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

1% solution of Sequestrene Na_2. The platelets were isolated according to STEFANINI et al.[7], then resuspended in saline. The final yield from 150 ml of blood was 10–15 ml of a suspension containing 0.5 to 2.0×10^6 platelets per mm^3. A control of the preparation showed that the contaminating leucocytes were less than 300 per mm^3 and the erythrocytes were practically absent. Platelet suspensions were lysed by 5 repeated freezing and thawing. Part of lysate, containing the membranes, was incubated for 2 h at 45 °C with (sample E) and without (sample D) NADP added (final concentration was $1.0 \times 10^{-4} M$ in Soerensen buffer pH 7.4); then centrifuged at $15,500 \times g$ for 30 min at 4 °C in a Lourdes refrigerated centrifuge. The remaining of the lysate was first centrifuged for 30 min at $15,500 \times g$; the supernatant was then incubated for 2 h at 45 °C with (sample C) and without (sample B) added NADP ($1.0 \times 10^{-4} M$), except for a sample that was not incubated (sample A) and served as 100% activity control.

In 2 cases, part of the supernatant collected after centrifugation was further centrifuged at $105,000 \times g$ for 2 h at 4 °C in a Spinco ultracentrifuge. A yellowish pellet, present after centrifugation, was discarded, while the supernatant, treated as described for sample B and C, was tested for enzyme activity and electrophoretic mobility (sample F and G, respectively).

G6PD and 6PGD were measured according to GLOCK and MCLEAN[8], GSSGR according to LONG and CARSON[9]. Electrophoresis of the samples was performed as previously described[6].

Results and discussion. The activity of 3 enzymes (G6PD, 6PGD, GSSGR) from human platelet lysates is presented in Table I. The electrophoretic pattern of 6PGD in untreated platelet lysates was always of the PdA type[10]. Only a single fast band is present in platelets.

The incubation of platelet lysates, with and without membranes, resulted (Table II) in the inactivation of G6PD when NADP was omitted from incubation mixture. The temperature and duration of incubation, previously selected in order to enhance the effect on 6PGD[5], did not permit us to differentiate between stromal and heat effect on G6PD. However NADP ($10^{-4} M$) protected the activity (Table II, C-E) and the electrophoretic mobility of the enzyme, both in the presence or absence of membranes (Figure).

The incubation of whole lysate (membranes not removed) in the presence of NADP resulted in the inactivation of 6PGD (Table II, E) and change in electrophoretic mobility (Figure, E). When membranes were removed by centrifugation at $15,500 \times g$ for 30 min, the incubation of lysates in the presence of NADP *did not* produce a significant loss of activity: the electrophoretic pattern, however (Figure, C), was not as sharp as in the case of untreated lysates or in the whole lysate incubated without NADP (Figure, A and D respectively). When ultracentrifugation was performed ($105,000 \times g$ for 2 h), the electrophoretic pattern of 6PGD was again similar to the pattern of the untreated sample (Figure, G).

Glutathione reductase activity, measured after incubation of platelet whole lysate (membranes not removed), was not increased (Table II, C–E); on the contrary, a

Table I. Enzymatic activity of G6PD, 6PGD and GSSGR in lysates from human platelets

Case No.	Platelets ($\times 10^6$)	Enzymatic activity		
		G6PD	6PGD	GSSGR
1	1.72	0.513	0.172	0.477
2	2.00	0.566	0.158	0.441
3	0.57	0.502	0.286	0.397
4	0.79	1.366	0.570	0.783
5	1.03	0.683	0.250	0.345
6	1.31	1.554	0.622	0.876
7	1.07	1.609	0.639	0.824

The enzymatic activities, expressed as Δ O.D.$_{340}$/1'/1.0×10^6 platelets, represent the activity of the untreated, membrane free lysates (sample A, see text). The activity was assayed at 37°C in a Gilford 2000 recording spectrophotometer. The final pH was 8.0 for G6PD and 6PGD and 7.0 for GSSGR. Samples from cases No. 4, 6 and 7 are from patients with polycytemia vera.

