period of starvation was nearly three weeks. These results are in agreement with those of *Limnaea*. Apparently, the free amino-acid pools obtained from catabolic processes (or biosynthesis from precursors in the body) are again forced to assume the original pattern. Thus the pools which defy starvation may be regarded as a stable biochemical index (gene-determined) rather than purely as building blocks for protein. This rather curious finding is, however, in agreement with that of ROBERTS and SIMONSEN[7] with rat tissues[8,9].

Résumé. Dans la capsule des œufs de *Pila* (Gastéropode), le fluide alimentaire contient à peu près tous les acides-aminés libres qui existent aussi dans l'embryon bien développé. L'auteur a déterminé le nombre des acides-aminés libres qui se trouvent dans le corps de ces escargots après l'éclosion. Le modèle-type des acides-aminés libres reste inchangé chez les individus affamés.

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[7] E. ROBERTS and D. G. SIMONSEN, in *Amino Acids, Proteins and Cancer Biochemistry* (Academic Press, 1960), p. 123.

[8] I take this opportunity of thanking Mr. N. CHATTERJEE and Mr. S. K. DE for their indispensable help.

[9] *Note added in proof:* Recently, AWAPARA[10] noted that the amino acid pattern of the land snail *Otala lactea* remains unaltered after a starvation of six months. This result obtained by two-dimensional chromatography is more convincing than my findings.

[10] J. AWAPARA, in *Amino Acid Pools* (Ed. Holden, 1962).

Free Amino Acids in *Limnaea* III

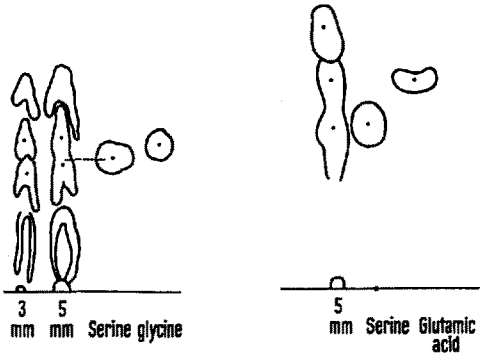
It has been shown in the earlier communications[1,2] inspired by the trail-blazing work of HADORN and MITCHELL[3], that in *Limnaea* there is a constant pattern of amino-acid pools, increasing in intensity with age and size of the snails and that these pools are resistant to prolonged starvation. This constancy and increasing intensity are explicable if all or most of the cells in the growing organism continue to prepare the same type of pools, even during the catabolic process in starvation. Some changes in amino-acid pools in the course of morphogenesis were, however, observed[1].

As, however, the intrinsic amino-acid content of the near-hatching eggs and small snails which have just hatched is very low, the less intense pools escaped notice.

A large number of very small snails (only a few days old), were therefore crushed one after another directly onto the filter paper and chromatographed as earlier[1,2]. The faint band of ninhydrin positive spots having higher Rf values which are characteristic of the adult snails[1] was now revealed. Thus, even at this early stage, practically all the pools are present and the relative intensities are also apparently already fixed. The present finding, therefore, all the more supplements and confirms the view expressed earlier[1]. It is now established that from about the hatching stage onwards only the absolute value of the amino-acid pools increases with the size. The relatively higher intensity of some pools over others, that is constantly maintained either by using up some pools very quickly or by replenishing the more intense pools alone by lysis of older tissue or by a consistent higher rate of biosynthesis, can be regarded as a genetically fixed biochemical index.

In view of the above result it now also seems possible that all the amino-acids of the snail are present even in the prehatching stage and that the two streaks[2] are only four unresolved spots, while the amino-acids with higher Rf values are too small to be detected.

As it would be of some interest to identify the amino-acid pools, pure known amino-acids were chromatographed with the solvent, *n*-butanol:acetic acid:water (4:1:1). With the same degree of caution as expressed by HADORN and MITCHELL[3], it can now be suggested that the four most prominent and clearly resolved amino-acid pools of *Limnaea*[1] are probably histidine or asparagine, serine or glutamic acid, or glycine, threonine and β-alanine. The spots due to pure amino-acids were compared with those due to direct crushing of small snails on the same paper. It is seen from the Figures that direct crushing of 3 and 5 mm long snails yielded streaks with some resolution. Of the four spots due to the 5 mm snail compared with serine and glutamic acid, the second and third are extremely close to the two known acids. In the other Figure also serine is suggestive. (Here the two lowest spots have a very peculiar shape due to the trapping effect of the solid material.)



It may be mentioned that Simpson et al.[4] made a detailed study of free-amino acids in some aquatic invertebrates. They found alanine, β-alanine, glycine, arginine, aspartic acid, glutamic acid, taurine and glutamine in snails. Taurine was found only in the marine snails, and not in fresh water snails such as *Limnaea*.

Résumé. L'auteur a constaté que les acides aminés libres chez les gastéropodes du genre *Limnaea*, immédiatement après l'éclosion, sont les mêmes que chez les adultes. Il a essayé aussi d'identifier chez des sujets adultes les quatre acides-aminés libres les plus caractéristiques. Deux d'entr'eux sont probablement l'acide glutamique et la sérine.

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[1] R. L. BRAHMACHARY and A. BHATTACHARYA, Exper. *19*, 143 (1963).

[2] R. L. BRAHMACHARY and A. BHATTACHARYA, Exper. *19*, 225 (1963).

[3] E. HADORN and H. K. MITCHELL, Proc. Nat. Acad. Sci. *37*, 650 (1951).

[4] J. W. SIMPSON et al., Biol. Bull. *117*, 371 (1959).