In the observation of the corion-allantoic membranes of the injected eggs, after 72 h of incubation, the capillary vessels appear oedematous, swollen, the intercellular spaces are augmented so as to render possible the exit of the red cells from the vessels.

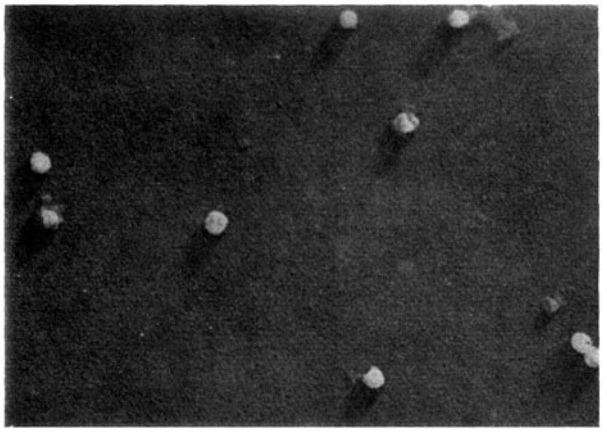

Fig. 2.

The findings to which the most essential data refer are reproducible in sets: on the surface of the red cells of blood of scarlet fever subjects in the acute stage of the malady, we obtained findings somewhat more variable than those in microphotograph No. 1. Nevertheless in the observation performed on the blood cultures, essentially similar findings were obtained in numerous cases.

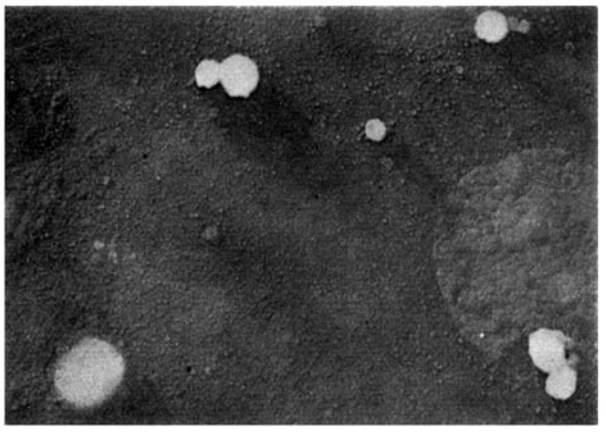

Fig. 3.

Fig. 2/3.—From the culture of blood of scarlet fever subject withdrawn the second day of the malady, observation performed after thirty days of incubation in thermostat. Numerous elementary single corpuscles of the same (Fig. 3) and different size (Fig. 4), are noted (shadows with chromium vapours 18,000 × 1).

The changes of the capillaries of the corion-allantoic membranes of chick embryos infected with cultures of blood of scarlet fever subjects can be reproduced by inoculating the allantoic fluid of these embryos in the allantoic cavity of other embrionated eggs.

Considerations. The morphological aspects of a pathogenic agent barely visible to the optical microscope can be studied only by means of the electron microscope.

From the analysis of results obtained in the performed experiments, it follows that the elementary corpuscles observed for the first time by DI CRISTINA, CARONIA, and SINDONI[1] in culture of blood from scarlet fever subjects can clearly be demonstrated in the electron microscope.

If there be any relations between the adult element visible to the optical microscope and the streptococcus, they do not appear from our observations.

I wish to express my thanks to the Superior Institute of Health in Rome for the hospitality accorded to me for the execution of the researches reported here.

F. MULÈ

Institute of 1st Pediatric Clinic and of Infectious Diseases, University of Rome, February 16, 1954.

Riassunto

È stato eseguito uno studio al microscopio elettronico su culture di sangue di ammalati affetti da scarlattina. Sono stati osservati dei corpuscoli elementari sulla superficie dei globuli rossi ed al di fuori di essi.

Nelle membrane corion allantoidee delle uova inoculate con le culture di sangue di scarlattinosi, i capillari appaiono ademato si, rigonfi, gli spazii intercellulari sono aumentati così da rendere possibile la fuoriuscita dei globuli rossi.

