Although measurements of the amino acids in the urine and balance studies to ascertain the amount of any amino acid that is retained by the body are of great value, it should be recognized that alterations in the degree of metabolism of any amino acid are not necessarily determined by the microbiological methods used. Thus, when diets deficient in a specific amino acid or a vitamin are fed and no large differences in the excretion of the unchanged amino acid is observed, it may be quite possible that the amino acid is only partially metabolized and these partial metabolites excreted in the urine are microbiologically inactive. That marked changes in the amounts of the amino acids excreted in the urine do occur with mice fed various deficient diets has already been observed. Further studies on the specificity of these amino acid methods and their reliability with respect to blood and urine analysis will aid in obtaining more information on the metabolism of specific amino acids by various species.

Summary. Rats have been fed diets deficient in tryptophane, riboflavin or nicotinic acid and the amounts of microbiologically available histidine, arginine, threonine, phenylalanine and tryptophane excreted in the urine were determined. No large changes were noted in the amounts of these amino acids excreted in the urine when rations deficient in nicotinic acid were fed. Rats fed a riboflavin or a tryptophane deficient diet excreted approximately twice as much of the ingested amino acids as animals fed adequately supplemented diets. In all cases, however, the amounts excreted were small, less than 2.5 per cent of those ingested. After acid hydrolysis of the urine samples, a 2-4 fold increase was observed in the values for histidine, arginine, threonine and phenylalanine.

[15] Albanese, A. A., Holt, L. E., Frankston, J. E., and Irby, V., *Fed. Proc.*, 1946, **5**, 118.

16078

Vasoconstrction Elicited by Addition of Plasma to Vasoinactive Tissue Extracts.*

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Perfusion experiments on the rabbit's ear have shown that several vasoinactive, renin-containing protein fractions of hog kidney became vasoconstrictive when the perfusion fluid (Ringer-Locke) was supplemented by minute amounts of epinephrine.[1]

Identical effects were observed when the kidney fractions were replaced by similarly prepared fractions of other organs—especially liver.[2]

A change from vasoinactivity to vasoconstriction in the rabbit's ear was previously reported[3] when plasma instead of Ringer-Locke was used for perfusion with kidney fractions (renin). This constriction was not attributed to the direct combined effect of the kidney fraction and plasma, but to the formation of a special vasoconstrictive substance, angiotonin (hypertensin).

Since plasma is known to contain traces of epinephrine,[4] it seemed desirable to investigate whether this epinephrine content might have contributed to the constrictive effect elicited by kidney- or other tissue-fractions.

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

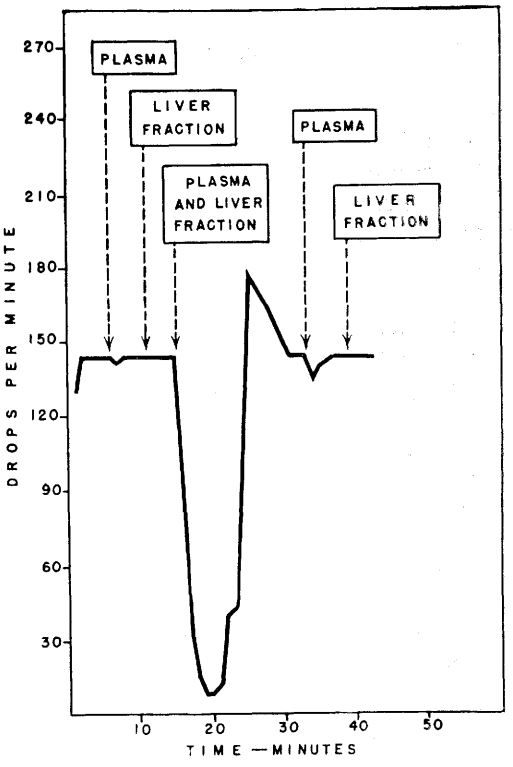
[4] Shaw, E. H., *Biochem. J.*, 1938, **32**, 19.

The following experiments were carried out to clarify this point.

Materials and Methods. Rabbit plasma was prepared as described by Landis[5] with especial care in the heparinization of the animal 5 minutes before drawing the blood. This was then spun down at +5°C for 45 minutes, the plasma siphoned off and stored at the same low point until used. Fractions of liver extracts were prepared in accord with previously reported methods.[2] Epinephrine (Parke-Davis 1:1000) was freshly diluted several times a day to 1:100,000 with distilled water and used as stock solution. Further dilutions made from this concentration were used within 2 minutes. The subthreshold dose was usually between 1 cc and 1.5 cc of a 1:5,000,000 solution. The isolated rabbit's ear was chosen for perfusion. The volume of each separate substance to be tested was brought up to a constant amount by the addition of Ringer-Locke.

