between norepinephrine content and the number of granulated vesicles in the hypothalamus observed in the present study suggests that the granulated vesicles contain only norepinephrine. It is also suggested that the sedation induced by Win 18501-2 or reserpine is closely related to loss of norepinephrine, which is contained within the granulated vesicles in the synapses and the axons of the hypothalamus[13].

Zusammenfassung. Der Hypothalamus von Kaninchen wurde nach Win 18501-2-Injektion biochemisch und elektronenmikroskopisch untersucht. Die Behandlung erzeugte auffallende Veränderungen im Noradrenalingehalt und in der Anzahl der Katecholamine enthaltenden granulierenden Vesiculae im Hypothalamus. Die Resultate scheinen zu zeigen, dass diese charakteristischen Vesiculae nur Noradrenalin enthalten und dass die Sedation des Kaninchens nach der Injektion von Win 18501-2 auf der Verminderung des Noradrenalins und der charakteristischen Vesiculae beruht.

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Effects of Starvation on the Free Amino Acid Pools of *Ciona intestinalis*

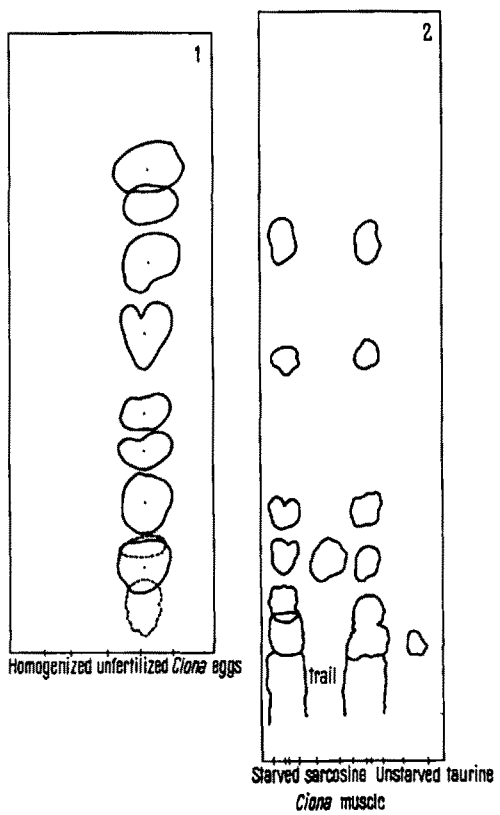
Ferrini et al.[1] have recently given quantitative data on the free amino acids of ovarian eggs of *Ciona intestinalis* with the help of amino acid analyser. This line of investigation, using such a modern and quantitative technique, has surpassed the method of paperchromatography, which gives only a qualitative indication of the more abundant amino acids present and is misguiding when the amount of material is small[2-4]. However, even this provides certain clues in connection with certain specific questions.

Ferrini et al. found a high concentration of taurine and sarcosine, unusual amino acids, in eggs of *Ciona*. Earlier, Ackermann and Janka[5] had also detected taurine in *Ciona*. Taurine has also been detected in other marine invertebrates[6]. The excessive quantities of certain amino acids present in marine invertebrates is an interesting fact which has not yet been fully explained, but Awapara[6] has discussed the various possibilities. It is not clear whether the pattern is dependent on environment, i.e. if it can change with altered concentration of salt in the medium. On the other hand, there is some evidence by Roberts and Simonsen[7], Brahmachary[8], and Awapara[6] that some organisms maintain a starvation-resistant genetically-fixed pattern of amino acids. In tadpoles of the toad *Bufo melanostictus* and in the Sicilian frog Discoglossus[9,10], Brahmachary found a considerable loss in the quantity of free amino acids in tails following starvation, though there was no appreciable change due to metamorphosis, and by comparison with a larger quantity of tails from starved tadpoles, he detected that the pattern remained qualitatively the same. Other workers detected a loss of amino acids in starving insect larvae[11].

In view of these facts, it may be of some interest to follow the free amino acid patterns, especially taurine and sarcosine, in starved and unstarved *Ciona*.