I risultati di questi esperimenti sono riproducibili in serie.

[1] G. DI CRISTINA, La Pediatria *31*, 1 (1923). – G. CARONIA and M. B. SINDONI, La Pediatria *31*, 745 (1924).

Darkfield Microscopy of Living Neurosecretory Cells

During a study of the transport of secretory material in the axons of the neurosecretory cells of the blow-fly, *Calliphora erythrocephala*, it was found that the neurosecretory material could be seen in the living axons when using darkfield illumination (E. THOMSEN[1]). This observation prompted us to examine whether neurosecretory material could also be observed in the pericaryon of the neurosecretory brain cells.

The two medial groups of neurosecretory cells, situated in the pars intercerebralis of the protocerebrum, are recognizable in the living adult fly by their bluish white colour (E. THOMSEN[2]). These cell groups were exised, together with as small and thin a sheet of the surrounding brain tissue as possible, and were then examined in RINGER'S solution in the darkfield using ZEISS' darkfield equipment.

Figure 1 shows a photograph of the two groups taken with a Zeiss "Phoku" camera. The neurosecretory cells

[1] E. THOMSEN, Pubbl. Staz. Zool. Napoli *24*, suppl., 48 (1954); J. Exp. Biol. 1954 (in press).

[2] E. THOMSEN, Nature *161*, 439 (1948); J. Exp. Biol. *29*, 137 (1952).

stand out very clearly in contrast to the adjacent tissue. The dark spot in the centre of the cells represents the nucleus, and the number of cells in each group is about 8–10. The diameter of the cells is 20–24 μ. In the photograph the neurosecretory cells appear white on

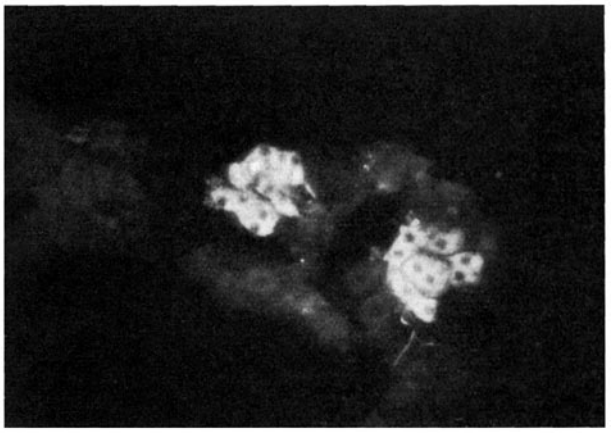

Fig. 1.—The two medial groups of neurosecretory cells (white) surrounded by other brain cells (grey). 120 ×.

account of their content of numerous refractive granules or spheres, visible in some of the cells of Figure 1 and more clearly in Figure 2. On direct observation in the darkfield microscope the granules show a bluish tinge. These granules correspond in appearance to those observed in sections of the neurosecretory cells when stained with the chrome-hematoxylin phloxin stain of GOMORI, and there can hardly be any doubt that they

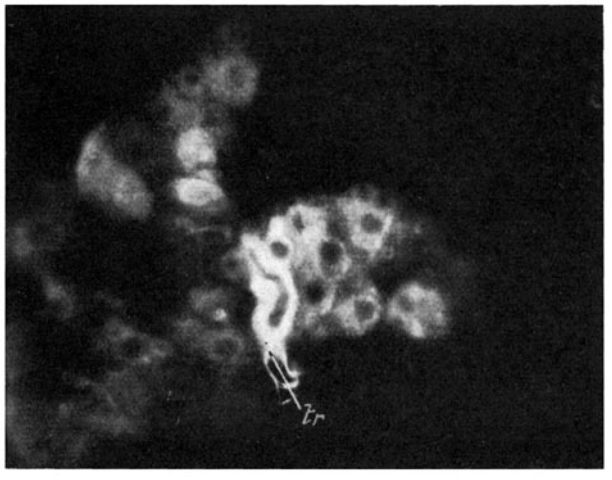

Fig. 2.—Neurosecretory cells with refractive spheres (= aggregates of granules). *tr* trachea. 240 ×.

are identical. This observation was briefly mentioned in a paper read to the first International Symposium on Neurosecretion held in Naples in May 1953 (M. THOMSEN[1]).