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Table II. Residual enzymatic activity of G6PD, 6PGD and GSSGR of human platelets lysates after various tretments

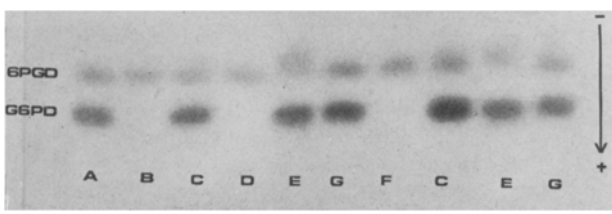
Sample		Enzymatic Activity		
		G6PD	6PGD	GSSGR
Membrane free lysate, no NADP	B	1.5± 2.2	82.5±18.1	94.4± 8.1
Membrane free lysate + NADP	C	84.4±11.4	77.7±15.1	85.9±17.5
Whole lysate, no NADP	D	2.1± 2.2	77.8±13.9	84.1±17.9
Whole lysate + NADP	E	80.4±13.3	46.0±11.2	73.7±28.6

The values represent % of the intitial activity (±S.D.) of the samples in Table I, taken as 100%. The preparation of samples B, C, D and E is described under 'Materials and methods'.

slight loss of activity was present, although not significantly different from the decrease found in the membrane-free lysate (Table II, B-D). This result is in contrast with the findings obtained on red blood cell lysates, in which erythrocyte membranes induce an activation on cytoplasmic GSSGR[2].

Our results show that incubation of whole platelet lysate in presence of added NADP induces changes in activity and in structure of 6PGD. This result indicates that the interaction between NAD(P)ase and 6PGD is similar in platelets and in red cells.

On the other hand, ultracentrifugation with complete removal of corpuscolate particles known to contain NAD(P)ase is necessary to prevent minor structural changes in 6-PGD.



Electrophoretic pattern of G6PD and 6PGD from platelet lysates after various treatments. Slot symbols from A to G correspond to the sample preparations symbol described under Materials and Methods. A, untreated, membrane free lysate; B, membrane free lysate, after incubation at 45°C without NADP; C, same as B, with NADP; D, whole lysate, after incubation at 45°C without NADP; E, same as D, with NADP; F, same as B, but after centrifugation of the lysate at 105,000 × *g*; G, same as B, but after centrifugation of the lysate at 105,000 × *g* + NADP.

NAD(P)ase activity and location in the cell could therefore be relevant in the control of the activity of some cytoplasmic enzymes. Although NAD(P)ase is present on the outer surface of the red blood cell[11], indirect evidence suggests that the enzyme is active also in the inner surface of the membrane[4]. Furthermore in other cells, NAD(P)ase is bound to the corpuscolate parts (mitochondria, microsomes) and its activity has been related to the regulation of the activity of several glycolytic enzymes, with specific differences in Ehrlich ascites tumor cells[12,13]. Our results on platelets further strengthens the hypothesis of a regulatory role for NAD(P)ase. Finally, activation of GSSGR by cell membranes is probably unrelated to their NAD(P)ase activity, and other mechanisms seem to be involved[14].

Riassunto. L'incubazione di lisati piastrinici con NADP provoca inattivazione della 6PGD e ne modifica la migrazione elettroforetica. Non si osserva invece attivazione della GSSG-R.

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Effects of 2-Br-α-ergocryptine on Plasma Prolactin Level and Milk Yield in Cows

The ergot alkaloids ergocornine and 2-Br-α-ergocryptine (CB 154) inhibit certain reproductive functions dependent on prolactin in rats[1–4] and also reduce serum and pituitary prolactin levels in rats and mice[5–7]. The anterior pituitary is considered the target of the inhibitory action on prolactin release[6]. Furthermore first clinical studies with CB 154 gave successful inhibition of galactorrhoea in several patients[8].

These results motivated our studies using the bovine species, which is distinct concerning reproductive physiology in the following points: a) In contrast to the rat LH rather than prolactin is assumed to be luteotropic. b) Our breeds are specially selected for milk yield. c) Prolactin blood levels and milk yield are exactly and continuously measurable over longer period in the same individuals, but the prolactin level has not been convincingly correlated with the stage of lactation or the milk yield[9]. There are two most distinct phenomena of the cows' prolactin blood level, i.e. the short increase during the milking stimulus and the longer lasting high peak before parturition[9–11]. In our first experiments we tried to examine the action of 2-Br-α-ergocryptine on these phenomena.

Material and Method. Animals. We used 4 non-pregnant cows and 1 pregnant cow around parturition. 4 cows were of the Brown Swiss and 1 cow of the Holstein-Frisian breed. The animals were 6–13 years old and they were kept in an open stable with pasturing.

Blood Collection. Blood plasma was collected in centrifuge tubes from the jugular vein; in experiment No. 1 by means of an inserted catheter and in the other experiments by needle puncture; the heparin preparation 'Liquemin' (Hoffmann-La Roche) was applied as an anticoagulant and the plasma samples were kept frozen (−18°C) until assay.

Inhibitor substance. The ergot alkaloid 2-Br-α-ergocryptine-methane-sulfonate = CB 154 (kindly supplied by SANDOZ, Basel) was used. The substance was dissolved

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