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Chromatography of α-ε-Diaminopimelic Acid on Starch Columns.* (20408)

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In connection with studies on the amino acid composition of certain proteins it was desirable to know the behavior on starch chromatographic columns of the recently discovered amino acid, α-ε-diaminopimelic acid‡ (called in this paper DAP) isolated by Work (1,2). It was of interest also to note its mobility by means of paper electrophoresis.

Procedure. The starch columns (0.9 x 30 cm) were prepared as recommended by Moore and Stein(3), and the effluent values of the DAP and certain amino acids added to the solution as markers were followed by means of the ninhydrin reaction on fractions collected by an automatic fraction collector and drop-counting apparatus(4). Two solvents were used in this study, 1:2:1 (n-butyl-n-propyl, 0.1 N-HCl) and 2:1(n-propyl-0.5N HCl). The DAP was dissolved in 0.1 N-HCl to which was then added 4 volumes of 1:2:1 solvent, giving a final concentration of 3 mg DAP per ml. Proper aliquots were added to the columns to give the quantities designated in Table I. For paper electrophoresis§ the different mixtures of amino acids as used in the chromatography experiments were placed on strips of Whatman No. 1 filter paper and run at 130 volts and 25 milliamperes for 2 hours in barbiturate buffer pH 8.6. The buffer was made as follows. Sodium barbiturate, 29.428 g, sodium acetate 3 H_2O, 19.428 g and N/10 HCl 180 ml were dissolved in 3000 ml distilled water and then diluted with an equal amount of distilled water. The same amino acids as mentioned above were also dissolved directly in the buffer before placing them on paper strips. At the end of the experiment the strips were dried in an oven, sprayed with ninhydrin and heated 4 minutes at 90°C.

Results. In the first chromatographic experiment leucine and DAP were put on the column with the 2:1 solvent and the DAP was found to emerge at an effluent value of 80 ml, as shown in Table I. It remained on the column in experiment 2 when the 1:2:1 solvent was used and emerged only after the solvent was replaced with 2:1 solvent and then at an effluent value about 65 ml past this change. These values were suggestive of the position for lysine. Therefore, in a third experiment lysine was added to DAP and the mixture placed on a column. At the same time a mixture of proline and DAP was run in tandem. As shown in Table I, DAP emerged identically with lysine as a single sharp peak and the recovery was close to the theoretical value. (Fig. 1).

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‡ For these studies some of the amino acid was generously given to us by Dr. Work.

§ Paper strip model electrophoresis apparatus obtained from Arthur H. Thomas Co., Philadelphia, Pa.

TABLE I. Effluent Values and Recovery of α-ε-Diaminopimelic Acid on Starch Columns. 4 experiments.

Solvents used	Changed solvent at effluent value, ml	Amino acid	Effluent value, ml	mg amino acid Added	mg amino acid Recovered
2:1		Leucine	10	.3	.25
		+ DAP	80	.5	.55
1:2:1 & 2:1	104.4	Leucine	14	.3	.31
		+ DAP	169	.5	.55
1:2:1 & 2:1	101.4	Lysine	171	.3	.59
		+ DAP	171	.3	
1:2:1 & 2:1	99.5	Proline	62	.3	.31
		+ DAP	173	.3	.32

When studied by means of paper electrophoresis at 25 milliamperes for 2 hours in barbiturate buffer, pH 8.8, as described above, the DAP was found to migrate only slightly slower 42 mm, than proline 45 mm, considerably slower than lysine 80 mm, and faster than glutamic acid 0 mm. See Fig. 2. These relative migrations were found to be the same whether the amino acids were dissolved in the 1:2:1 solvent or in the buffer solution (pH 8.6) used for the paper electrophoresis.

