Zusammenfassung. Es wird gezeigt, dass im Cytotoxizitätstest mit Mäuselymphocyten der cytotoxische Titer durch Neuraminidasevorbehandlung stark gesteigert werden kann, falls Kaninchenserum als Komplement verwendet wird. Durch Trypsin- oder Ficin-Vorbehandlung wird nur eine schwache Steigerung erzielt. Im Gegensatz zum Cytotoxizitätstest mit unbehandelten Lymphozyten wird der stärkste Titeranstieg bei der «one step»-Methode beobachtet. Um die spezifische Titererhöhung mit Kaninchen-Komplement zu erklären, wird ein «enhancing factor» mit einer Spezifität gegen Neuraminidase behandelte antikörperbeladene Lymphocyten postuliert.

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Anti-Lymphocyte Serum and Suppression of Interferon Production

Although the effect of heterologous anti-lymphocyte serum on interferon production has been studied by several investigators, there is no general agreement regarding the nature of this effect. HIRSCH et al.[1] reported no significant depression in interferon levels of mice given 3 injections of rabbit antimouse lymphocyte serum before stimulation with vaccinia virus. BORDEN et al.[2] obtained a slight reduction (2-fold) in interferon titers of mice treated with anti-lymphocite serum. In contrast to these findings, a study by BARTH et al.[3] reported a significant reduction in interferon titers of mice given 3 injections of burro antimouse lymphocyte serum. The present study reports additional data on suppression of interferon production by antilymphocyte serum.

Methods. Anti-mouse lymphocyte serum (ALS) was produced by immunizing rabbits with two i.p. injections of mouse thymocytes, according to a modified procedure of LEVEY and MEDAWAR[4]. Rabbits were bled 1 week after the last injection. Sera were pooled, inactivated at 56 °C for 20 min, and adsorbed with 20% mouse red blood cells for 1 h at 37 °C and overnight at 4 °C. Agglutination tests with mouse red blood cells were negative after adsorption. Pooled serum was tested for antibody to mouse lymphocytes by the cytotoxicity test: incubation of ALS with mouse spleen cells for 30 min at 37 °C resulted in 32% cell mortality, compared to 6% mortality in normal rabbit serum (NRS) controls. Mice were injected i.p. with 1 ml of either ALS or NRS, followed 24 h later by 1 ml Newcastle disease virus (NDV) (640 hemagglutinating units). Animals were bled 8, 16 and 25 h after injection of NDV. Sera were collected and assayed for interferon in L-929 cells by inhibition of cytopathogenic effect, using vesicular stomatitis virus as challenge[5].

Results. Treatment of mice with ALS produced a 4-fold reduction in the amount of interferon produced, when compared to NRS-treated controls. Suppression could be demonstrated 8 h after stimulation with NDV. After 24 h, interferon could not be detected in ALS – treated mice (Table).

These results are in general agreement with those of BARTH et al.[3] who reported a 4- to 5-fold reduction in the amount of interferon produced in ALS-treated mice.

Depression of interferon response in ALS-treated mice constitutes indirect evidence for involvement of lymphoid cells in interferon production.

Résumé. L'administration de 1 ml ALS, 24 h avant l'introduction de l'interféron avec le virus de la maladie de Newcastle, provoqua une réduction au quart du taux de l'interféron.

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Effect of treatment with ALS or NRS on induction of interferon in mice by NDV

Treatment	Interferon titer (units/3 ml) h after induction		
	8	16	24
NRS	1500	550	280
ALS	390	200	0

