It is of special interest that the "acid" phosphatase, in contrast to the other enzymes, decreased in total amount but not in concentration (units per gram of tissue). It would seem, therefore, that this enzyme is necessary for the integrity of the cell and perhaps its endocrine function while the "alkaline" phosphatase and arginase are necessary only in connection with urine formation.

Summary. In "endocrine kidneys" there is a marked indeed almost a complete disappearance of both arginase and "alkaline" phosphatase. On the other hand the "acid" phosphatase decreases in total amount but not in concentration.

16205

Activation of Hypertensin and Tyrosine by Subthreshold Amounts of Epinephrine.*

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Fractions of hog kidney containing renin do not produce constriction of the vessels of the rabbit's ear until subthreshold amounts of epinephrine are added to the perfusate.[1] Similar vaso-activation of liver fractions by epinephrine has been noted when they are combined with subthreshold amounts of epinephrine.[2] Fresh plasma is also capable of activating liver fractions.[3] This last observation is of interest because of the report that vaso-inactive renin-containing kidney fractions become vasoconstrictive when plasma is added to the ear perfusate.[4] This was ascribed to the formation of hypertensin rather than to the activation of renin by plasma. Since a similar constriction occurs following the addition of plasma to liver fractions under circumstances that would seem to be incompatible with the formation of hypertensin, a reinvestigation of the action of hypertensin on the vessels of the rabbit's ear was undertaken.

The experiments that follow show that hypertensin, a protein fraction, constricts the vessels of the rabbit's ear only when the perfusate is supplemented with traces of epinephrine or fresh plasma. This suggested the possibility that parts of the protein molecules, *e. g.*, peptides or even amino-acids, are responsible for the observed effect. Accordingly, the studies were extended to encompass a large majority of the known amino-acids. Special attention was directed to tyrosine, tyrosine-containing peptides, and a tyrosinamide in accord with Cruz-Coke's[5] hypothesis that tyrosine molecules are necessary for the *in vivo* action of hypertensin.

Materials and Methods. Hypertensin was prepared from purified hog renin with the bovine globulin Fraction IV-1 (Armour) serving as substrate. Three hundred cc of a 1% solution of substrate were buffered at pH 7.5 and incubated for 10 minutes with 2-3 Swingle units of renin. N1 HCl was then added to bring the pH to 5.2 and the mixture immersed in boiling water for 10 minutes. The coagulated proteins were then removed by filtration and the almost water-clear filtrate lyophilized. The lyophilized material was taken up in 16 cc of distilled water and the small residuum of undissolved particles was

* Aided by a grant from the Commonwealth Fund.

1 Mylon, E., Horton, F. H., and Levy, R. P., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 375.

2 Mylon, E., Horton, F. H., and Levy, R. P., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 378.

3 Mylon, E., and Heller, J. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 319.

4 Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

5 Cruz-Coke, E., New York Academy of Sciences, Section of Biology, February 9 and 10, 1945; *Science*, 1946, **104**, No. 2691.

