogen units of ACE per 24 hours added to the above solution. The remaining 16 rats did not receive ACE. The level of blood glucose was determined at 2-hour intervals during the first 14 hours. The data of Fig. 1 show that the work output of the rats treated with ACE was enhanced above that of the untreated rats and that there was an accompanying elevation in blood glucose. At the end of 24 hours the average total number of recorder revolutions for the rats treated with ACE was 26429 $\pm$ 1470 and the corresponding value for the untreated rats was 21756 $\pm$ 1047. The difference (4673 $\pm$ 1805) meets the usual requirements for statistical reliability.

In Exp. 2, 35 adrenalectomized-eviscerate rats were each given 20 ml of solution per 24 hours containing the same loads of glucose, insulin and antibiotics as in Exp. 1. Seventeen of the rats each received 12 glycogen units of ACE per 24 hours added to the above solution. The remaining 18 rats did not receive ACE. The level of blood glucose was determined at 2-hour intervals during the first 8 hours. The data of Fig. 2 show that the work output of the rats treated with ACE was greater than that of the untreated rats and that there was an accompanying elevation in blood glucose. At the end of 24 hours the average total number of recorder revolutions for the rats treated with ACE was 20364 $\pm$ 997 and the corresponding value for the untreated rats was 14451 $\pm$ 866. The difference (5913 $\pm$ 1320) meets the usual requirements for statistical reliability.

In Exp. 3, 36 adrenalectomized-eviscerate rats were each given 20 ml of solution per 24 hours representing a glucose load of 200 mg per 100 g of rat per hour and the same loads of insulin and antibiotics used in Exp. 1 and 2. Eighteen rats received 12 glycogen units of ACE per 24 hours added to the above solution and the remaining 18 rats did not receive ACE. In this experiment both back legs were stimulated. The level of blood glucose was determined at 2-hour intervals during the first 8 hours. The data of Fig. 3 show that the work output of the group treated with ACE was greater than that of the untreated group. By the 6th hour, muscular responsiveness was lost in all of the animals of the un-

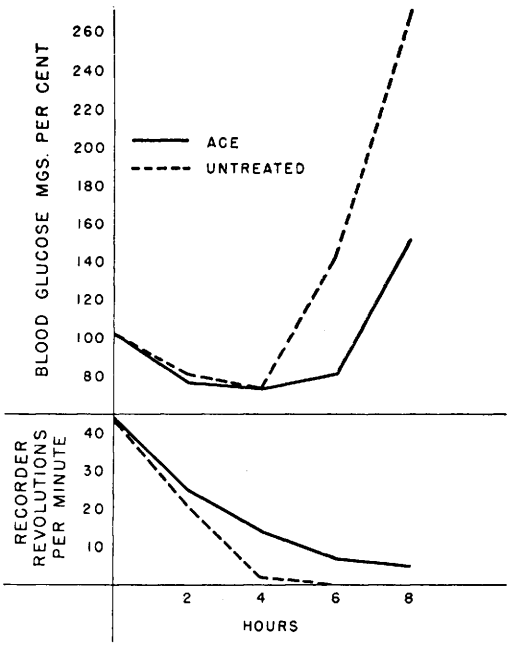

FIG. 3. Effect of ACE upon work output and glucose tolerance of adrenalectomized-eviscerate rats given a fluid load of 20 ml per rat per 24 hr. Stimulate both back legs. Averages.

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.

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

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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.