Using an oil-immersion objective (100 ×) it is seen that what appears as granules with a lower magnification are in fact aggregates of very small particles, about 0·4 μ in diameter, but probably somewhat varying. These particles are most easily demonstrated in the axons of the neurosecretory cells (Fig. 3), while (in the photographs) they are difficult to distinguish in the pericaryon owing to its thickness. At present it cannot be decided whether all granules observed represent "the neurosecretory material" or if part of them are mitochondria

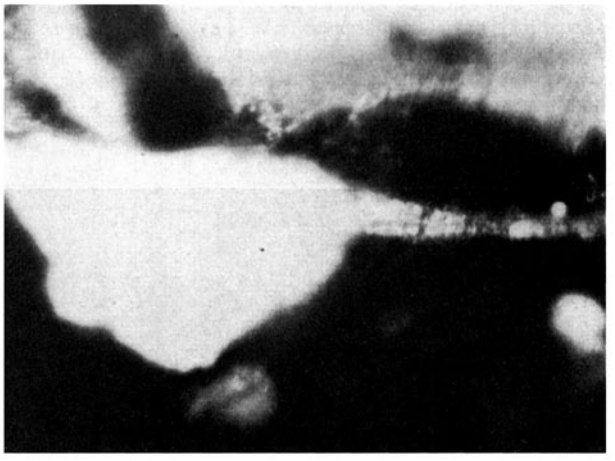

Fig. 3.—A single neurosecretory cell filled with refractive granules, separately visible in the proximal part of the axon (right). 1000 ×.

and other elements. Our finding is in accordance with the observation by PASSANO[1], who examined the X-organ cells of the crab *Sesarma* with phase-contrast microscope; he states that the cytoplasmic spheres are in reality "spheroid systems" composed of small granules (0·3 μ). CARLISLE[2] has observed the proximo-distal movement of "droplets, granules, spherical systems, and mitochondria" in the living X-organ connective of the crustacea *Dromia* and *Lysmata*.

Considering the discussion on the nature of the cytoplasmic inclusions characteristic of the neurosecretory cells, it is of some importance that such structures are not only demonstrable in fixed and stained preparations but are visible in the living neurosecretory cells and their axons.

ELLEN THOMSEN and M. THOMSEN

Zoological Laboratory of the Royal Veterinary and Agricultural College, Copenhagen, January 18, 1954.

Zusammenfassung

In anderen Arbeiten von ELLEN THOMSEN wurde gezeigt, dass neurosekretorisches Material in lebenden Axonen der neurosekretorischen Zellen von *Calliphora* mit Dunkelfeldmikroskopie beobachtet werden konnte. Im Dunkelfeld sieht man ebenfalls, dass die Pericarya der lebenden neurosekretorischen Zellen der beiden medialen Gruppen in der Pars intercerebralis mit refraktilen Körnern gefüllt sind, die den in Schnittpräparaten mit Chrom-Hämatoxylin dargestellten ganz ähnlich sind. Diese Beobachtungen zeigen die Naturtreue des fixierten Präparates. Durch Anwendung von Ölimmersion konnte festgestellt werden, dass die genannten "Körner" von kleineren Partikelchen (0,4 μ) zusammengesetzt sind, entsprechend den mit Phasenkontrast beobachteten «spheroid systems» in X-Organzellen von Krustazeen (PASSANO, CARLISLE).

[1] M. THOMSEN, Pubbl. Staz. Zool. Napoli 1954 *24*, suppl., 16 (1954).

[1] L. M. PASSANO, Anat. Record. *112*, 460 (1952).

[2] D. B. CARLISLE, Pubbl. Staz. Zool. Napoli *24*, 135 (1953).