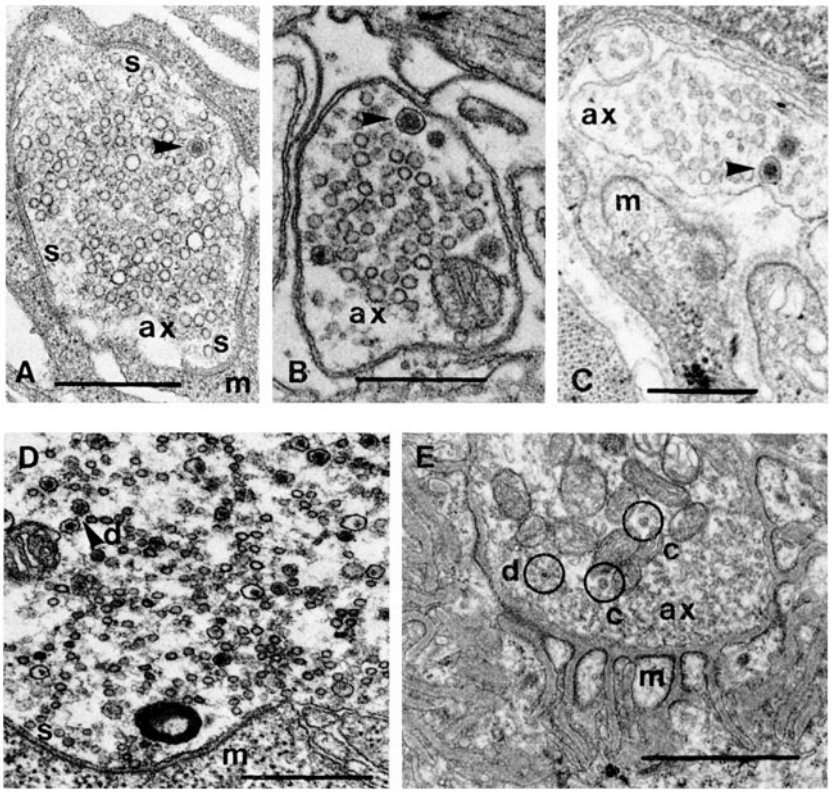

Examples of dense-cored vesicles in arthropod and vertebrate presynaptic nerve terminals. Fixation was accomplished using glutaraldehyde and osmium tetroxide; staining was done with lead citrate and uranyl acetate. A) Fresh-water crayfish (*Procambarus clarkii*) leg opener muscle, excitatory axon (ax). Several synaptic contacts (s) with the muscle fiber (m) are evident. The axon terminal is filled with clear synaptic vesicles, and contains a prominent dense-cored vesicle of 800 Å diameter (arrow). B) Excitatory nerve terminal in the depressor muscle of the tarsal claw of a tarantula spider, *Eurypelma marxi*. The presynaptic axon (ax) contains many synaptic vesicles, and a dense-cored vesicle of 1050 Å diameter (arrow). C) Developing neuromuscular junction of a new-born mouse (soleus muscle). An axon (ax) in close contact with the muscle fiber (m) contains clear synaptic vesicles and a dense-cored vesicle (arrow) of 1050 Å diameter. D) Stomach muscle of the spiny lobster (*Panulirus argus*). An excitatory nerve terminal forms a synapse (s) with a muscle fiber (m). Numerous dense-cored vesicles (d) are present, in addition to clear synaptic vesicles. E) Mouse neuromuscular synapse (extensor digitorum longus, adult muscle). The axon terminal (ax) contains numerous synaptic vesicles and mitochondria. A dense-cored vesicle (d, circled) of 750 Å diameter, and two 'coated' vesicles (c, circled) appear in this terminal. Calibration marks: 0.5 μm in A, B, C, D; 1 μm in E.

