greatly influence the count. If results are expressed as number per million nucleated erythrocytic cells, megakaryocytes are actually more numerous than results would indicate in diseases characterized by erythrocytic hyperplasia such as blood loss anemia due to purpura hemorrhagica and actually less numerous than the results would indicate in erythrocytic hypoplasia. The same objections apply when the result is expressed as number per million nucleated marrow cells and, in addition, leukocytosis, leukemia, and other hyper- or hypoplastic diseases of the leukocytic system would distort the apparent number of megakaryocytes. For these reasons, it would be preferable to determine the absolute number per ml.

Study by this thick drop technic of the number of megakaryocytes found in normal and pathologic marrows is under way, but not yet complete. Preliminary results would seem to indicate that the normal range is between 500 and 4000 megakaryocytes per ml; between 20 and 150 per million nucleated marrow cells; and between 50 and 300 per million nucleated erythrocytic cells.

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Influence of Amino-Acids on Adrenal Enlargement, Nephrosclerosis and Hypertension by Anterior Pituitary Preparations.*

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Previous investigations carried out in this laboratory have shown that overdosage with lyophilized anterior pituitary (LAP) can produce nephrosclerosis and hypertension in rats fed a 30% casein diet.[1] It was also observed that different kinds of proteins[2] and protein hydrolysates[3] are as effective as casein in the production of nephrosclerosis and hypertension by LAP. This paper is concerned with the nephrosclerotic activity of LAP in animals fed a diet in which the protein component was provided by a mixture of crystalline amino-acids.

Material and methods. 25 hooded black-and-white male rats weighing 110 to 130 g were divided into 4 groups. Groups I and II received a "22%" amino-acid‡ diet and Groups III and IV a "22%" Amigen‡ (enzymatic casein hydrolysate) diet, both rations containing 4% sodium chloride. In order to maintain equal food intake, all animals received their diet as a 70 g % aqueous suspension, 3 times daily at 6-hour intervals through a stomach tube. First Groups I and II were adapted to this procedure by forced-feeding mixtures of Amigen and amino-acids in which the proportion of Amigen was gradually decreased so that on the 10th day only amino-acids were fed. At the same time, Groups III and IV were adapted to forced feeding with the Amigen diet. The volume and caloric value of the food given was the same in all groups. The amount of food was so adjusted that the animals slightly increased in weight.

After the adaptation period, all animals

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1 Dontigny, P., Hay, E. C., Prado, J. L., and Selye, H., *Am. J. Med. Sciences*, 1948, **215**, 442.

2 Hay, E. C., Prado, J. L., and Selye, H., *Canad. J. Research* (Section E, Med. Sc.), 1948, **26**, 212.

3 DeGrandpré, R., Prado, J. L., Dontigny, P., Leduc, J., and Selye, H., *Fed. Proc.*, 1948, **7**, 27.

‡ "22%" means a nitrogen content corresponding to 22% casein. For composition of diets see Tables I and II.

TABLE I.
Composition of Amino-acids Mixture.

	g
l-(+)-arginine hydrochloride	5.8
l-(+)-histidine ''	11.9
l-(+)-lysine ''	30.0
dl-isoleucine	24.0
l-(—)leucine	21.6
dl-methionine	14.4
dl-phenylalanine	16.8
dl-threonine	28.8
dl-tryptophane	4.8
dl-valine	33.6
l-(+)-glutamic acid	137
l-(—)-tyrosine	20.0

TABLE II.
Composition of Diets (Parts %).

	Amigen	Amino-acids diet
Amigen	30*	—
Amino-acids mixture	—	37*
Corn starch	28	28
Sugar	30	30
Cod liver oil	1	1
Fat	1	1
Steenbock's mineral mixture	4	4
Sodium chloride	4	2.5
Cellu flour	1	1
Charcoal	5	5
Vitamins[4]	10 ml	10 ml
Sodium bicarbonate†	—	21.3

* These amounts correspond in nitrogen to 22 g of casein.

† Used for neutralization of the amino-acids hydrochlorides.

were castrated and unilaterally nephrectomized. LAP treatment was started the following day in Groups I and III, and continued for 27 days. LAP was administered in 2 daily subcutaneous injections of 0.1 cc of 20 mg/day during 15 days, this dose being raised to 30 mg for 6 days and to 40 mg during the last 6 days.