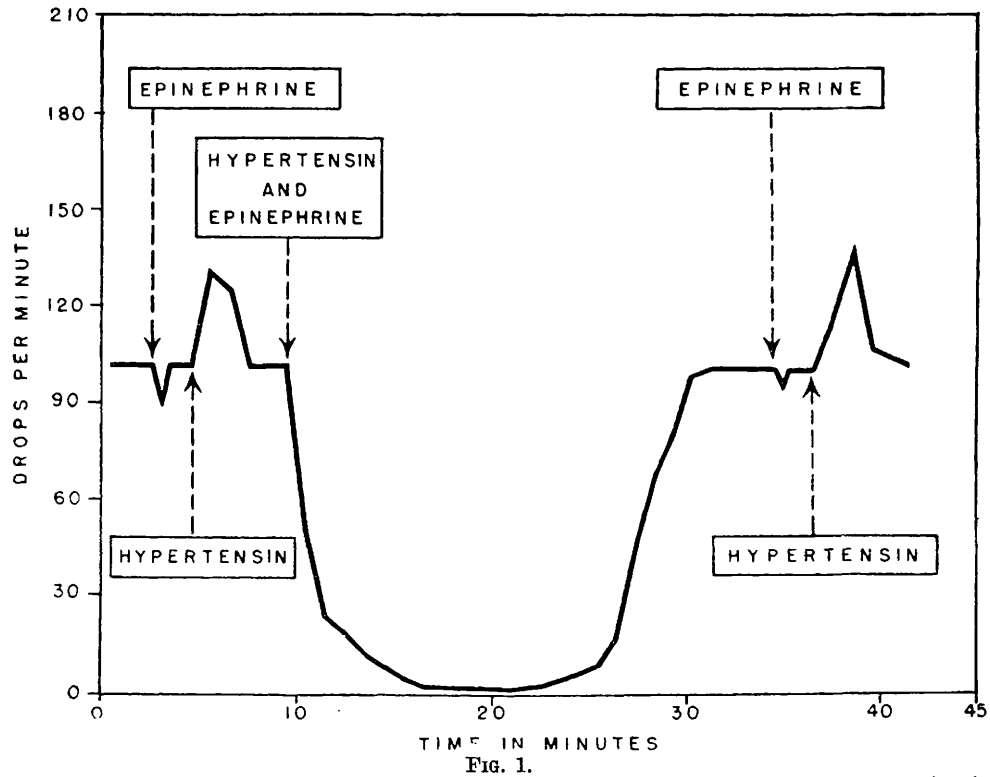

FIG. 1.

While the effect of 1 cc of epinephrine 1:5,000,000 is minimal and the effect of 1 unit of hypertensin is slightly dilating, combination of the two causes a strong and sustained constriction of the vessel of the rabbit's ear.

removed by centrifugation. Four cc of each lot was found to contain between 6 and 8 dog units of hypertensin as tested in the living animal. The remaining 12 cc of each lot were used in perfusion experiments, in amounts equivalent to from one-third to 2 dog units. The ear perfusion technic has been described.[1]

Ears of young albino rabbits seemed to be more sensitive than those of older rabbits and of other types. A preparation was judged to be suitable when the initial drop rate per minute was between 100 and 160, and in addition when an injection of 1 cc of a 1:5,000,000 or 1 cc of a 1:10,000,000 solution of epinephrine caused minor and transient constrictions. Experiments were considered valid only when a terminal injection of epinephrine reproduced a reaction similar to the initial one. The order of the injections with the different materials was varied.

The plasma was prepared according to the technic of Landis.[6] The epinephrine used was Parke Davis 1:1000, and the dilutions were prepared as described previously.[1,2]

The amount of an amino-acid, amide or dipeptide used for a single injection was between 1/100 and 1/200 of a millimole; for *l*-tyrosine it was always 1/200 of a millimole. The pH of the Ringer-Locke solution (7.3) was not changed by the addition of the small amounts of the test substances.

The following amino-acids were used: *dl*-phenylalanine, *dl*-alanine, *dl*-serine, *l*-valine, glycine, *l*-cystine, *l*-tryptophan, *dl*-glutamic acid, *l*-aspartic acid, *dl*-lysine, *l*-leucine, *dl*-isoleucine, *dl*-norleucine, *dl*-threonine, *l*-proline, *l*-tyrosine, *l*-hydroxyproline, *l*-histidine, *dl*-arginine and *dl*-methionine.

The tyrosine-containing dipeptides glycyl-*l*-tyrosine and *l*-tyrosyl-glycin, and also the

[6] Landis, E. M., Wood, J. E., Jr., and Guerrant, J. L., *Am. J. Physiol.*, 1943, **139**, 26.

