It is readily apparent that the standard stimulus is most effective at the lowest [NaCl] tested (50 mM),and is still more effective at an intermediate (100 mM) than at the normal concentration (150 mM). This graded response was obtained with all possible permutations of the experimental design and cannot be ascribed to the addition of choline, since the replacement of NaCl by LiCl gave similar results.

These observations could be explained by assuming that both Na^+ and Ca^+ have an affinity for the hypothetical cellular binding sites (X), but that only the complexes Ca-X are able to promote release. In media containing a high NaCl concentration, Na ions might combine with either or both of postulated anionic groups of X so as to form inactive complexes.

If such an antagonism did exist, the release of hormones might depend on the $[Ca^{2+}]/[Na^+]^2$ ratio over a wide range of Ca^{2+} concentrations. In order to test this possibility, experiments were done in which this ratio in the bathing solution was either kept constant, or varied deliberately, ionic strength and iso-osmolarity being maintained in each case by the addition of choline chloride.

Results are summarized in the Table and in Figure 2, and show that for $CaCl_2$ concentrations between 0.28 and 2.2 mM, the release of hormones increases linearly with the rises of the $[Ca^{2+}]/[Na^+]^2$ ratio.

Whereas release is indeed determined by this ratio over an approximately 10-fold range of $[Ca^{2+}]$, the relationship does not hold for $CaCl_2$ concentrations above approximately 3 mM, which is in keeping with the observation of DOUGLAS and POISNER[1] that vasopressin release decreases in this range. Similarly, BROWN and FELDBERG[7] have described that the release of acetylcholine from the perfused cervical superior ganglion by raised [KCl] is depressed by high Ca^{2+} concentrations; and GAGE and QUASTEL[8] have reported that the frequency of miniature end plate potentials in rat diaphragma increases with $[Ca^{2+}]$ in the range of 0.32 to 2.0 mM, but diminishes with a further rise in $CaCl_2$. It has been proposed that the partial inactivation of the release process in high $[CaCl_2]$ may be due to the formation of inactive Ca_2-X complexes.

[NaCl] (mM)	$[CaCl_2]$ (mM)	$[Ca^{2+}]/[Na^+]^2$ (M^{-1})	Mean hormonal output (mU oxytocin) Mean S.D.M.	No. of experiments
150	1.24	0.055	8.6 ± 0.6	5
100	0.55	o.055	8.8 ± 1.5	5
150	2.48	0.11	9.3 ± 1.5	5
100	1.10	0.11	11.6 ± 1.5	5
50	0.28	0.11	10.2 ± 1.6	6
150	4.95	0.22	7.4 ± 0.7	5
100	2.20	0.22	14.9 ± 2.3	5
50	0.55	0.22	15.0 ± 3.1	4
150	9.90	0.44	2.0 ± 0.0	5
100	4.40	0.44	20.9 ± 3.5	6
50	1.10	0.44	23.3 ± 1.6	5

The cellular localization of the ionic binding sites X is still unknown. Our finding that release is governed by the $[Ca^{2+}]/[Na^+]^2$ ratio in the bathing solution suggests the existence of sites not far removed from the cell surface, viz. within the membrane, which is readily accessible to the incubation medium. If the sites were intracellular, release ought to be depressed by an increase in intracellular $[Na^+]$; no such depression was found by DICKER[9] when active sodium transport was blocked with cardiac glycosides.

It remains to be determined whether the inhibitory action of Na ions on neurohypophysial hormone release is specific or is shared by other univalent cations. In frog cardiac muscle, an increase in the tension of contracture occurs when NaCl is replaced by sucrose, and lithium, choline or *tris* chlorides[4], suggesting that the effect of lowering $[Na^+]$ is a specific action of Na ions. In contrast, KELLY[10] has suggested that at frog neuromuscular junctions the competition between Na^+ and Ca^{2+} may not be chemically specific, but charge specific.

The fact that the release mechanism does inversely depend on the external Na^+ concentration – in contrast to the height of the action potential[11] – suggests that the release process for octapeptide hormones can function independently of the generation of action potentials (cf. ref.[2]). Moreover, since under normal conditions, a major part of the action currents flowing at the level of the axon terminals in the neural lobe are in all likelihood carried by Na ions, it may be concluded that, both in vivo and in standard Locke solution, the release mechanism may not be fully operational[12].

Résumé. La secrétion hormonale de neurohypophyses isolées de rat a été estimée à l'aide d'un test d'éjection du lait. Au moment de la dépolarisation des terminaisons neurosecrétrices, il y a compétition entre le sodium et le calcium externes pour d'hypothétiques sites membranaires. Pour des concentrations de calcium inférieures ou égales à la concentration physiologique, la libération hormonale est fonction du rapport $[Ca^{2+}]/[Na^+]^2$ dans le milieu externe.

J. J. DREIFUSS, J. D. GRAU and RITA E. BIANCHI

Department of Physiology, University of Geneva Medical School, CH-1211 Genève 4 (Switzerland), 18 June 1971.

