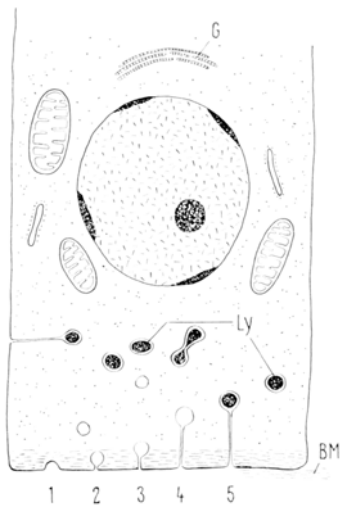
Über die Bildung von Lysosomen im fetalen Dünndarm

Bei der Bildung der primären Lysosomen sollen nach den gegenwärtigen Anschauungen Golgi-Apparat und endoplasmatisches Reticulum führend sein[1,2]. Offenbar kann die Lysosomenbildung in fetalen Dünndarmepithelzellen der Ratte vom 17.–18. Schwangerschaftstag auch vom Plasmalemm ausgehen. Wir beobachteten im basalen Zytoplasma dieser Zellen zahlreiche runde, ovale bzw. polymorphe, von einer Membran umgebene, saure phosphatase-positive Granula von 1500–3000 Å Durchmesser, die wir für Lysosomen halten.



Schematische Darstellung einer Dünndarmepithelzelle der Ratte vom 17. Embryonaltag. 1–5 Plasmalemminvaginationen, die als Vorstufen primärer Lysosomen (Ly) gedeutet werden. BM, Basalmembran; G, Golgi-Apparat.

In unserem Untersuchungsmaterial haben wir keine Hinweise dafür gefunden, dass diese ausschliesslich im basalen Zytoplasma vorkommenden Lysosomen im supranucleär gelegenen Golgi-Apparat oder – wie vermutet wurde[3] – im endoplasmatischen Reticulum entstehen. Wir nehmen stattdessen an (Figur), dass sie sich aus bläschenförmigen Einsenkungen und Abschnürungen des basalen und seitlichen Plasmalemms entwickeln, die wir häufig in unmittelbarer Nähe der Lysosomenanhäufungen beobachten. Für diese Annahme spricht, dass die Abschnürungen etwa denselben Durchmesser wie die Lysosomen besitzen und dass einige Vesikel, die durch einen etwa 4000 bis 5000 Å langen Plasmalemmstiel mit der Zellmembran in Verbindung stehen, bereits vor ihrer Ablösung von der Zellwand elektronendichtes, osmiophiles Material in ihrem Zentrum enthalten. Weitere Untersuchungen müssen zeigen, ob diese Art der Lysosomenbildung auch für andere embryonalen Gewebe zutrifft[4].

Summary. Electron microscopical and enzyme histochemical investigations suggest that in the fetal small intestine of the rat primary lysosomes originate from invaginations of the cell membrane and not from the Golgi-apparatus or the endoplasmic reticulum.

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Phagocytosis of Zymosan Particles by Mast Cells

Despite the voluminous work that has appeared dealing with the mast cell[1–4], the physiologic functions of this cell are still poorly understood. Although mast cells are thought not to be phagocytic, some suggestive but inconclusive evidence has appeared to indicate otherwise. Dougherty and Schneebeli[5,6] have recorded, by time-lapse cinematography, the in vitro behavior of mouse mast cells exposed to glucan particles. They concluded that some mast cells attempted to engulf the glucan. However, this resulted only in surface indentation, and lysis of the mast cells followed before ingestion occurred. Roberts[7], by the use of autoradiography, described the incorporation of a tritiated soluble sulfanylazo derivative of porcine γ-globulin and noted a decreased basophilia of the cytoplasmic granules of the labeled mast cells. This was attributed to binding of the antigen to the granules which prevented subsequent binding of the dye.

In the course of studies on chemotaxis[8], it was noted that zymosan injected i.p. into rats appeared to become embedded in mast cells. It was not possible by the techniques used to establish with certainty whether the particles were intracellular or were merely indenting the mast cell surface. Therefore, electron microscopic techniques were used to re-examine this finding.

Male rats of the Sprague-Dawley Holtzman strain, 35–61 days old, were injected i.p. with 0.1 ml of a zymosan preparation (Standard Brands Inc., New York, N.Y., Lot OB-298) suspended in 0.9% NaCl solution. After various time intervals, rats were decapitated and peritoneal fluid was harvested for both light and electron microscope studies. Smears were stained with Wright-Giemsa. In addition, wet mounts of both living and dead cells, with and without staining, were also examined. For

