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On the amino-acid content of involuting frog-larvæ.

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In order to test the hypothesis that in the metamorphosis of the larvæ of the frog autolysis is the principal factor, estimation of the amino-acid content was made by the gasometric method of Van Slyke and by the formol titration method of Sörensen. Beckmann freezing point and conductivity determinations were also made since Bayliss,[1] Wells,[2] Sjöqvist[3] and others have shown that the curve of proteolysis follows that of increase of conductivity and lowering of the freezing point.

Individual tadpoles were used at first, the tails being cut from the bodies, dried in an air-blast at 40° C., pulverized in a mortar, dissolved in water to make a one per cent solution, the estimations being made upon the coagulable protein-free filtrates from boiling with basic lead acetate. About 0.40 g. dried material could be obtained from one individual, but this amount became in the neighborhood of 0.10 g. in specimens which were absorbing their tails. The determinations mentioned above gave no characteristic differences between absorbing and non-absorbing animals.

Attention was then directed to larger amounts and accordingly the dried material from a number of larvæ in the stages preceding metamorphosis was mixed and another mass was obtained from a number of individuals undergoing involution. The former gave 3.84 g. dried material, the latter 2.50 g. Upon these amounts, determinations were made as above, but again no perceptible differences in amino-acid content was found, nor were the freezing point and conductivity determinations different in the two cases. As typical examples, in conductivity experiments, with $R = 500$, $a = 58.1$ for non-absorbing and 50.3 for absorbing; for freezing points, $D = 0.72$ for non-absorbing and 0.73 for absorbing. For formol titration with 20 cm.^{3} aliquots, non-absorbing gave 0.1

[1] *Journ. Physiol.*, Vol. 36, p. 221, 1907.

[2] *Journ. Biol. Chem.*, Vol. 3, p. 35, 1907.

[3] *Skand. Arch. f. Physiol.*, Bd. 5, p. 364, 1895.

cm.^{3}, absorbing 0.3 cm.^{3} of $n/5$ $Ba[OH]_2$, in average of several determinations. VanSlyke's gave with $T = 23°$ C. and barometer = 734 mm., non-absorbing 0.34 g. nitrogen and absorbing the same. The samples were in all cases positive to ninhydrin, showing that some alpha-amino acids were present in the filtrates, but in such small amounts that they were not recognizable by the methods used.

It may be well argued, as it was in the earlier work with amino-acids in the blood, that these compounds are so quickly removed from the sphere of action that at any one time they are present in only minute quantities. It is well known that phagocytes crowd into the tissues of the metamorphosing organs after the earlier stages of dissolution are under way and it may be through their agency that the products of proteolysis are removed. Mercier[1] has been able to trace the circulation of phagocytes throughout the metamorphosing organs and the body by causing the cells to engulf carmin granules and it may be that the end products of the action of proteolytic enzymes which we must imagine to be developed at the beginning of dissolution of the muscles, etc., are taken up and carried to the body proper by these cells. This conception, however, does not give any support to the so-called phagocytic theory of involutionary phenomena, for it is quite certain that dissolution has begun before the wandering phagocytes have entered the tissues affected.

In vitro studies of autolysis of normal and involuting larvæ are in progress.

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A simplified method for cultivating spirochaetes on liquid media.

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The method I wish to describe is a modification of the original method of Noguchi[2] for cultivating spirochaetes, which I think

[1] *Arch. zool. expér. et gén.*, T. 5, Ser. IVe, 1, p. 151, 1906.

[2] *Journ. Exp. Med.*, 1912, XV, p. 211.