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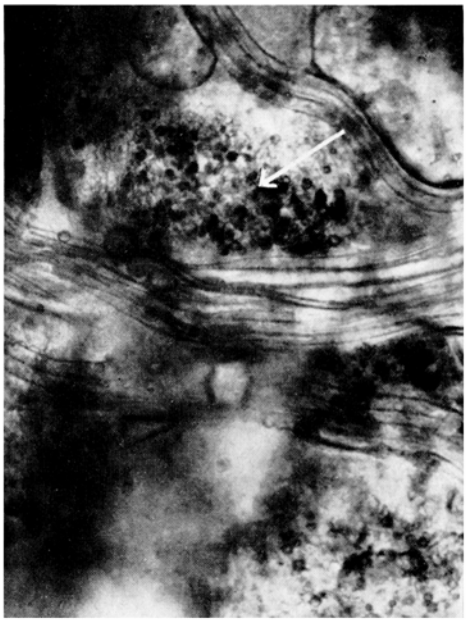
Peroxidase Uptake by the Fat Body of a Millipede *Spirostreptus asthenes* (Diplopoda, Myriapoda)

A recent study on the fat body of a millipede *Spirostreptus asthenes* shows the appearance of tyrosine-rich protein granules in the premolt stage and their disappearance following molt.[1]. That this protein appears prior to cuticle formation and disappears during the formation of cuticle may suggest its utilization in the cuticle formation.

Studies on insect fat body show that the fat body protein could be formed either by synthesis or sequestration.



Dark brown granules in the cytoplasm of the fat body. The arrow shows the site of the peroxidase activity.

Results obtained from incubation procedures and radio isotopic studies indicate the protein synthetic capabilities of the fat body and that these synthesized proteins are for the most part secreted into the blood without prior storage[2–5]. However, the protein granules stored in the fat body of *Calpodes ethlius* are formed by the sequestration of haemolymph proteins, as indicated in the work of LOCKE and COLLINS[6] using plant peroxidase as the tracer protein. In the light of these observations, it is of interest to study the origin of tyrosine-rich protein granules appearing in the fat body at the premolt stage of a millipede *Spirostreptus asthenes*.

Millipedes of the species *Spirostreptus asthenes* were used in the present study. Stages of the moult cycle were assessed according to the method of VERHOEFF[7] and JOLY[8], using claw development as the main criterion. The tracer protein employed was horse radish peroxidase, and the procedure followed was that of LOCKE and COLLINS[6] with appropriate modifications. The peroxidase solution was perpared by dissolving 1 mg of peroxidase in 0.1 ml of millipede saline containing sodium chloride 0.63 g, potassium chloride 0.025 g, calcium chloride 0.025 g, and glucose 0.2 g in 100 ml of distilled water[9]. Five premoult millipedes were injected with 0.5 ml of peroxidase solution and 4 h were allowed for incorporation. For visualizing the sites of peroxidase activity, the animals were sliced by placing them in ice-cold 4% buffered neutral formaldehyde and kept in the same for 4 h for fixation. The fat body was then separated and washed with ice-cold 10% sucrose solution 3 times at intervals of 30 min. Subsequently the material was washed repeatedly in phosphate buffer (pH 7) and then 9 ml of benzidine reagent (0.3% benzidine in phosphate buffer at pH 7) was added with gentle shaking. After 2 min, 1 ml of 0.3% hydrogen peroxide was added and shaken vigorously at room temperature for 10–30 min. The tissue was dehydrated, cleared in xylene and mounted in canada balsam. Control animals were injected with 0,5 ml of millipede saline and left for 4 h.

The whole mounts of the fat body of the experimental millipedes show the presence of dark brown granules in the cytoplasm (Figure) indicating the presence of peroxidase[6]. No such granules were detected in the fatbody of the control animals.

The presence of an endogenous peroxidase has been reported in several insect tissues such as gut epithelium, flight muscle and epidermis[10,11]. But the presence of this enzyme has not so far been reported in insect fat body. As the whole mounts of the fat body taken from the control millipedes do not give a benzidine positive reaction, it may be assumed that an endogenous peroxidase enzyme is absent in the fat body of *Spirostreptus asthenes*.

In this experiment, the uptake of a foreign recognizable proteinlike plant peroxidase by the fat body from the blood has been taken to indicate the capacity of the fat body to sequester proteins from the blood. LOCKE and COLLINS[12] observed the course of peroxidase uptake by fat body at various stages in the development of the larva of *Calpodes ethlius* and noted that the peroxidase uptake is maximum at the approach of pupation when the protein granule formation is at its peak, suggesting the direct incorporation of blood protein into the fat body protein granules. In *Spirostreptus asthenes* the storage protein granules are formed at the premoult stage. The peroxidase uptake at this period of moult indicates that the proteins might be sequestered from the blood for the formation of granules. In insects like *Malacosoma americanum* and *Rothschildia orizaba*, a general migration of blood proteins into fat body at the time of pupation, when the storage protein granules are formed, has been observed by electrophoretic means[13]. These observations are consistent with the idea of sequestration of blood proteins by the fat body of insects like *Calpodes ethlius* [6,12] and of millipedes like *Spirostreptus asthenes* as indicated in the present work. A similar condition is also present in the grasshopper *Melanoplus differentialis*, where the pericardial cells play some role in intermediary protein metabolism by taking up protein from the haemolymph but releasing it in an altered form[14]. But in the present study it is not known whether the sequestered protein is stored as such or undergoes change before storage. This aspect deserves further investigation.

Résumé. On a observé chez un mille-pattes (*Spirostreptus asthenes*) que la péroxidase injectée dans le sang a été trouvée plus tard dans le corps gras, ce qui suggère que celui ci est capable d'isoler la protéine du sang.

T. SUBRAMONIAM[15]

Department of Zoology, University of Madras, Madras-5 (India), 20 April 1971.

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[15] I am thankful to Prof. Dr. G. KRISHNAN for guidance and helpful criticism. I am greatly indebted to Dr. LOCKE, Case Western Reserve University, USA, for the gift of horse radish peroxidase and for independent verification of the results.