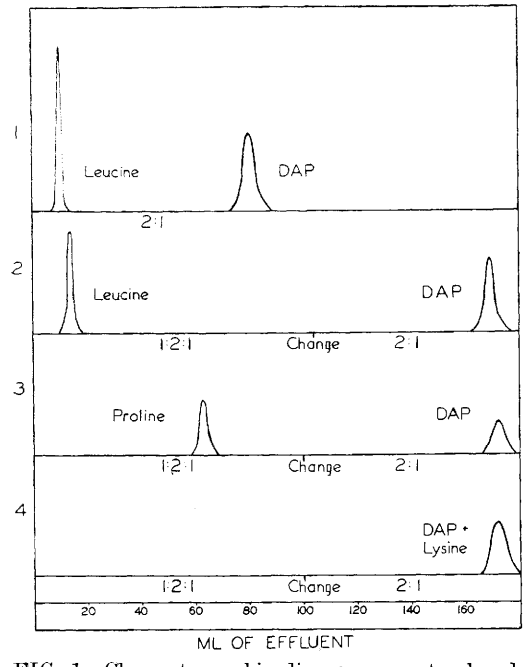

FIG. 1. Chromatographic diagrams on starch columns (0.9 × 30 cm) of DAP mixed with leucine (1 and 2), with proline (3), and with lysine (4). Solvents were 1:2:1 n-butyl-n-propyl –0.1 N-HCl and 2:1 n-propyl –0.5 N-HCl.

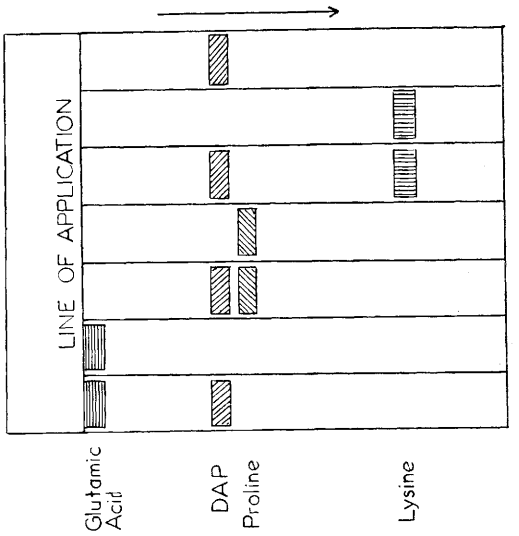

FIG. 2. Paper electrophoretic diagrams of DAP and DAP mixed with glutamic acid, proline or lysine. Barbiturate buffer, pH 8.6, for 2 hr at 25 milliamperes.

Discussion. The fact that the DAP is eluted from starch at the same effluent value as lysine is of interest in view of the suggestion of Work(5) that this amino acid may be a biological precursor of lysine and also of the finding(1) that the R_F of the DAP was similar to that of lysine by paper chromatography in collidine. In phenol, on the other hand, she found DAP and glutamic acid to travel more closely.

These chromatographic absorption properties by starch or by paper are obviously not dependent upon electrical charge, since DAP does not migrate in electrophoresis like either lysine or glutamic acid. Instead, its migration is similar to that of the neutral amino acid

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).

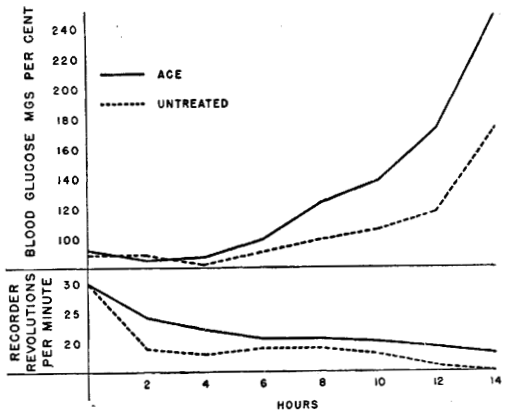

FIG. 1. Effect of ACE upon work output and glucose tolerance of adrenalectomized-eviscerate rats given a fluid load of 40 ml per rat per 24 hr. Stimulate one back leg. Averages.