then 3 times weekly, the longer period being found sufficient for statistical purposes. The pressures for each week were averaged and the rise or fall recorded. Increases of 20 mm. of mercury or greater were considered as significant.

Fourteen animals injected with neutralized aspartic acid (doses varying between 20 and 250 mg. per kilo of body weight) all showed a rise in systolic blood pressure, the maximum rise being 25 mm. after 6 weeks and 73 mm. after 22 weeks. (See chart 1). The minimum dose of aspartic acid to produce an elevation of blood pressure was 40 mg. per kilo. Further increases of aspartic acid did not cause a more rapid or higher elevation of blood pressure. Blood urea and creatinin were within normal limits. The urine showed albumin and casts in 4 animals. Newburgh and Marsh[2] report that aspartic acid is mildly nephrotoxic when injected intravenously into rabbits. They also state that the smallest dose that produced renal injury was 0.5 gm. per kilo. Our dosage was considerably less. The histological changes observed were thickening of the elastic tissues of the aorta, congestion of the glomeruli of the kidney, and in a few instances tubular degeneration.

Two rabbits injected with guanidine carbonate, neutralized with HCl, showed a maximum rise of 42 and 46 mm. in systolic blood pressure after 5 weeks. No abnormal changes in the blood urea or creatinin or in the urine were noted. No histological change in the kidney was observed in one animal after 7 weeks of injection.

Rabbits injected with glutamic acid and succinic acid showed no definite rise in blood pressure.

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Studies in Hypertension. II. Changes in Blood Proteins in Experimental Hypertension.

ADOLPH BERNHARD AND I. J. DREKTER.

From the Achelis Laboratory and the A. Jacobi Division for Children, Lenox Hill Hospital, New York City.

It has been shown[1] that in rabbits the daily injection of aspartic acid and guanidine produces a definite rise in blood pressure. The blood proteins of these animals were analyzed both before and after

[2] Newburgh, L. H., and Marsh, P. L., *Arch. Int. Med.*, 1925, **36**, 682.

[1] Rafsky, H. A., Bernhard, A., and Rohdenburg, G. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1243.

a definite and prolonged elevation of the blood pressure was obtained. Control animals injected with putrescin, tyrosine, and tryptophane were observed for a similar length of time and their blood proteins were analyzed.

The red cells were separated from the oxalated blood by hypertonic sodium sulphate as suggested by Folin[2], and the protein precipitated from the supernatant fluid with sulphuric acid. This procedure precipitates all the serum proteins from a definite volume of blood. The Van Slyke[3] method of protein analysis was adapted to micro methods.

The whole blood, the red cells, and the serum proteins from 1 cc. of blood were hydrolyzed with 10 cc. of concentrated HCl in a small Erlenmeyer flask covered with a funnel and placed on a sand bath. The hydrolysis was continued for 4 hours and the mixture evaporated to dryness on the water bath. The contents of the flask were diluted to 15 cc., transferred to a large centrifuge tube and magnesium hydroxide added, then aerated for 1 hour for the determination of the amide nitrogen. The residue is centrifuged, filtered, and washed, and the filtrate and washings diluted to 40 cc. Five cc. is removed for the determination of nitrogen after hydrolysis, and to the remaining filtrate, 5 cc. of 10% phosphotungstic acid in 10% H_2SO_4 is added, the mixture allowed to stand overnight, then centrifuged and washed with phosphotungstic acid solution. The entire precipitate obtained by centrifugation was digested and the total nitrogen determined, which gives basic amino nitrogen precipitated by phosphotungstic acid. The filtrate from the phosphotungstic acid precipitation was extracted with amyl alcohol and ether, neutralized with NaOH, 10 cc. removed for total nitrogen, and 2 cc. for the manometric determination of monoamino nitrogen.