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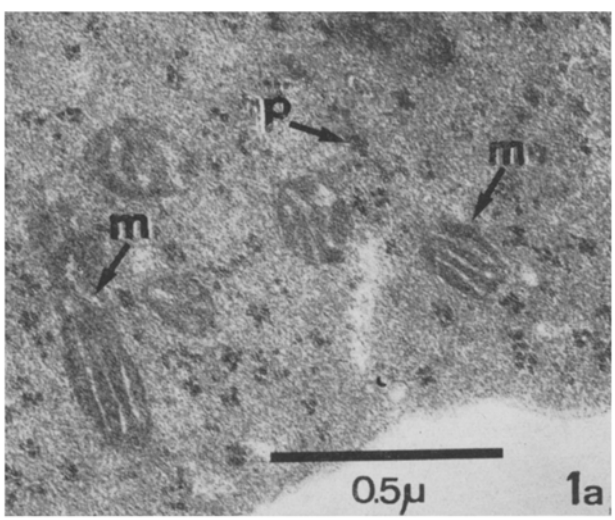
Hemoglobin in Immature Erythrocyte Mitochondrion-Like Organelles

An increase in number of mitochondria occurs in reticulocytes after nuclear extrusion by erythroblasts in the peripheral blood of rabbit-embryos[1]. Mitochondrion-like organelles (MLO) in cells fixed in hypotonic medium and stained by phosphotungstic acid were shown from pinocytotic vesicle formation onwards[2]. Reticulocyte MLO are formed concomitantly with the transformation of iron containing material, for heme biosynthesis. Besides their higher number in relation to the later erythroblasts, reticulocyte MLO, constituted predominantly by longitudinal cristae, are highly electron dense, suggesting a high protein content in these organelles[2].

Heme biosynthesis occurs in mitochondria[3], and ferritin or ferruginous material was found within the interlamellar mitochondrial space of erythroblasts in hypochromic anemias[4,5]. Benzidine reaction is also positive for reticulocyte MLO as seen in hemolyzed blood smears[2]. These facts and the supposed presence of proteins in relatively high amount within the reticulocyte MLO[2] lead us to investigate the possible hemoglobinic nature of this protein.

Material and methods. Blood was obtained from the umbilical cord of 19-day-old rabbit-embryos. As examined in Giemsa stained smears, the peripheral blood presents, besides its high number of reticulocytes and a lower number of polychromatic and orthochromatic erythroblasts, primitive erythroblasts practically without organelles. Blood from newborn rabbits was also utilized and immature erythrocytes are only the annucleated ones. For electron microscopy, blood was fixed in 4% glutaraldehyde in isotonic phosphate buffer, for 1 h, followed by osmic acid fixation (15 min), uranyl acetate staining (30 min), dehydration, and embedding in Araldite. Thin sections were obtained in a Porter-Blum microtome, stained by lead citrate[6] and photographed in an Elmiskop I electron microscope at 60 KV, with magnifications from $\times 8{,}000$ to $\times 40{,}000$.

For the demonstrations of hemoglobin within the MLO through electrophoresis, blood cells from 40–50 embryos or from 6–8 new-born rabbits were fractionated, and the



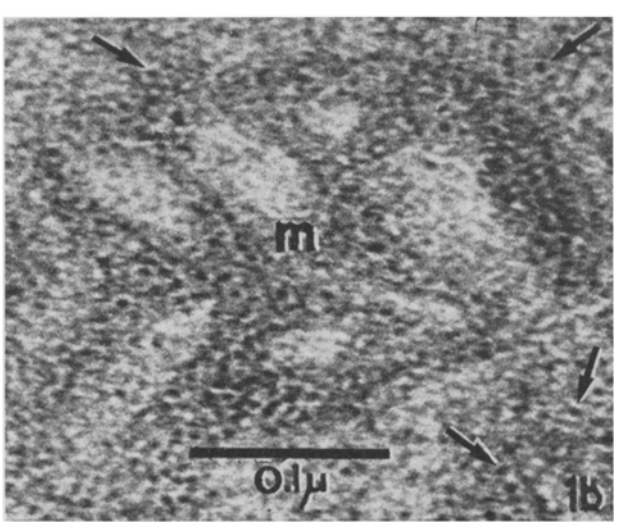
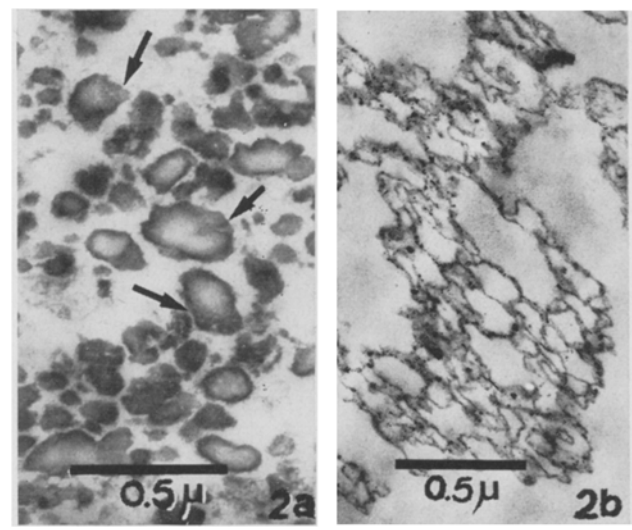