[1] T. Hökfelt, Acta physiol. scand. *76*, 427 (1969).
[2] H. M. Gerschenfeld, Z. Zellforsch. *60*, 258 (1963).
[3] I. Zs.-Nagy, Symposium on Neurobiology of Invertebrates (Ed. J. Salanki; Plenum Press 1968), p. 69.
[4] B. Katz, *Nerve, Muscle and Synapse* (McGraw-Hill, Maidenhead 1966).
[5] F. E. Bloom, L. L. Iversen and F. O. Schmitt, *Macromolecules in Synaptic Function*, Neurosciences Res. Program, Bulletin *8*, 325 (1970).
[6] H. L. Atwood, Experientia *24*, 753 (1968).
[7] H. L. Atwood and W. A. Morin, J. Ultrastruct. Res. *32*, 351 (1970).
[8] F. J. Barrantes, Z. Zellforsch. *104*, 205 (1970).
[9] T. Komuro, Z. Zellforsch. *105*, 317 (1970).
[10] T. Miller and W. W. Thomson, J. Insect Physiol. *14*, 1099 (1968).
[11] W. A. Morin, personal communication.
[12] N. N. Osborne and M. R. Dando, Comp. Biochem. Physiol. *32*, 327 (1970).
[13] E. Maynard, personal communication.
[14] A. H. Bunt, J. Ultrastruct. Res. *28*, 411 (1969).
[15] D. J. Curtis and G. A. Kerkut, Comp. Biochem. Physiol. *30*, 835 (1969).
[16] L. Guth, Physiol. Rev. *48*, 645 (1968).
[17] Supported by grants from the National Research Council of Canada and the Muscular Dystrophy Association of Canada.
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vesicles than the neuromuscular junctions of adult animals. 'Coated' vesicles, which may be precursors to synaptic vesicles[5,14], were also observed (Figure E).

From these results, it appears that dense-cored vesicles are a common feature of arthropod and vertebrate motor and peripheral inhibitory nerve terminals. As noted above, there is some evidence from flourescence microscopy and reserpine treatments, that these vesicles may contain monoamines as in other systems[1,3,15]. The function of the dense-cored vesicles in neurons which are known to release acetylcholine, GABA, or other substances as transmitter agents is obscure. They may mediate some 'trophic' effect[16] on the muscle fibers[17].

Résumé. Les vésicules synaptiques granulées se trouvent dans les jonctions neuromusculaires de mammifères, des crustacés, et des araignées. On ne connait pas leur fonction.

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Isolation of Intracellular Yolk Granules in Early Chick Embryos and Estimation of their DNA Content

Quantitative analysis of DNA in early chick embryos has demonstrated the presence of unusually high amounts of DNA in the early embryo cells during the first 18 h of development[1]. Since photometric determinations of the nuclear DNA content showed values similar to those in older embryo stages, it was concluded that the excess DNA was localized to the cytoplasm, the most likely site being the intracellular yolk granules. The latter represent ovocyte material segregated at the formation of the blastoderm and they differ markedly from the yellow granules which make up most of the yolk in the hen's egg[2-4]. A later investigation has confirmed the presence

of DNA in the intracellular granules[5] and similar conditions have been shown to exist in invertebrate and amphibian embryos[6,7], the yolk granule DNA of the latter being so far the only nucleic acid of its kind which has been analyzed more closely[8]. No definite information exists as to the role of yolk granule DNAs. A nuclear origin for such DNA has, however, been demonstrated in the polychaete Ophryotrocha[9] and possibly it represents informational DNA, coding for developmental processes during early embryogenesis.

All observations were made on embryos from freshly laid White Leghorn eggs; incubation was performed at 37.5 ± 0.5 °C. All procedures were carried out at +4 °C.

Preparation of embryos was carried out in 0.93% NaCl. Analyzed material comprised the embryo area (area pellucida) of 0-, 12- and 18-h blastoderms and for the 0-h stage also the area opaca. 30–40 embryo areas were pooled and homogenized in a glass-teflon homogenizer at low speed. The suspension was then centrifuged on a sucrose gradient, which was found to give maximum yield when prepared as shown in Figure 1. Homogenization was performed in 0.93% NaCl and the solution was thereafter changed to 0.5 *M* sucrose (= the upper phase of the gradient). The sucrose gradient was centrifuged for 80 min at 1300 *g* in an MSE centrifuge at +4 °C. The gradient material was removed in 3 separate phases, containing, respectively, mitochondria, yolk granules and nuclei. Samples of the yolk granule phase were frozen as soon as small amounts had been taken to control the purity. Nuclear and mitochondrial contamination as revealed in the phase microscope was always very low. The samples were thawed for analysis and ultracentrifuged at 200,000 *g* in an MSE SS 50 centrifuge for 2 h. Each DNA and protein analysis was made on 3 combined samples, i.e. with material from about 100 embryos. For each stage at least 3 determinations were made which gave identical results. The yield of yolk granules was controlled for each sample. In all cases it was estimated to be 80%; scanning electron microscopic analysis (Figure 2) showed the preparations to be fully representative and without contamination. Seasonal variations were never observed.

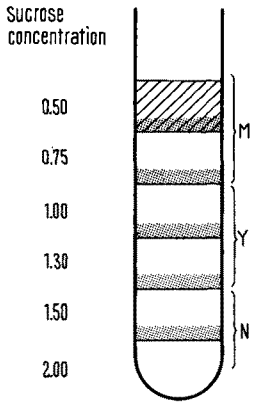

Fig. 1. M, Y and N represent phases containing, respectively, mitochondria, yolk granules and nuclei.

