Effects of anaerobiosis upon morphology and energy metabolism of alveolar macrophages cultured in gas phase

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ABSTRACT: Metabolic and morphological effects of anoxia were studied in alveolar macrophages obtained by lung lavage from guinea-pigs by means of an original method of cell culture allowing direct contact with air without interposition of liquid medium. After selection by glass adherence, alveolar cells were layered on a porous membrane applied to the surface of a reservoir filled with nutrient medium. Alveolar macrophages were then cultured in gas phase under either aerobic or anaerobic conditions for 24, 48 and 72 h. Cellular adenosine triphosphate (ATP) content, an indicator of cell vitality, significantly decreased by 68 and 88% after 48 and 72 h of exposure to anaerobic environment, respectively. Significant increases in lactate production (68% at 24 h) and in glucose uptake (125% at 24 h), evidence of marked glycolytic activity, occurred before these falls in intracellular ATP and parallel decreases in culture medium pyruvate level (76 and 85% at 48 and 72 h, respectively). The shift of energy metabolism resulted in cell death after 72 h, as noted by morphological degeneration and decreased cellular ATP content. Twentyfour hour re-exposure to normoxic atmosphere showed that recovery was possible when duration of anaerobiosis did not exceed 48 h. This reversibility in anoxic cell injury has been related to plasma membrane integrity. The results of these studies indicate that alveolar macrophage resistance to anaerobiosis is limited as ATP content falls and morphological degeneration occurs after 48 h. This novel approach of anaerobic effects at the cell level should be adaptable to investigations of activity and, in particular, the mechanisms of metabolic activity of antianoxic drugs.
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Macrophages are widespread in the body where their surrounding conditions can vary considerably from one organ to another. Such environmental circumstances appear to modulate them or otherwise are linked to their functional and metabolic activities [1, 2]. For example, alveolar macrophages (AM), which reside practically in direct contact with free oxygen in the alveoli, show increased oxidative phosphorylation and decreased glycolytic activities compared to macrophages residing in the peritoneal compartment [3–5].

However, AM offer remarkable resistance to hypoxia *in vitro* which involves effective adaptation mechanisms in response to environmental O_2 tension variations, including increase in pyruvate kinase activity, a key enzyme in glycolysis, and diminution in cytochrome oxidase activity, a key enzyme in oxidative phosphorylation [4, 6]. The latter is associated with decreased total mitochondrial structure available for respiratory activity [6]. Increase in phosphofructokinase activity and corresponding reduction in adenylate energy charge have also been reported as adaptive

responses [7]. The considerable adaptability to hypoxia, or even anoxia, suggested by these findings appears to be amazing for those alveolar cells that are well adapted to aerobiosis. In actual fact, the works referenced above have been performed using classical monolayer cultures with cells adhering to the flask and covered with nutrient medium, under conditions very far from the *in vivo* situation. Consequently, it seemed worthwhile to ascertain whether or not the AM adaptability to anoxia as noted by many investigors is specific to this kind of culture.

The aim of the present study was to investigate the effects of anaerobiosis upon morphology and energy metabolism of AM under conditions approximate to those existing in the lower respiratory tract by means of gas phase cell culture previously described by VOISIN and co-workers [8, 9]. This method has also proved to be appropriate for developing a reliable model of experimental anoxia, as it allows a direct contact of AM with the atmosphere and is, therefore, adaptable to a precise evaluation of gas effects on cells.

Materials and methods

Animals

Experiments were performed in female albino Dunkin-Hartley guinea-pigs (Mepal, Montmedy, France), each weighing approximately 300 g, which had been carefully housed conforming to the guidelines of the Official Journal of European Communities. They had been allowed to acclimatize in our quarters for at least one week before use.

Alveolar macrophages gaseous phase culture. According to MYRVICK *et al.* [10], AM were obtained by lung lavage with Hank's solution from guinea-pigs. After centrifugation (800 g for 10 min), cells were resuspended in 10% calf serum Basic Eagle's medium (BEM) (Gibco Laboratories, Grand Island, NY, USA) within glass flasks to obtain purified AM. After 24 h, non-adherent cells were eliminated by rinsing, whereas macrophages adhering to glass were removed by 0.125% (w/v) edetic acid (EDTA) solution in Dulbecco's phosphate buffer saline (PBS). Cell numbers were determined manually with a standard haemocytometer. The cells were then suspended in BEM with glutamine (2 mM) and 10% fetal calf serum to obtain 10×10^6 cells·ml^{-1}. The cell cultures were performed according to the method of VOISIN and co-workers [8, 9]. The AM were layered on porous cellulose triacetate membrane Metricel GA 8 0.2 µm (Gelman Sciences Inc., Ann Arbor, Michigan, USA). This membrane was applied to the surface of a reservoir filled with nutrient medium so as to be saturated by capillarity. A quantity of cells varying from 0.5–2 million in a volume of 50–200 µl were placed on the membranes. Under these conditions, cells were in direct contact with air without any interposition of liquid medium. In all studies, the cell cultures were maintained at 37°C in a water saturated environment within an appropriate chamber (Lequeux Ltd, Paris, France). Thus, when incubated under normoxic conditions, AM were able to maintain their metabolic and functional activities for 4–6 days [11].