Fig. 1. Thin sections of intact reticulocytes. a) m, mitochondrion-like organelles; p, polyribosomes. b) Particles similar to hemoglobin molecules (arrows) showing a higher concentration within m than in cytoplasm.



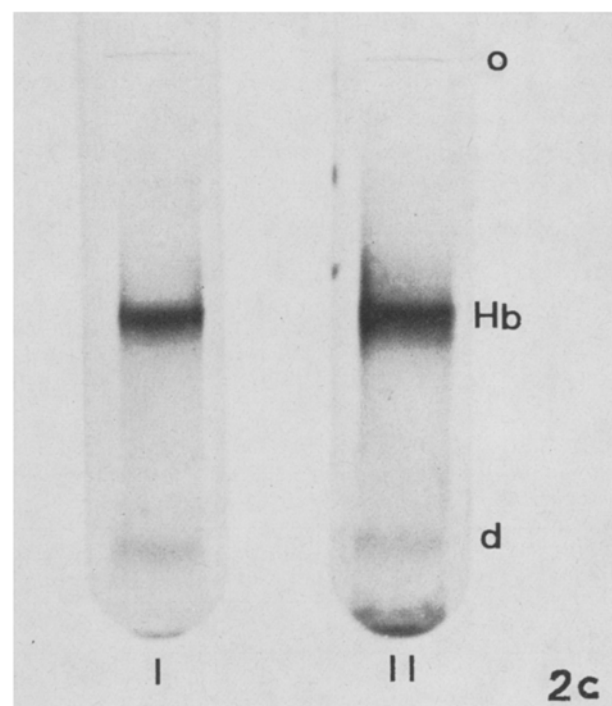


Fig. 2. Sections of mitochondrion-like organelles obtained from reticulocyte fractionation. a) After 5 washings, showing traces of lamella (arrows). b) After osmotic lysis for electrophoresis of the supernatant. c) Electrophoretic patterns of hemoglobin from fetus (I) and from mitochondrion-like organelle content (II); o, origin; d, dye band.

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MLO isolated and lysed according to the following procedure: 1. Blood is poured on 12 ml of a 0.153 *M* NaCl, 0.005 *M* KCl, 0.005 $MgCl_2$ and 0.03 *M* phosphate buffer (pH 7.2). 2. Centrifugation of the cell suspension for 10 min at 200 g, discarding of the supernatant, and resuspension of the sedimented cells (0.7–1.0 ml) in 10-fold their volume of a 0.32 *M* sucrose and 0.03 *M* phosphate buffer. 3. Homogenization in a Potter-Elvehjem tube at 1,000 rpm for 1 min at 4°C. 4. Centrifugation of the homogenate at 1,350 *g*, for 10 min (generally steps 3 and 4 were repeated, resuspending the 1,350 *g* sediment). 5. Centrifugation at 4°C of the 1,350 *g* supernatants for 10 min at 10,000 *g* 6. Resuspension of the nuclei-free MLO sediments in 0.32 *M* buffered sucrose, and 5-fold washings of the fraction by successive resuspensions and centrifugations at 10,000 *g* for 10 min. The sediment was lysed by resuspension in 3.0 ml distilled water; 7. After the lysis is completed, the suspension was centrifuged at 19,000 *g* for 20 min. The supernatant was used for electrophoresis, and the sediment was fixed and embedded for electron microscopic examination.