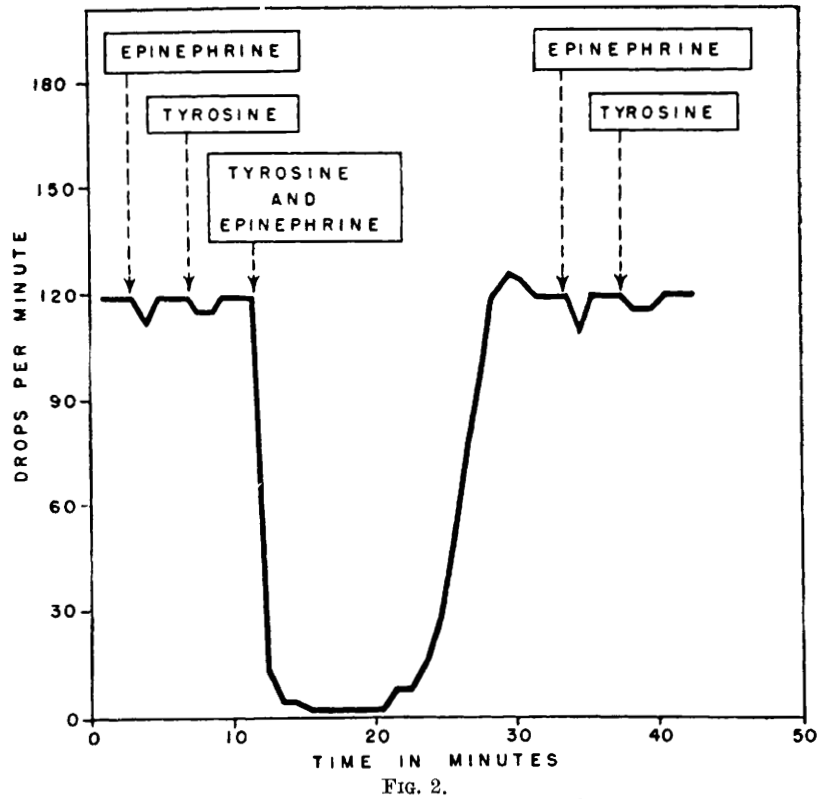

FIG. 2.

Insignificant effect of epinephrine and *l*-tyrosine alone in contrast to the strong vasoconstriction elicited by a combination of both.

Amounts injected:

Epinephrine: 1 cc of a sol. 1:5,000,000.

l-Tyrosine: 0.005 millimole.

tyrosine-amide acetate were tested.†

Iodine was introduced into the tyrosine molecule by slowly adding n/10 aqueous iodine solution to n/1000 solution of *l*-tyrosine until a faint yellow color remained. After keeping the mixture in the dark for several hours, the excess iodine was removed by heating.

Results and Brief Discussion. Hypertensin alone: Neither the initial injection of hypertensin nor its subsequent introductions into the perfusion circuit produced vasoconstriction as determined in numerous experiments with 15 different lots. A slight vasodilation was the usual sequel of the addition of hypertensin to the perfusion fluid.

Hypertensin plus epinephrine: On the other hand, when hypertensin was combined with subthreshold amounts of epinephrine, powerful and sustained vasoconstriction followed. A typical result can be seen in Fig. 1. A total of 28 perfusion experiments were carried out to test the effect of the combination of hypertensin with subthreshold amounts of epinephrine. The vasoconstriction that followed was so intense in 14 instances that the vessels of the ear reduced the drop rate 90 to 99% for 4 to 18 minutes and 100% for 20 minutes. In 10 of the other 14 tests the drop rate was reduced 60 to 90% for from 3 to 8 minutes and in the remaining 4 experiments a more moderate reduction of 50% was observed, lasting from 1 to 8 minutes.

Hypertensin plus plasma: When 1 cc of fresh rabbit plasma was used in place of epinephrine in combination with hypertensin,

† We are indebted to Dr. J. S. Fruton for the generous supply of glycyl-*l*-tyrosine (*l*-tyrosylglycine), and tyrosine-amide-acetate.