Normoxic conditions. Normoxic conditions consisted of purified reconstituted air (Alpha Gaz S.A., France), saturated with water (37°C) and enriched with 5% CO_2.

Anaerobic conditions. Anaerobic conditions were achieved by flushing the chamber with a 95% N_2 and 5% CO_2 mixture. Slight traces of oxygen were eliminated by the GasPak system (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Maryland, USA) which consisted of an envelope containing one piece of filter paper, one tablet of sodium borohydride and one tablet of sodium bicarbonate plus citric acid. H_2 and CO_2 were given off by addition of tap or distilled water into the envelope. Palladium chloride placed within the chamber promoted a H_2 and CO_2 reaction to produce H_2O (Laidlaw principle), resulting in the disappearance of O_2 and a water saturated environment. Anaerobiosis was verified by means of a very sensitive colorimetric indicator: resazurin which became colourless when reduced (E_0 = -51 mV); *i.e.* when anaerobic conditions were achieved [12, 13]. Several experimental trials were performed in which AM were exposed to anaerobic conditions for 24, 48 and 72 h. Following exposure to anaerobic atmospheres, some cell samples were exposed to normoxic atmospheres for 24 h in order to estimate their recovery ability.

Cell morphology. Morphological analysis was performed by electron microscopy, after fixing in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 for 12 h at 4°C and staining with 0.5% uranyl acetate, pH 5 for 1 h at 4°C, as described previously [9]. The cells were examined under a Hitachi type HV 12 electron microscope.

Energy metabolism. Total cell energy metabolism was studied by assay of intracellular adenosine triphosphate (ATP) through a bioluminescent method using luciferin-luciferase [9, 14, 15] on a Berthold Biolumat LB 9500T (C.L.V. Interbio®, Villeurbanne, France). The analysis was carried out on cellular extract in dimethyl sulphoxide (DMSO).

Culture medium pyruvate and lactate were assayed, as glycolysis indices, by classic enzymatic methods (kits no. 726 U.V. and no. 826 U.V. from Sigma Chemical Co., Saint Louis, Missouri, USA) using lactate-dehydrogenase, at the end of each trial [12]. Measuring was performed after incubation with a UV spectrometer at 340 nm.

Glucose uptake. Measurement of ^{3}H-2-deoxy-D-glucose uptake by cells is regarded as a suitable method for determining the uptake of hexoses by cells [16, 17]. Deoxyglucose crosses the cell membrane by the same carrier mechanism characteristic of facilitated diffusion as glucose, but it accumulates in cells as deoxyglucose-6-phosphate [16–18].

In our study, measurements were made in triplicate in normoxia at 37°C after either aerobic or anaerobic 24 h exposures [12]. Each study sample was randomly assigned to 4 equivalent groups (according to the schedule 10, 20, 30 and 40 min) for kinetic examination. Radiolabelled deoxyglucose [^{3}H] (specific activity: 30.4 Ci·mmol^{-1}; Amersham International plc, Amersham, Buckinghamshire, UK) was added to cells (1 µCi×10^6 cells) by replacing BEM nutrient medium with phosphate buffered saline (PBS) enriched with 0.9 mM Ca^{++}, 0.5 mM Mg^{++} and the radioactive sugar so that all the samples were under standardized conditions. Uptake was stopped by gently washing the membranes supporting the macrophages with cold PBS at 4°C two times. This washing made it possible to reduce non-specific radioactivity. Radioactivity of both cells and membranes was measured on a Beckman LS 1800 liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, California, USA). Results were expressed as cpm·10^{-6} macrophages in comparison with blank cellulose triacetate membranes without any cells and treated   under identical conditions. Where appropriate,

cytochalasin B (Sigma Chemical Co., Saint Louis, Missouri, USA) was added at zero time, along with the tritiated deoxyglucose, at a concentration of 5 $\mu g \cdot ml^{-1}$. Radioactivity measurements were processed as a function of time on an Apple III microcomputer (Business Graphic Software) using a regression program which allowed calculation of transport rate [12].