As control, a mitochondrial fraction of Hep_2-cell tissue cultures was suspended in the organelle-free supernatant of the 10,000 *g* reticulocyte homogenate centrifugation. This suspension of Hep_2-cell mitochondria in embryo-blood hemoglobin, was submitted to the same procedure as used for immature erythrocyte MLO isolation and lysis, from step 5 to 7.

The supernatants of the lysed organelles and of the last washing medium of both cell type organelles were concentrated about ten-fold in a low vacuum chamber, to be submitted to spectrophotometric determination and electrophoresis. Diluted fetal hemoglobin was used for comparison through those methods.

Spectrophotometry was done in a BECKMAN DBG, according to the method of KAMPEN and ZIJLSTRA[7]. Hemoglobin was run on disc electrophoresis in polyacrylamide gels as described by DIETZ and LUBRANO[8]. A current of 2.5 mA was applied for 40 min at 5°C, and the hemoglobin band was identified by benzidine reagent.

Results and discussion. Reticulocyte MLO are typical with regard to their structure, in which the lamellas dispose themselves predominantly along their axis. Since fixation was done in glutaraldehyde and osmic tetroxyde, they are little more electron dense than the hemoglobinized cytoplasm (Figure 1a) when compared with the dense MLO of reticulocytes fixed in hypotonic medium and stained by phosphotungstic acid[1,2]. This is due only to the disposition of the particles, being more agglomerated within the organelles than within the cytoplasm. During maturation, besides the decrease of polyribosomes, the electron density of the cytoplasm increases and tends to equal that of MLO. The enhanced concentration of cytoplasmic hemoglobin prevents clear visualization of the organelles. Cytoplasmic particles, identical to the ones within the MLO (Figure 1b), have about the same dimensions of the hemoglobin molecule ($64 \times 55 \times 50$ A[9]). Spectrophotometric determinations showed absorbance in all supernatants, as that of the washed MLO (Figure 2a), due to traces of the heme group. However, only the supernatant of the lysed MLO (Figure 2b) showed a characteristic hemoglobin band in disc gel electrophoresis (Figure 2c). Neither the control nor the last washing supernatant showed any visible band.

These results suggest that the final hemoglobin synthesis may occur in reticulocyte MLO, after their formation and concomitant heme biosynthesis[2]. Globin, synthesized in polyribosomes[10,11], could be enveloped by pre-MLO structures before the formation of the pro-MLO[2], combining to heme within the organelles. Since this newly formed organelle of immature erythrocytes can be considered a specialized structure whose first function is heme biosynthesis, followed by a possible hemoglobin biosynthesis, it could be termed hemoglobinosome or simply hemosome.

Zusammenfassung. Es wurde das Vorkommen von Haemoglobin in mitochondrienähnlichen Organellen unreifer Erythrocyten aus peripherem Kaninchenblut elektrophoretisch aus der abgetrennten Fraktion nachgewiesen und ergänzt durch elektronenoptische Beobachtungen an Dünnschnitten ganzer Retikulozyten. Für die Organellen wird die Bezeichnung «Hemosome» vorgeschlagen.

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Normal Macrophage Migration Inhibition in Dexamethasone-Treated Guinea-Pigs

Suppression of in vivo delayed hypersensitivity skin tests is one of the reported effects of corticosteroid therapy[1]. In the present experiments the macrophage migration inhibition test is used to investigate whether treatment of guinea-pigs with dexamethasone (DEX) abolishes established in vitro delayed hypersensitivity.

Normal Hartley albino guinea-pigs of both sexes weighing 300–500 g were immunized with 0.1 cm^3 of complete Freund's adjuvant (CFA) into each foot pad and 0.6 cm^3 into the nuchal fat pad. A random sample of these guinea-pigs tested intradermally with 50 µg purified protein derivative of tuberculin (PPD) 3 weeks after immunization gave reactions of more than 10 mm induration. Beginning 2 to 6 weeks after immunization the guinea-pigs and their

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