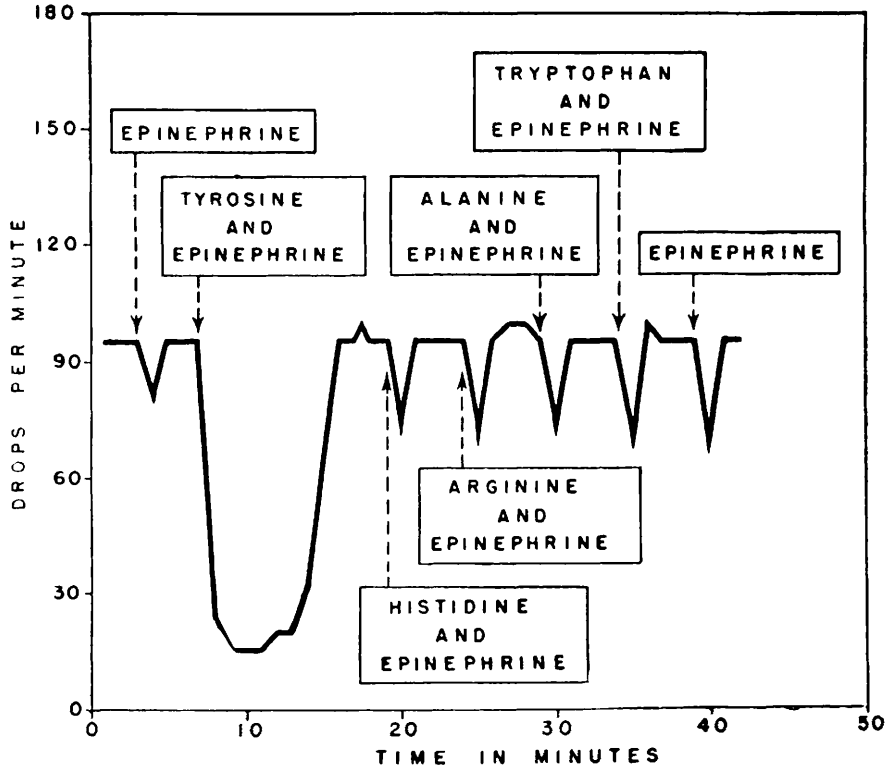

FIG. 3.

Strong vasoconstriction is solely elicited by *l*-tyrosine plus epinephrine and not by a combination of other amino acids and epinephrine.

Amounts injected:

Epinephrine: 1 cc of a sol. 1:5,000,000.
l-Tyrosine: 0.005 millimole.
The other amino acids: 0.01 millimole.

a strong vasoconstriction was elicited. This effect was only obtainable with fresh plasma and incubating the plasma for 8 hours abolished its ability to activate hypertensin. These results are analogous to those previously reported, in which liver fractions were combined with fresh and incubated plasma.[3]

On the basis of the identical results obtained with plasma plus renin[4] and plasma plus hypertensin, it could not be decided whether the observed constriction followed a direct action of plasma on renin or an action of plasma on hypertensin. The specificity of the renin-hypertensinogen reaction on the other hand suggested additional experiments in which it was found that renin itself is being activated. Vasoinactive pig renin, unable to catalyze hypertensin formation with human hypertensinogen, in combination with vasoinactive human plasma produced a significant constriction in the vessels of the rabbit's ear.

Amino-acids: A total of 95 experiments involving one or another of 20 different amino-acids failed to demonstrate any effect when these were added individually to the rabbit's ear perfusate. More important is the fact that with one exception, tyrosine, there was only an inconstant, slight and transient response, for any one compound when traces of epinephrine were added to the perfusate. Addition of epinephrine to the tyrosine-containing perfusate was followed by a sharp and sustained decrease in the drop rate, 45% to 90% for from 3 to 9 minutes (Fig. 2 and 3).

These findings led to the exploration of other tyrosine-containing compounds including glycyl-*l*-tyrosine, *l*-tyrosyl-glycine, and tyrosine-amide-acetate. Needless to say they had no influence when added to the Ringer-Locke perfusate. This status persisted when

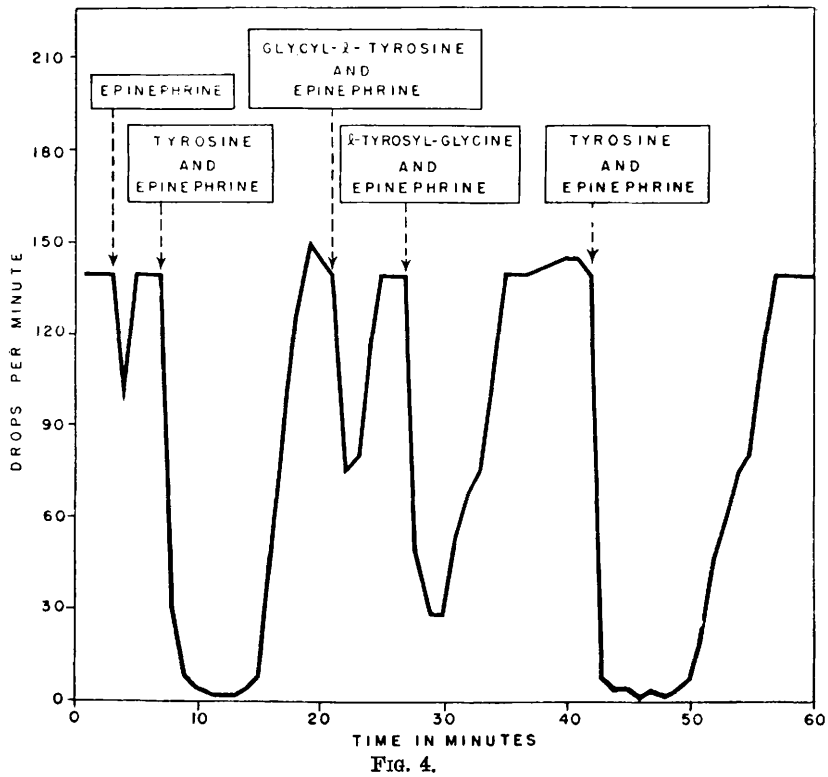

FIG. 4.

