immediately. The reaction mixture contained: 0.1 ml of 0.21 *M* *tris*-0.21 *M* glycerolphosphate buffer, pH 8.6; 0.1 ml of solution of phosphorylase B (crystallized with 10^{-4} *M* AMP, centrifuged and dissolved in 0.015 *M* cystein, pH 7.0, to 25 mg/ml concentration); 0.05 ml of phosphorylase B kinase (muscle extract); 0.05 ml of 0.1 *M*/mg acetate-0.03 *M* ATP, pH 7.0, and 0.02 ml of 1 *M* NaF. After 5 min of reaction at 30 °C an aliquot of 0.1 ml was removed and diluted 1:20 in 0.02 *M* glycerolphosphate-0.03 *M* cystein buffer, pH 6.8, and the phosphorylase activity was assayed in the absence of AMP, according to CORI et al.[7].

The use of fluoride during the procedure was intended to prevent the activation of ATPase[12] caused by DNP and also to prevent the reaction phosphorylase A to B by inhibition of the PR enzyme.

Table II shows that, actually, the drug activates the in vivo phosphorylase B kinase. Experiments to verify the in vitro effect were, up to date, unsuccessful, the little activation observed in the kinase could not explain the results in vivo. Experiments performed in vitro by several authors in order to verify the activation of the phosphorylases, were also unsuccessful, so we suppose that DNP has, in fact, an indirect effect on the glycogenolysis, only observed when it is injected into the animal.

Our results show that DNP in vivo increases the content of phosphorylase A from phosphorylase B by activation of the phosphorylase B kinase and, at the same time, also increases the content of lactic acid. This strongly suggests that DNP actuates as do other glycogenolytic agents, and corroborates the results of other authors who consider phosphorylase B kinase as one of the most important enzymes in the control of the glycogenolysis in skeletal muscle.

Résumé. Des rats empoisonnés par le DNF ont une teneur de phosphorilase A trois fois plus grande que les animaux normaux. Cette augmentation est due à l'augmentation de la quinase de la phosphorilase B. Nous n'avons pas décelé d'action directe in vitro du DNF sur cette quinase, de sorte que l'action in vivo paraît plutôt indirecte. Les résultats suggèrent que le DNF a une action semblable à celles des autres agents glycogènolytiques.

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Intracellular pH of the Peritoneal Macrophage Effects by Silica and by Drugs Affecting Cell Metabolism

Silica is thought to damage the phagocytes by rupture or permeation of the phagolysosomal membranes due to the powerful hydrogen-bonding activity of surface SiOH groups[1]. Silica has been shown to cause the extrusion of lysosomal content into the extracellular space[2], and this might follow a previous discharge into the cytoplasm. In this case cell acidification[3,4] and inhibition of cell metabolism might occur with initiation of autolytic processes.

In an attempt to verify this possibility, the changes intervening in the pH of phagocytes under such conditions were investigated. Cell pH was calculated by the distribution between extra- and intra-cellular water of the weak acid 5,5-dimethyl-2,4-oxazolidinedione-2-^{14}C (DMO)[5,6]. The effects of metabolic poisons and uncouplers were also analyzed.

Peritoneal macrophages were obtained and handled as previously indicated[2] and the experiments carried out at 37 °C in a Warburg apparatus as indicated in the Tables. DMO-2-^{14}C (New England Nuclear, Boston, Mass.) specific activity 7.76 mc/m*M* was used in amount of

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Table I. Intracellular pH of peritoneal macrophages incubated in Ringer *tris*-HCl buffer initial pH 7.23 ± 0.017

	Incubation (min)	O_2 uptake (μl/mg dry wt.)	Inhibition (%)	Extracellular pH	Intracellular pH
Control (5)	0–30	9.7 ± 1.23	–	7.16 ± 0.045	6.85 ± 0.031
	90–120	6.4 ± 0.66	32.5 ± 4.99	7.09 ± 0.035	6.79 ± 0.068
Silica (6)	0–30	9.0 ± 0.97	–	7.17 ± 0.037	6.98 ± 0.067
	90–120	5.1 ± 0.53	41.8 ± 5.98	7.13 ± 0.033	6.55 ± 0.083

Figures represent means and the standard errors. O_2 uptake measured by the WARBURG direct method in air as gas phase and CO_2 trapped by KOH. Silica (tridymite batch 67-70-72M[2]) used in amount of 2.0 mg/10^7 cells. The figures in parentheses indicate the number of observations.