The blood pressures were determined on the 27th day of treatment by the direct method, as previously described,[1] the animals being killed next day.

The severity of nephrosclerosis was designated in terms of a scale of 0 to +++. The criteria employed microscopically were arteriolonecrosis, capillary tuft hyalinization and cast formation with resultant tubule dilatation and atrophy.

Discussion. As may be seen from Table III, adrenal enlargement, nephrosclerosis and hypertension can be produced by LAP, on a

TABLE III.
Effect of Amino-acids Content of the Diet on Hypertension Produced by LAP.*

Groups	No. rats	Treatment	Body wt		Nephrosclerosis‡		Adrenal wt, mg	Kidney wt, mg/100 cm² b. surface	Blood pressure, mm Hg.
			Initial	Final	Incidence, %	Severity, %			
1	6	"22%"† amino-acids + LAP	128	189 ± 3.5	50	22	72 ± 6.1	663 ± 51	143.8 ± 20
2	6	"22%" amino-acids	126	169 ± 2.2	0	0	32 ± 1.2	420 ± 18	115.5 ± 13.9
3	6	"22%" Amigen + LAP	126	225 ± 3.0	100	75	92 ± 11	629 ± 18	140.6 ± 6.3
4	7	"22%" Amigen	124	194 ± 2.2	0	0	39 ± 1.3	324 ± 11.3	129.4 ± 10.8

* Lyophilized anterior pituitary.
† "22%" means a nitrogen content corresponding to 22% casein.
‡ Nephrosclerosis as observed by microscopic examination.

diet in which amino-acids completely replace proteins. This finding indicates that the constituent amino-acids are responsible for the conditioning, by dietary proteins, to the above toxic effects of LAP overdosage. Our data also show that the growth, adrenal enlargement and nephrosclerosis produced by LAP were less pronounced on the amino-acid diet than on the Amigen diet of equal nitrogen content. It may incidentally be mentioned that the usual myocarditic changes[5] which accompany this type of hypertension were also less obvious in the amino-acid than in the Amigen groups. This could possibly be explained by the fact that the animals of Groups I and II received an unnatural mixture of amino-acids. Yet the existing difference between Groups I and III is of sufficient magnitude to suggest that equal amounts of amino-N are not necessarily equally conducive to the production of nephrosclerosis by LAP.

Summary. Adrenal enlargement, nephrosclerosis and hypertension were produced by a lyophylized anterior pituitary (LAP) preparation in rats kept on a synthetic diet containing no protein, but an adequate amount of amino-acids. The severity of the lesions was less marked, however, than in rats receiving an equivalent amount of nitrogen in the form of a casein-hydrolysate. It appears that amino-acids suffice to sensitize the organism to the production of hypertensive disease by LAP. This is noteworthy since no such pathologic changes can be produced by LAP on other diets deficient in proteins.

[4] Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, The Blakiston Co., Philadelphia, 1947.

[5] Selye, H., *J. Clin. Endocrinol.*, 1946, **6**, 117.

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Comparative Assays of Rodenticides on Wild Norway Rats. II. Acceptance.*

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In the previous paper of this series,[1] 8 common rodenticides were compared on the basis of their acute toxicities to recently trapped wild Norway rats. As was pointed out in that paper, a high toxicity does not, however, guarantee effectiveness under field conditions; of at least equal importance are those properties, such as a bitter taste or the lack of it, which influence the voluntary consumption of poisoned baits. It is the purpose of the present work to reexamine the same 8 rodenticides in the light of their acceptability and effectiveness when offered to wild Norway rats in a standard bait under controlled conditions in the laboratory.

Methods. The 565 wild Norway rats on which data are here given were trapped in the city of Baltimore during the period from July 1946 to December 1947. Before use they were kept in the laboratory, in large holding cages, for a few days; this delay proved necessary to allow for occasional deaths or for signs of debility to become evident. They were maintained on Purina fox chow and water during this time. The healthy specimens were then transferred (in almost every instance within 2 weeks after trapping) to individual wire mesh cages provided with a metal food cup and a water bottle. The food cup had fitted over it an aluminum

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[1] Dieke, S. H., and Richter, C. P., *Pub. Health Rep.*, 1946, **61**, 672.