The constrictor effect of *l*-tyrosine plus epinephrine is much greater than that of *l*-tyrosyl-glycine plus epinephrine. The effect of glycyl-*l*-tyrosine and epinephrine is insignificant.

Amounts injected:

Epinephrine: 1 cc of a sol. 1:5,000,000.
l-Tyrosine: 0.005 millimole.
The dipeptides: 0.01 millimole.

glycyl-*l*-tyrosine was supplemented with traces of epinephrine. On the other hand, *l*-tyrosyl-glycine and tyrosine-amide-acetate combined with minimal amounts of epinephrine had a moderate constrictive effect on the vessels of the rabbit's ear (Fig. 4).

The vasoconstrictive effect of tyrosine, it would seem, is abolished when the amino group is linked to a carboxyl of a second amino acid. When the amino group remains free and the carboxyl group is bound by another amino-acid or transformed into an amide, the vaso-constrictive effect persists to about 50%.

Another finding still difficult to correlate with the coverage of the amino group of tyrosine-like compounds is the effect of iodinization. This also abolished the vasoconstrictive effect. These results are of interest in relation to Cruz-Coke's suggestions[5] that the pressor action of hypertensin *in vivo* might be related to its tyrosine content.

Summary. Hypertensin has no effect on the vessels of the perfused rabbit's ear. It becomes strongly vasoconstrictive, however, when supplemented with traces of epinephrine and also when fresh plasma is added to the perfusate. None of 20 different amino-acids tested in perfusion experiments on the rabbit's ear had any constrictor effect. When traces of epinephrine were included in the perfusion fluid, tyrosine was the only one of the 20 that exhibited a strong and sustained vasoconstriction. Glycyl-*l*-tyrosine was inactive with and without epinephrine. *l*-tyrosyl-glycine and tyrosine-amide-acetate, both inactive when perfused alone, constricted the vessels if combined with minute amounts of epinephrine. The activity of these two compounds, however, was definitely less than that of tyrosine. Introduction of iodine into the

tyrosine molecule abolished the constrictor action entirely.

The conclusion drawn from these findings is that constriction of the blood vessels of the rabbit's ear is dependent to a large degree upon traces of epinephrine and tyrosine-containing compounds of special configuration.

16206

Prevention of Experimental Dietary Hepatic Cirrhosis by Goitrogenic Substances.

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In previous observations[1] thiouracil, and to some extent thiourea, exerted a beneficial effect in the prevention of dietary cirrhosis of the liver in rats. In animals receiving thiourea the food intake was generally very low and their weights showed a rapidly progressive decline. In contrast, the general well being of rats fed the same basal ration mixed with thiouracil (0.1% in the diet) remained undisturbed. The weight losses sustained were smaller than in the control animals without any appreciable difference in the average food intake in the two groups.

The beneficial effect of thiourea and thiouracil in the prevention of experimental dietary cirrhosis has been related to their effect on thyroid function, specifically to the decreased metabolic rate including the metabolism of protein. Decreased turnover of proteins pertains also to that of their constituents, including methionine, which is one of the key substances in the pathogenesis of dietary hepatic injury.[2]

It is difficult to furnish direct proof for the correctness of this assumption. The demonstration of strict parallelism between goitrogenic activity and preventive effect on hepatic cirrhosis may serve as indirect circumstantial evidence. The superior effect of thiouracil over thiourea is in good accord with these considerations. In this connection it seemed advisable to include propyl-thiouracil and aminothiazole in these investigations. Propyl-thiouracil is at present probably the most effective goitrogenic agent available. Aminothiazole, when first used, was reported to be very effective in the treatment of hyperthyroid conditions.[3] However, pharmacological[4] and more recent clinical studies[5] are at variance with this conclusion and support the view that aminothiazole is less effective and more toxic than propyl-thiouracil or even thiouracil.

Experimental. During the course of our studies extending over several years it became evident that even without any change in the basic composition of the experimental cirrhosis-producing diet the results, such as incidence and severity of cirrhosis, food intake, weight changes, and survival time, may vary at various times. These variations—admittedly within narrow limits—are probably due to changes in the composition of the fat used (perhaps in addition to obscure climatic influ-

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