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

[1] D. S. DAVID, M. H. GRIECO and P. CUSHMAN JR., J. chron. Dis. *22*, 637 (1970).

nonimmunized controls were treated with daily i.m. injections of either 80 μg or 200 μg of DEX in 0.1 or 0.2 ml of normal saline for a minimum of 6 (high dose) and a maximum of 20 (low dose) consecutive days. A sample of 4 animals skin-tested before steroid treatment and again prior to sacrifice demonstrated diminution but not complete abolition of their positive skin tests. Animals made anergic were too ill to test. Results of treatment with both doses of DEX were similar and have been pooled.

Induction, preparation and migration of peritoneal exudate cells were performed according to techniques previously described[2]. For direct migration inhibition (DMI) tests the cells migrated directly into media containing 20% fetal calf serum and additives as follows: no additive, 10 μg/ml excipient-free PPD (a gift of Dr. H. B. DEVLIN, Parke-Davis & Co., Detroit, Mich.), 8 μg/ml DEX, or 10 μg/ml PPD and 8 μg/ml DEX. For indirect migration inhibition (IMI) tests lymphocytes were isolated from 3-day peritoneal exudates by elution from cotton columns[2] and cultured in serum-free media, containing the same additives, for two days following which PPD and/or DEX was added to control media (after centrifugation to remove cells). Supernates were dialyzed against TC 199, serum was added, and these media were used unconcentrated for migrations of normal peritoneal exudate cells.

The oil-induced peritoneal exudates of treated guinea-pigs were significantly lower in volume but not in per cent lymphocytes compared to controls. DMI, using PPD as the antigen, for normal, DEX-treated, CFA-immunized, and DEX-treated, CFA immunized animals are detailed in the Table. Inhibition of macrophage migration occurred with CFA animals whether or not DEX treatment was given. Addition of DEX in vitro abolished the inhibitory response (Table). In vitro DEX often caused a marked stimulation of migration, but controlling for this by adding DEX to control cultures did not uncover inhibitory activity. In IMI experiments, addition of DEX after culture to supernates containing migration inhibitory factor (MIF), abolished MIF activity (IMI before DEX = 0.67, after DEX = 0.97). Non-specific toxicity interfered with an attempt to assay for MIF in supernates of lymphocytes cultured simultaneously with PPD and DEX.

The implication of these data is that MIF production to a previously encountered antigen is normal during short-term corticosteroid treatment, a conclusion compatible with the occasionally observed positive IMI tests in clinical states of cutaneous hyporeactivity[2,3], and with the findings of other authors[4,5]. Since MIF was not specifically purified (other than by dialysis) in the present experiments, this conclusion is only presumptive.

Whether the absence of inhibition in the presence of in vitro dexamethasone is the result of interference with MIF or with the assay cells is not answerable from these experiments. The work of COHEN, STAVY and FELDMAN[6] would suggest that the latter possibility is true.

DMI to PPD and to PPD and DEX by in vivo treatment

In vivo treatment	In vitro test media			
	PPD		PPD + DEX[a]	
	No. of animals	Mean MI	No. of animals	Mean MI
None	22	1.03	7	1.16
DEX	9	0.96	8	1.45
CFA	14	0.52	4	1.00
DEX-CFA	5	0.59	5	1.07

[a] All animals tested with PPD + DEX are also represented in the PPD column.

Résumé. L'inhibition de la migration des macrophages se voit chez les cobayes immunisés à la tuberculine, même au cours d'un traitement in vivo au dexaméthasone. In vitro le dexaméthasone abolit cette inhibition de même que la réponse au facteur inhibitif de la migration des cellules des cobayes témoins.

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Effect of Platelet Membranes on the Electrophoretic Pattern of 6-Phosphogluconic Dehydrogenase from Platelet Lysates

The interactions between membranes (stromata) and cytoplasmic enzymes were studied in red blood cells in vitro[1]. Three enzymes of human hemolysates, glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconic dehydrogenase (6PGD) and glutathione reductase (GSSGR) are modified in their activity by stromal factors: while erythrocytic GSSGR is activated by red cell stromata[2], the inactivation of G6PD and 6PGD is related to the activity of stromal NAD(P)ase, which inactivates G6PD directly by splitting the stabilizing NADP,[3,4]; 6PGD is instead indirectly modified in its structure and activity by one of the products of NAD(P)ase reaction, PADPR[5,6].

In the present work we compared the alterations of 6PGD and GSSGR in platelet versus red cell whole lysates.

Materials and Methods. Blood was collected with siliconized needle and syringe from 4 normal volunteers and 2 patients with polycytemia vera, then transferred to polypropylene centrifuge tubes containing $^1/_2$ vol. of a