[1] H. EMANUELSSON, Acta physiol. scand. *52*, 197 (1961).
[2] V. D. MARZA and R. V. MARZA, Q. J. microsc. Sci. *78*, 134 (1935).
[3] A. L. ROMANOFF and A. J. ROMANOFF, *The Avian Egg* (Wiley and Sons, New York 1949).
[4] R. BELLAIRS, J. biophys. biochem. Cytol. *11*, 207 (1961).
[5] H. EMANUELSSON and C. v. MECKLENBURG, Ark. Zool. *22*, 155 (1968).
[6] W. A. ANDERSON, J. Ultrastruct. Res. *26*, 95 (1969).
[7] J. BRACHET and A. FICQ, Expl. Cell Res. *38*, 153 (1965).
[8] E. BALTUS, J. HANOCQ-QUERTIER and J. BRACHET, Proc. natn. Acad. Sci., USA *61*, 469 (1968).
[9] H. EMANUELSSON, Z. Zellforsch. *95*, 19 (1969).

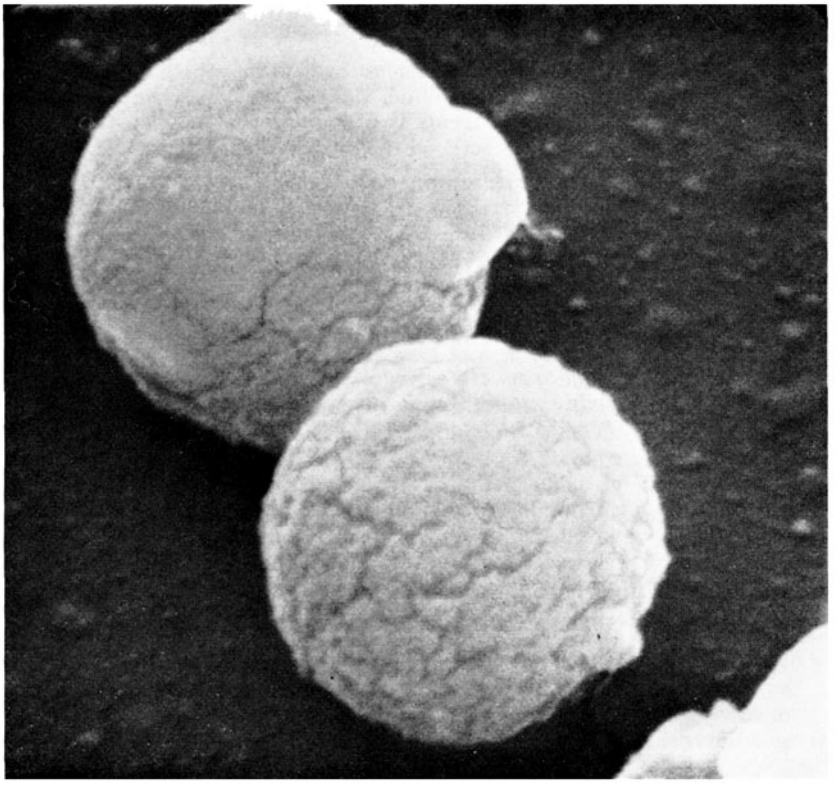

Fig. 2. Scanning electron micrograph of yolk granules, prepared as described in the text, mounted on a cover glass and acetone dried. ×21,000.

The amount of DNA was determined by the diphenylamine method[10]. Calf Thymus DNA Type 1 Sigma was used as a standard. In samples treated with DNase (Bovine Pancreas DNase I, Sigma) DNA was no longer detectable by the same method.

The protein content of granules was determined by the LOWRY method[11]. Bovine serum albumin (Fraction V, Sigma) was used as a standard. Estimations of the number of cells in analyzed areas refer to counts made on fixed and stained control preparations.

Owing to non-removal of the area opaca, the 0-h stage does not exactly correspond to the more advanced ones. However, this addition was necessary because of the difficulty in defining the areas exactly at this stage, and preliminary analyses had shown the DNA content/cell to be the same in both areas. Judging from electron micrographs of the stage in question, the resemblance between cells from the two areas is still manifest at this time, especially with regard to yolk granule content. Furthermore, the area pellucida comprises at least 55% of the total number of cells of the 0-h blastoderm, which implies that dissimilarities between the areas in the features analyzed here are likely to be minimal.

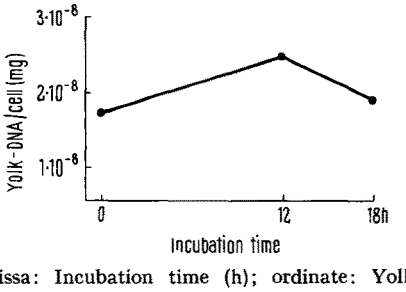

Fig. 3. Abscissa: Incubation time (h); ordinate: Yolk-DNA/cell (mg). Each point represents an average value from at least 300 embryos.