Osborne and Harris[4] and Van Slyke[3] have shown that under suitable conditions the solubility of the phosphotungstic acid precipitate is very small. We have observed that the precipitation of basic amino nitrogen by phosphotungstic acid is much more complete in solutions diluted as stated above. The results reported in this communication represent the total nitrogen of the basic amino nitrogen precipitated, duplicate determinations checking within 0.1 mg. of nitrogen. Therefore it seems that differences obtained in this fraction between normal rabbits and those in which hypertension has been induced are significant.

[2] Folin, O., *J. Biol. Chem.*, 1930, **86**, 173.

[3] Van Slyke, D. D., *J. Biol. Chem.*, 1911, **10**, 15.

[4] Osborne, T. B., and Harris, I. F., *J. Am. Chem. Soc.*, 1903, **25**, 323.

TABLE I.

Blood serum proteins of normal and hypertensive rabbits in mg. per 100 cc.

Blood Pressure M. M. of Hg.	Total N.	Amide N.	Humin N.	Total N.* of Filtrate	Basic Amino N precipitated by Phospho-tungstic Acid	Total N in Phospho-tungstic Acid Filtrate	Monoamino N.	Remarks.
102	700	27	123	550	140	410	400	Control Animal
110	630	17	93	520	110	400	380	Control Animal
98	830	20	280	530	100	400	400	Control Period
104	830	20	260	550	130	420	400	After Tryptophane injections
100	900	24	336	540	120	420	400	Control Period
102	900	24	326	550	140	400	400	After Tyrosine injections
106	800	23	203	570	100	470	400	Control Period
108	800	20	210	570	130	440	400	After Putrescine injections
90	850	21	289	540	110	430	400	Control Period
130	850	21	199	630	30	600	600	After Aspartic Acid injections
107	900	22	348	530	130	400	300	Control Period
128	780	22	368	390	40	350	340	After Guanidine injections
104	800	40	260	500	100	400	350	Control Period
140	850	40	170	640	40	600	600	After Guanidine injections

*From hydrolysis after removal of humin and amide N.

The completeness of the hydrolysis of the proteins was determined by experiment for periods of 2, 4, 8, and 24 hours and checked against Van Slyke's procedure. It was found that there was no further hydrolysis at the end of 24 hours than at the end of 2 hours, but to insure complete hydrolysis of the various blood samples the 4-hour period was employed.

The formation of humin nitrogen from the basic amino nitrogen is rather small. Roxas[5] showed that less than 10% of the basic amino nitrogen played any rôle in humin nitrogen formation. In the results reported here the differences in basic amino nitrogen between normal and hypertensive animals is far too great to be explained by the possibility of humin nitrogen formation from this fraction.

In the control animals there were no appreciable changes in the total nitrogen, amid nitrogen, or amino-nitrogen fractions or a rise of blood pressure after injection of putrescin, tryptophane, or tyrosin.

In those animals in which hypertension was produced by the injection of aspartic acid and guanidine very definite changes were observed in the blood serum fraction. (Table I.)

The basic amino nitrogen precipitated by phosphotungstic acid is about 20% of the total nitrogen after hydrolysis for normal animals, and the monoamino nitrogen is between 70-80%. In those animals with hypertension the basic amino nitrogen varied between 4.9-10%, while the monoamino nitrogen varied between 80-90%. There were no changes in the amide nitrogen either before or after injection of aspartic acid or guanidine.

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Studies in Hypertension. III. Blood Proteins in Children with Hypertension.

ADOLPH BERNHARD, JEROME S. LEOPOLD AND I. J. DREKTER.

From the Achelis Laboratory and the A. Jacobi Division for Children, Lenox Hill Hospital, New York City.

In rabbits with hypertension changes in the basic amino nitrogen, and the monoamino nitrogen fractions of the blood proteins have been described by Bernhard and Drekter[1]. The investigation has

[5] Roxas, M. L., *J. Biol. Chem.*, 1916, **27**, 71.

[1] Bernhard, A., and Drekter, I. J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1245.