Two batches of *Ciona* were starved for one month and three weeks respectively by keeping them in filtered sea water which was changed every day. The water was not pasteurized and some microorganisms might have dropped into it from the air, and thus a very small amount of nourishment might have been available to the organisms. But as the creatures became perceptibly very much emaciated (some of them died), the process of catabolism had far exceeded that of anabolism. The free and bound amino acid patterns in this condition were compared with those of normal *Ciona*. *The patterns turned out to be consistently very similar.*

The muscle tissues of *Ciona* were cut and removed and, after drying, homogenized in 70–80% methanol or ethanol. One-dimensional chromatograms developed in the solvent *n*-butanol : acetic acid : water (4 : 1 : 1) showed three very intense spots with some trailing. This condition was highly



reproducible. (It may be mentioned that the chromatogram developed from the homogenized unfertilized eggs with the same solvent, gives a much better resolution and shows a different pattern, Figure 1.) The chromatograms developed from hydrolysed muscle tissues gave a clearer resolution of the spots (Figure 2). Although specific tests for amino acids were not carried out, comparison with standard amino acids suggests the presence of sarcosine, taurine, glycine, and glutamic acid as the most intense spots. Taurine, glycine, and glutamic acid were also compared in another solvent system, namely methanol:water:pyridine (20:5:1). Two-dimensional chromatograms (*n*-butanol:acetic acid:water and phenol) of the material and the material plus sarcosine and taurine and only sarcosine and taurine were compared. Although resolution was poor there were enhanced intensities in the proper places[12].

Zusammenfassung. Nach den vorliegenden Befunden scheinen Taurin und/oder Sarcosin, Glycin und/oder Glutaminsäure die wesentlichen Aminosäuren der *Ciona*-Muskulatur zu sein. Das charakteristische Chromatogramm lässt sich in nahezu gleicher Intensität auch in den Extrakten von ausgehungerten Versuchstieren nachweisen.

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The Fractionation of Arginine-Rich Histones from Calf Thymus

Histones from calf thymus and other mammalian tissues can be divided into two major groups – arginine- and lysine-rich histones. The arginine-rich histones are heterogeneous as was shown by JOHNS and BUTLER[1] who, on the basis of solubility in absolute ethanol obtained two arginine-rich fractions coded as F2a and F3[2]. The F3 histones are electrophoretically slow (in starch gel electrophoresis) and contain alanine as the sole NH_2 terminal amino acid. The F2a histones are fast in starch gel electrophoresis and do not possess any definite NH_2 terminal amino acid. Proline, alanine and glycine are usually recovered in small amounts, and PHILLIPS[3] has shown recently that the NH_2 terminal amino acids in this fraction are acetylated to a great extent. Because the starch electrophoresis of the F2a and F3 histones shows the presence of at least two distinct bands in each of the two fractions, attempts were made to fractionate the arginine-rich histones.

The arginine-rich histones were prepared from ethanol washed crude calf thymus nucleohistone[4] by extraction with a mixture of absolute ethanol and 1.25 *N* HCl[5,6]. Extract containing the F2a and F3 histones was precipitated with 6 Vol of cold acetone and the precipitated protein was recovered by centrifugation, washed with acetone and with ether and dried in vacuo. In another series of experiments, the ethanol-HCl extract was evaporated in dialysis bags in a stream of cold air approximately to $^1/_3$ of the original volume and dialyzed against distilled water. The small amount of precipitate which formed during evaporation and dialysis was removed by centrifugation and the F2a and F3 histones were then precipitated with trichloroacetic acid (5% final concentration), converted to hydrochlorides and dried[5].

The precipitate formed during evaporation and dialysis was washed with cold acetone, then by ether, and dried. Part of the material became soluble in acetone and after removal of acetone by flash evaporation a brown lipid-like material was obtained. This material contained no protein or amino acids and no further characterization was attempted. The protein part of the precipitate was hydrolyzed in constant boiling HCl (5.7 *N*) and analyzed for amino acid composition, which is shown in the Table under 'lipo'.

The arginine-rich histones F2a and F3 were separated by filtration on Sephadex G 75 (Fine, in bead form) columns. The swollen gel suspended in 0.01 *N* HCl was poured into columns 50 × 1600 mm, 1500 mg of the F2aF3 histones were dissolved in 15 ml of 0.01 *N* HCl and applied to the column. The fractions were eluted with 0.01 *N* HCl saturated with chloroform. A flow rate of 48 ml/h was maintained and fractions were collected every 10 min. The elution pattern obtained is shown in Figure 1. The F3 histones were eluted from the column first, followed by the F2a fraction. Fractions comprising the peaks were pooled as indicated by arrows in Figure 1, and the proteins were recovered by dialysis against distilled water and by lyophilization.

The F2a histones obtained in this way were further fractionated. 3.0 g of the F2a fraction were dissolved in

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