Results. The plasma from 5 of the 25 normal rabbits used in this study was in itself slightly and transiently vasoconstrictive on perfusion. The 25% constriction for 2 minutes observed with these 5 plasmas increased to 80% for 8 minutes on the addition of liver extract. This liver extract was completely vasoinactive if used for perfusion without plasma. Nineteen of the remaining 21 plasma samples had no effect on the isolated vessels until the liver extract was added to the perfusate. The degree of vasoconstriction recorded with these 19 samples varied from 60 to 99%, lasting for 3 to 10 minutes. The plasma samples of the two remaining rabbits elicited strong vasodilatation if added to the Ringer-Locke perfusate. Addition of the liver preparation abolished this vasodilatation.

The next experiments were designed to compare the effects of plasma and epinephrine on perfusion fluids containing liver preparations. Various combinations and sequences were tried. In none was it possible to differentiate between the effects of epinephrine and plasma on the liver containing perfusate. Typical experiments are recorded in Fig. 1 and Fig. 2.



FIGURE—1

Vasoconstriction caused by the combination of vasoinactive plasma with a vasoinactive liver fraction.

All of the previous observations were carried out with fresh plasma that had been stored for not more than 3 hours at +5°C.

In the light of the fact that the epinephrine content of plasma is destroyed on incubation,[6] a new series of experiments was initiated in which the plasma was incubated for 12 hours before its use. The effect of this preliminary incubation on the plasma was striking: in none of the 8 experiments was any constriction obtained with plasma alone or in the combination of the incubated plasma with the liver fraction.

Discussion. The similarity of action of fresh plasma and subthreshold amounts of epinephrine suggests that the epinephrine content of the plasma is responsible for the observed effect on the rabbit's ear.

The colorimetrically determined values for "true" blood epinephrine, however, are smaller than the subthreshold doses that were used with the rabbit's ear.

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

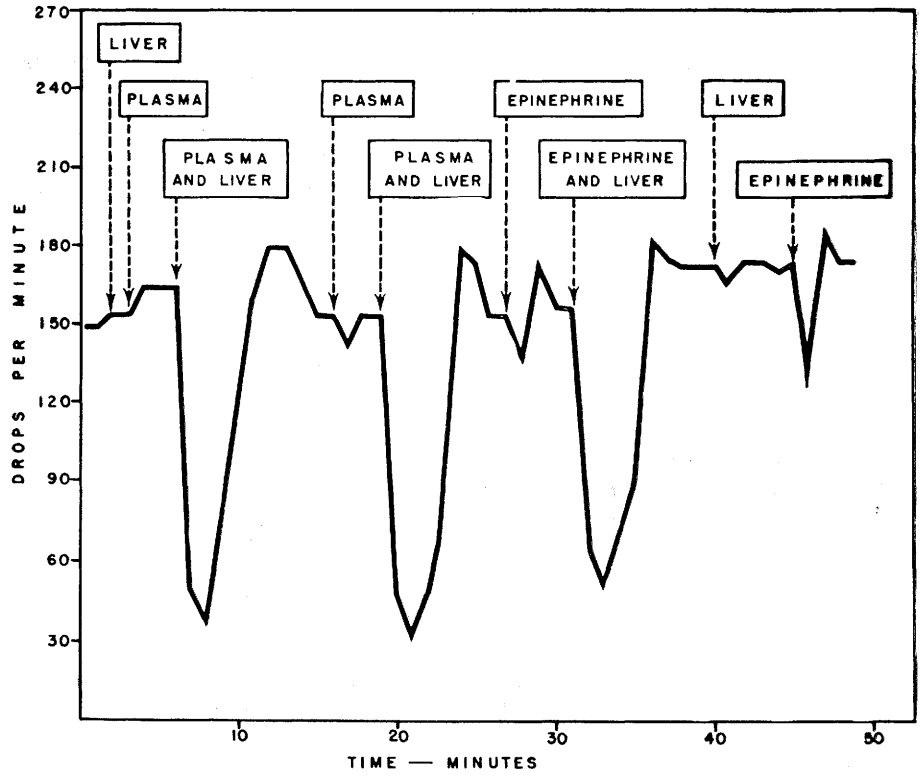


FIGURE II

Plasma or minute amounts of epinephrine if combined with a vasoinactive liver fraction act almost identically.

Recent studies[4,7,8,9] indicating that the blood contains substances structurally related to epinephrine point to the possibility that these compounds are biologically active and involved in the reaction reported in this paper.

Summary. Vasoconstriction of the isolated vessels of the rabbit's ear follows the addition of fresh plasma to perfusates containing liver preparations that are in themselves inactive. The activation caused by plasma is indistinguishable from the effect of subthreshold amounts of epinephrine added to these tissue fractions. Incubation of plasma, known to result in loss of its epinephrine content, also causes loss of its vasoconstrictive influence on perfusate containing liver fractions.

6 Bain, W. A., Gaunt, W. E., and Suffolk, S. F., *J. Physiol.*, 1937, **91**, 233.

7 Whitehorn, J. C., *J. Biol. Chem.*, 1935, **108**, 633.

8 Bloor, H. R., and Bullen, S. S., *J. Biol. Chem.*, 1941, **138**, 727.

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