detectable skeletal lesions, although the rats were stunted. γ-Aminobutyronitrile at the 0.2-percent level did not produce any lesions, and the animals developed normally during the 34 days of feeding.

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Purification of Angiotonin (Hypertensin)

Carl A. Kuether and Michael E. Haney, Jr.

Lilly Research Laboratories,
Eli Lilly and Company, Indianapolis, Indiana

Several reports have appeared recently describing the purification of angiotonin (*1–3*). Recent observations in these laboratories have provided evidence that angiotonin may be purified to yield preparations of higher purity than those previously reported. However, these preparations are unstable under all the conditions we have studied.

The specific activity of the various preparations varies widely (Table 1). The data also show that specific activities based on comparison with tyramine are significantly different from those based on direct comparison with angiotonin. The difference is undoubtedly the result of the fact that each investigator has used a different type of animal preparation for assay. Further, tyramine is not a suitable reference standard for angiotonin assay (*1, 4*). For comparative purposes, a standard method of assay and a stable, standard preparation of angiotonin would be of great value.

Table 2. Summary of purification steps.

Step	Yield		Specific activity (AU/mg)
	Grams	Percentage of starting activity	
Crude	1104	100	1.59
Methanol and phenol extractions	33.0	88.1	46.6
Partition column	2.02	17.4	151
Countercurrent distribution	0.8641	10.1	205
Electrophoresis	.0585	5.7	1710
Ether precipitation	.0042	16.4	68000

Crude angiotonin was prepared (*5*) by the method of Plentl and Page (*6*) from hog renin substrate and hog renin. The clear filtrate was concentrated in vacuum, autoclaved, filtered, and frozen-dried.

Purification of the material was effected by a series of steps involving (i) extraction into methanol, (ii) extraction from an aqueous solution into phenol, (iii) partition on a silica column using a solvent system composed of *n*-butanol, acetic acid, and water, (iv) counter-current distribution with a solvent system composed of acetate buffer *p*H 3.0, *n*-butanol, and methanol, (v) electrophoresis (*7*) in acetate buffer, *p*H 4.2, (vi) precipitation from methanol solution by addition of ether. As an illustration, data for one series of experiments are summarized in Table 2; 7090 lit of hog blood was treated with renin to obtain the 1104 g of crude angiotonin.

Angiotonin with high specific activity is unstable and loses pressor activity rapidly. The initial specific activity, 68,000 angiotonin units per milligram (AU/mg), was obtained when the sample (No. 221-194B-74-5) was assayed immediately the morning it was prepared. Assays that afternoon indicated an activity of 24,000 AU/mg, and the next morning, 424

Table 1. Specific activity of angiotonin preparations reported in the literature. The angiotonin unit, AU, is the pressor activity of 30 μg of a frozen-dried preparation of angiotonin (*11*). In the pithed rat preparation (*12*) it has a pressor activity equivalent to 0.155 Goldblatt unit, GU (*13*), to 0.863 of the unit used by Bumpus, BU (*13*), and to approximately 0.18 mg of tyramine hydrochloride. The nitrogen content of angiotonin is assumed to be 16 percent.

Preparation	Tyramine phosphate (mg/mg)	Tyramine hydrochloride (mg/mg)	AU/mg based on tyramine	AU/mg based on angiotonin	GU/mg *N*	BU/mg *N*
Edman 1945 (*4*)	39*		161			
Helmer 1950 (*11*)				37*		
Clark *et al.* 1954 (*1*)		5.8*	32	245		
Skeggs *et al.* 1954 (*2*)				5150	5000*	
Bumpus *et al.* 1954 (*3*)			402	2780		15000*
Kuether and Haney				68000*		

* The original datum used in the comparison.

AU/mg. This loss in activity was observed when the dilutions for assay were made in 0.9 percent NaCl solution. We have been unable to prevent these losses by making the dilutions in 0.2*M* sodium citrate at *p*H 6, 0.1 percent disodium ethylenediaminotetraacetic acid, 0.5 percent ascorbic acid in distilled water deionized with Amberlite MB-1 resin, or solutions saturated with nitrogen gas. The decrease in activity seems to continue until a specific activity of about 400 to 500 AU/mg is reached. In some preparations the activity had dropped to this level before assays could be completed.

The increase in total units observed following electrophoresis and methanol-ether fractionation has been observed each time one of these experiments has been run. We are not prepared to explain this observation, but a possibility is the separation of a substance that selectively combines with the active group of angiotonin.

Paper chromatography (*8*) of the acid hydrolyzate of this sample (No. 221-194B-74-5) showed the presence of the following amino acids: Asp, Glu, Gly, His, Ala, Pro, Ser, Tyr, Val, Thr, Phe, Leu, Ileu, Arg, and Lys. These results differ from those reported by Bumpus and Page (*9*) in that we have found threonine, and differ from Edman's (*4*) data in including threonine, phenylalanine, and arginine.

Paper chromatography (*8*) of the ether soluble and nonether soluble fractions of the acid hydrolyzate of the dinitrophenyl (DNP) derivative (*10*) showed the presence of ε-DNP lysine only. We are thus unable to confirm the observation (*9*) that angiotonin has a free amino group on aspartic acid.

An attempt to repeat this preparation differed from the first series of experiments in two ways: (i) the countercurrent distribution step was eliminated, and (ii) the electrophoresis was run in acetate buffer made up in a 0.1 percent ascorbic acid solution in deionized water. In this preparation (No. 221-194B-168-2) 2800 lit of hog blood gave 21.5 mg of angiotonin with a specific activity of 13,500 AU/mg.

These experiments provide evidence that angiotonin may be purified to obtain preparations of greater activity than hitherto reported. The marked instability of the highly purified preparations and the deterioration to a residual activity of about 500 AU/mg have not been explained. These observations suggest that caution be exercised in the interpretation of results of earlier purification efforts.

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Communications

Some Glass Apparatus Improvements

Explained and illustrated in this report are some improvements in design of several existing models of laboratory glass apparatus. These items of apparatus include solid-absorbent drying tubes employing sintered glass disks for a gas passage and an improved version of the standard Ten Broeck glass homogenizer. These units, shown in Figs. 1 and 2, have been used successfully in this laboratory within the past year.

Use of sintered glass disks in permanently sealed solid-absorbent drying tubes. An essential item found in many modern instruments is a container filled with anhydrous silica gel mounted strategically near a moisture-sensitive portion of the instrument. The container has to permit perfect access of the adjacent air for thorough drying by the silica gel and is usually easily detached for drying the spent agent. To avoid the necessity of emptying old drying agent and replacing with fresh gel, it is convenient to seal the gel in a tube equipped with sintered glass ends of the proper porosity which will permit the free passage of gases through the silica gel without the danger of tiny particles of the gel entering the remainder of the system.

Several models of such containers, designed for general application, are shown in Fig. 1. Tube *A* is of the standard form used for drying gases. The bulge represents the site of the opening used as an aid in initially filling the tube and later sealed off. Tube *B* represents a straight variation of this model, while tube *C* is a representative model used for drying stationary volumes of gases inside such moisture-sensitive instruments as the Beckman spectrophotometer and alpha-particle counting chambers.

The great advantage in the use of these tubes with the agent sealed in place is that the entire tube can be placed in an oven for drying the spent agent and then replaced in its original location. This completely eliminates the need for handling additional amounts