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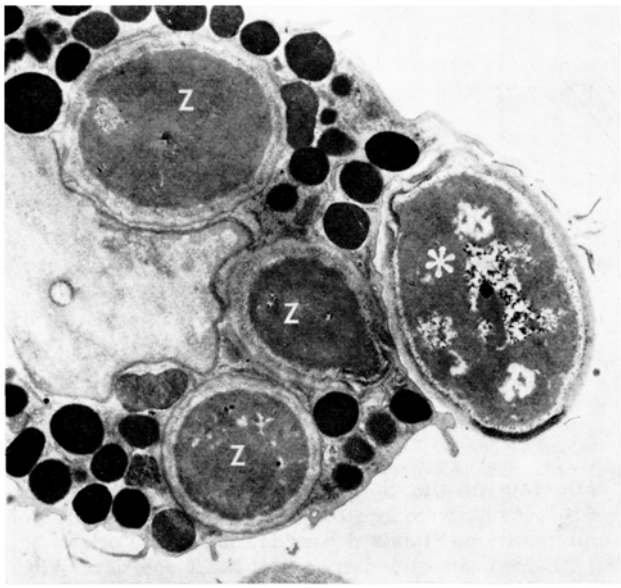
the electron microscope studies, peritoneal fluid was fixed for 3 h in Karnovsky's fluid diluted 1:2 with 0.1 *M* cacodylate buffer, pH 7.2. While in the fixative, the cells were collected into a pellet by centrifugation. After washing the pellet 15–18 h in sucrose-cacodylate buffer at about 4 °C, it was post-fixed for 1 h in 1% OsO_4 in veronal acetate buffer, pH 7.4, at room temperature. The pellet was dehydrated in ethanol at room temperature and was embedded in Epon according to the method of LUFT[9]. Thin sections were stained with solutions of either lead citrate or with both uranium acetate and lead citrate. An RCA EMU 3G microscope was used.

Some of the mast cells examined by light microscopy $1^1/_2$ h after the injection of zymosan appeared to contain zymosan particles and, at 3 h after zymosan, this close association between zymosan particles and mast cells was even more common. The resolution of the light microscope was not sufficient to determine with certainty whether the zymosan particles had become intracytoplasmic or whether they were merely indenting the surface and creating this illusion. 1–3 h after the injection of zymosan, the peritoneal macrophages were filled with these particles. Many eosinophils contained some particles, and some mast cells were associated with one or several particles.



A mast cell from rat peritoneal fluid. Lead citrate stain. One zymosan particle (*) is adherent to the cell surface. Three zymosan particles (Z) are within vacuoles. × 7800.

Electron micrographs supported the concept that mast cells took up zymosan particles by phagocytosis. The Figure shows a mast cell with several zymosan particles. One of these particles appears to be merely adhering to the cell whereas 3 particles are within vacuoles. One hour after the injection of zymosan, those mast cells associated with particles displayed large pseudopods, in contrast to their normal ovoid condition. More numerous and larger microvilli were often seen on the mast cell surface closest to the zymosan particles. Three hours after injection, neither pseudopods nor evidence of increased surface activity were noted. Mast cell granules, including those in the immediate vicinity of the ingested particles, appeared normal after both 1 and 3 h.

Experiments in progress indicate that zymosan particles are still visible in mast cells 8 days after injection and that, at the light microscope level, the particles still appear intact, contrary to their degraded condition in macrophages. The total number of peritoneal fluid mast cells decreased markedly following zymosan injection and remained low for at least 8 days.

This report demonstrates that mast cells can be phagocytic. Studies by others[10–12] have implicated the mast cells in certain immunological reactions. It remains to be determined whether phagocytosis by mast cells is an integral part of such phenomena[13].

Résumé. Des rats ont été injectés par voie péritonéale avec une suspension de zymosan, une préparation de parois de levure, *Saccharomyces cerevisiae*. Les cellules de la lymphe péritonéale furent étudiées après au microscope optique et électronique. Ces expériences ont démontré que les mastocytes changent d'aspect et que les particules de zymosan se retrouvent dans des vacuoles cytoplasmiques peu après l'injection. Les mastocytes sont donc des cellules capables de phagocytose.

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Small Myelinated Perikarya in the Cerebellar Granular Layer of Mammals Including Man

In the peripheral nervous system, myelinated neuronal perikarya have long been known to occur in spinal and some cranial ganglia, notably acoustic and vestibular, of a wide variety of vertebrates (cf. the review of ROSENBLUTH and PALAY[1]). In the mouse cerebellum cultured in vitro, perikaryal myelin was frequently observed around small cells, ca. 7 μ in diameter, with round chromatin-rich nuclei and scanty cytoplasm (SUYEOKA and OKAMOTO[2]) (Figure 1).

The present investigation was undertaken to determine whether these myelinated perikarya exist in the mouse cerebellum in situ. Adult mice, over 3 months old, were perfusion-fixed according to the method of CAMMERMEYER[3] and stained for myelin by WEIL's method. Small myelinated perikarya of similar size as observed in the cultures were also found not infrequently in the cerebellar granular layer[4] (Figure 2). Although the isolated perikarya were not rare, they were often closely associated