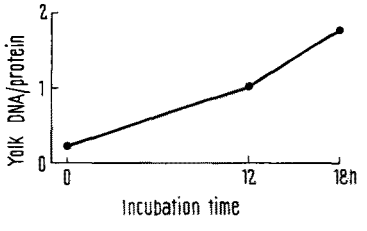

Fig. 4. Abscissa: Incubation time (h); ordinate: Yolk DNA/protein. Each point represents an average value from at least 300 embryos.

Figure 3 shows that during the period investigated the DNA present in the yolk granules undergoes an initial increase in amount followed by a decrease. This temporary increase at first seems puzzling, considering the cytological observation of a gradual disappearance of yolk granules from the developing embryo cells, but is in accordance with autoradiographical analyses which have revealed a considerable reduplication of yolk granule DNA during the very earliest hours of incubation[5]. As yet, however, there is no definite information as to whether this synthesis runs parallel with or is dependent upon nuclear DNA synthesis. Actually the amount of yolk granule DNA/cell at the 0-h stage corresponds to the total nuclear DNA content/cell at the same stage. It should be noticed that the decrease of yolk granule DNA is noticeable immediately before gastrulation in the chick embryo, which may indicate commitment in the morphogenetic events taking place during this process and/or in the subsequent organisation of early organ development. In Figure 4 is shown the result of protein analyses of the isolated yolk granules. The increase of the DNA/protein ratio – although large – seems natural and implies that, with degradation of the granules, utilization of the protein components proceeds more rapidly than the decline of DNA itself. This finding indicates some independence between DNA and protein in the granules. It may to some degree warrant the recent suggestion that yolk granule DNA may code for yolk catabolizing enzymes[8].

Zusammenfassung. Nachweis, dass der DNS-Gehalt der Dottergranula in Hühnerembryonalzellen in der frühesten Entwicklungsperiode (0–18 h) temporär ansteigt.

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Zoophysiological Institute, University of Lund, Lund (Sweden), 12 February 1971.

[10] K. BURTON, Biochem. J. *62*, 315 (1956).
[11] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[12] The skilled technical assistance of Miss INGER ANTONSSON is gratefully acknowledged.
[13] The scanning electron microscope was made available by Analytica, Sollentuna and the scanning micrograph was taken by Mr. G. ALSTERBORG.
[14] This work has been supported by the Swedish Natural Science Research Council (H.E.) and Kungliga Fysiografiska Sällskapet, Lund.

Histochemical Evidence for a Somatotopic Organization of the Rubrospinal Projection in the Rat

Loss of acetylcholinesterase (AchE) activity from the cell body of a neurone following severance or ligation of the axon has been reported by several authors [1–5, 7, 9, 11, 12]. The majority of such studies have involved the severance of axons in the peripheral part of the nervous system. The present study uses the thiocholine technique to demonstrate changes in the AchE content of neurones in the red nucleus of the rat following rubrospinal tractotomy.

Under Sodium Pentobarbital anaesthesia and using aseptic conditions, the rubrospinal tract was severed at the vertebral level C4 in one group of 11 rats and between the vertebral levels T9 and T13 in another group of 18 rats. After survival times ranging between 1 to 76 days the ani-

[1] L. W. CHACKO and J. A. CERF, J. Anat. *94*, 74 (1960).
[2] O. ERANKO and M. HARKONEN, Acta physiol. scand. *63*, 411 (1965).
[3] B. A. FLUMERFELT and P. R. LEWIS, J. Anat. *104*, 587 (1969).
[4] B. FREDRICSSON and F. SJOQVIST, Acta morph. neerl. scand. *5*, 140 (1962).
[5] M. HARKONEN, Acta physiol. scand. *63*, suppl. 237 (1964).
[6] P. R. LEWIS, Biblfia. anat., Basel *2*, 11 (1961).
[7] V. NAVARATNAM and P. R. LEWIS, Brain Res. *18*, 411 (1970).
[8] O. POMPEIANO and A. BRODAL, J. comp. Neurol. *108*, 225 (1957).
[9] H. G. SCHWARZACHER, Acta anat. *32*, 51 (1958).
[10] C. C. D. SHUTE and P. R. LEWIS, Brain *90*, 497 (1967).
[11] U. SODERHOLM, Acta physiol. scand. *65*, suppl. 256 (1965).
[12] H. A. WALDRON and D. G. GWYN, Brain Res. *13*, 146 (1969).