Table II. Intracellular pH of peritoneal macrophages incubated in Krebs-Ringer 25 m*M* bicarbonate buffer initial pH 7.41 ± 0.012

	Incubation (min)	O_2 uptake (µl/mg dry wt.)	Inhibition (%)	Extracellular pH	Intracellular pH
Control (5)	0–30	3.3 ± 0.56	–	7.50 ± 0.030	6.95 ± 0.052
		n.d.	–	7.79 ± 0.050	7.73 ± 0.098
	90–120	3.2 ± 0.42	3.0 ± 1.28	7.53 ± 0.022	6.92 ± 0.033
		n.d.	–	7.79 ± 0.104	7.30 ± 0.078
Silica (6)	0–30	3.4 ± 0.86	–	7.49 ± 0.021	6.90 ± 0.083
		n.d.	–	7.77 ± 0.045	7.36 ± 0.096
	90–120	2.8 ± 0.35	18.0 ± 2.20	7.51 ± 0.018	6.51 ± 0.122
		n.d.	–	7.68 ± 0.032	7.26 ± 0.169

O_2 uptake measured by the carboanhydrase, 3 *M* $KHCO_3$: K_2CO_3 (80:20), WARBURG[7] method. O_2:CO_2 (95:5) as gas phase.

Table III. Effect of drugs on the intracellular pH of peritoneal macrophages incubated as described in Table II

	Incubation (min)	O_2 uptake (µl/mg dry wt.)	Inhibition (%)	Lactic acid (m*M*/mg dry wt.)	Extracellular pH	Intracellular pH
Control (7)	0–30	3.6 ± 0.66	–	–	–	–
	30–60	3.5 ± 0.54	2.8 ± 1.41	0.97 ± 0.169	7.51 ± 0.023	6.98 ± 0.024
Iodoacetate 0.2 m*M* (10)	0–30	4.8 ± 0.20	–	–	–	–
	30–60	2.7 ± 0.21	37.6 ± 3.21	0.67 ± 0.073	7.48 ± 0.017	7.05 ± 0.065
Cyanide 1.0 m*M* (8)	0–30	6.2 ± 0.76	–	–	–	–
	30–60	2.5 ± 0.03	58.7 ± 8.81	2.03 ± 0.179	7.48 ± 0.027	6.95 ± 0.071
2,4-Dinitrophenol 0.1 m*M* (6)	0–30	4.0 ± 0.44	–	–	–	–
	30–60	2.2 ± 0.45	42.0 ± 6.62	1.72 ± 0.120	7.47 ± 0.025	6.86 ± 0.030

Lactic acid measured by the LDH Boehringer method at 340 nm.

0.2 µc/flask containing 2.5–3.0 × 10^7 cells. Inulin-carboxyl-^{14}C (inulin) (New England Nuclear) specific activity 18.25 mc/m*M* was employed in amounts of 3 µc/flask to measure the extracellular space. DMO distribution was determined at 30, 60 and 120 min incubation. Cells were rapidly sedimented at 6000 *g*, the supernates decanted and the tubes wiped with filter paper. Cell pellets were lysed in TCA 20% and the DMO and inulin concentration determined on the lysate. On the supernatants were determined the DMO and inulin concentration of the extracellular phase and the extracellular pH, measured at 37 °C with Beckman Expandomatic pH meter. Radioactivity was determined by liquid scintillation counting with Packard Tri-Carb spectrometer after samples were dissolved in PPO-toluene-ethyleneglycol monomethylether (0.4:70:30 or 0.4:93:7, w:v:v) solutions. Radioactivity was referred to 1 mg water. Cell water was determined by drying samples of control cells to constant weight. Cell pH was calculated according to POOLE et al.[5].

As is shown in the Tables, the results are somewhat different in *tris* and bicarbonate buffers. In *tris*, higher oxygen consumption by cells is observed due to absence of CO_2 which appears to interfere with cell respiration in bicarbonate[8]. Cell pH is, however, little affected by the different hydrogen ion activity of these buffers. This is in keeping with previous observations demonstrating the little sensitivity of cell pH to extracellular variations in the range 6.92–7.55[9]. In these conditions pH values near 6.9 are considered the more consistent in most cells and tissues[10,11]. When the pH of bicarbonate buffer is raised over 7.55 by lowering the CO_2 tension, the cell pH increases significantly.

The effects by silica on cell metabolism are slight compared with control cells. However, cell pH decreases significantly. Lesser decrease is observed when the pH of bicarbonate buffer is over 7.55, the alkalinity of the buffer hindering the cell acidification. Cell respiration appears to be inhibited by this drop of cell pH and possibly by the liberation of the lipolytic enzymes of lysosomes which cause rapid disruption of mitochondrial structure and uncoupling[12]. Uncoupling might further decrease the cell pH as it is observed with 2,4-dinitrophenol (Table III). This substance acts very probably by lowering the active proton extrusion coupled to oxidative phosphorylation and by stimulating the membrane ATPase and not through the inhibition of lactic acid oxidation[13], as it is also shown by cyanide. The slight increase of cell pH by iodoacetate appears, therefore, not attributable to the lowered production of lactic acid, and the suggested action by this acid on cell pH is doubtful (see also [14]), at least under the present conditions.

Riassunto. Viene esaminato l'effetto di silice cristallina sul pH intracellulare di fagociti. L'incubazione con i microcristalli determina una acidificazione delle cellule causante una inibizione del metabolismo. Ciò viene messo relazione con la liberazione del contenuto lisosomiale dalle cellule, previamente osservata.

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