Statistical analysis. All biochemical measurements were made in triplicate. Mean data are presented with standard deviation. Lack of variation of each metabolic parameter in AM cultured in normoxia was verified by a one-way analysis of variance, selecting time as classification variable, so as to demonstrate stability and reliability of cell preparations under the above-described conditions. Each individual result recorded under anaerobic conditions was compared with data obtained from the same cell batch (*i.e.* harvested in the same animal) cultured under similar conditions but in a normoxic environment. Thus, comparisons between means were made using paired Student's t-test and $p<0.05$ was considered to indicate significant differences. Since recovery consisted of additionally exposing AM to normoxic atmosphere for 24 h after an anaerobic trial, results of that investigation were compared with measurements made in anaerobiosis on the day before. Besides taking into account the perfect time stability of metabolic parameters in normoxia, these results were also compared with corresponding data obtained in normal atmosphere. The value obtained for lactate production represents overall production observed at the end of each trial. Thus, in case of recovery, this cumulative result must be compared with reference data of the preceding and same days.

Results

Morphological findings

The morphological characteristics of AM were observed after 24, 48 and 72 h in either aerobic or anaerobic conditions and their recovery ability was estimated after 24 h in normoxia following exposure to strict anaerobic environments. In AM cultured anaerobically for 24 h, only a few differences were found compared to control normoxia cells. There appeared to be little grey probably "lipidic vesicles". General ultrastructure remained unchanged: nucleus, pilopodia at the cell surface and mitochondria were all intact. In AM cultured anaerobically for 48 h, pilopodia became stunted and concentrated on the two poles of cell bodies which were obviously elongated as result of loss of cell volume regulation. Increase and spread of "lipidic vesicles" seemed to be in correlation with anaerobiosis duration (fig. 1). At this time, decrease in the number of mitochondrial cristae and even, on certain fields, progressive disappearance of mitochondria could be noticed. In AM cultured anaerobically for 72 h, vesicle number was greater and the nuclei became pyknotic. Some cells lost their plasmalemmal membranes

(fig. 2). Elongation of cell bodies persisted in the most resistant cells, the mitochondria of which had nearly totally disappeared. The completely degenerated cell population accounted for more than 60%.
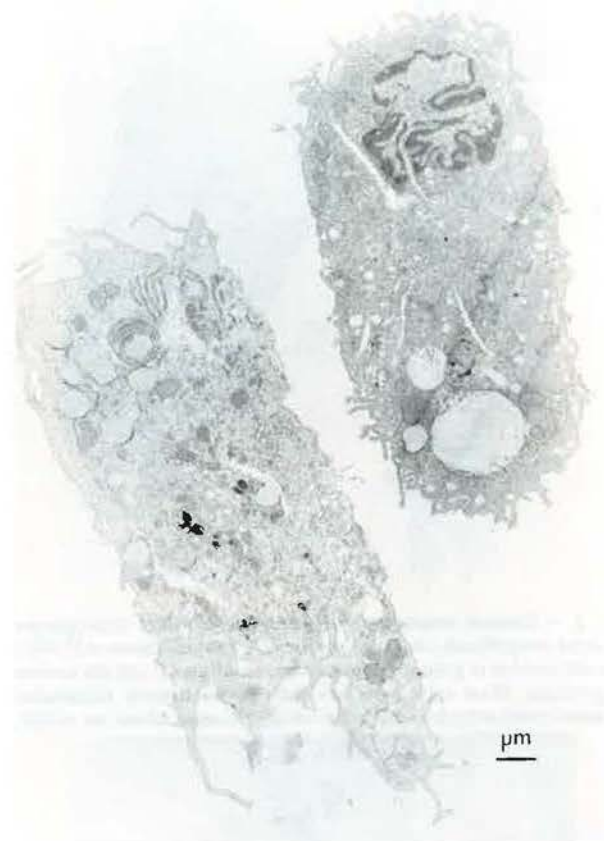

Fig. 1. – Electron micrograph of guinea-pig alveolar macrophages cultured anaerobically in gas phase for 48 h (magnification: ×12,000). Pilopodia are stunted and concentrated on the two poles of cell bodies which are elongated.

With regard to the recovery aspect, after 24 h of anaerobiosis, the recovery in normoxia was satisfactory and cells exhibited a nearly normal appearance. After 48 h of anaerobiosis, "lipidic vesicles" remained and the cells, although less elongated, did not recover their initial morphology. Owing to the different fields, the recovery of a few mitochondria was somewhat subjectively noted. In AM cultured anaerobically for 72 h prior to normoxic period, no morphological recovery was observed, as seen in figure 3. This finding indicates that, after 72 h in anaerobic environment, alveolar cells had entered an irreversible state of anoxic injury as they continued to degenerate and become necrotic despite reoxygenation.

Effects on energy metabolism

Anaerobiosis was maintained during 24, 48 and 72 h to test for adaptation and recovery abilities of AM. Table 1 shows that anaerobiosis merely entailed a significant
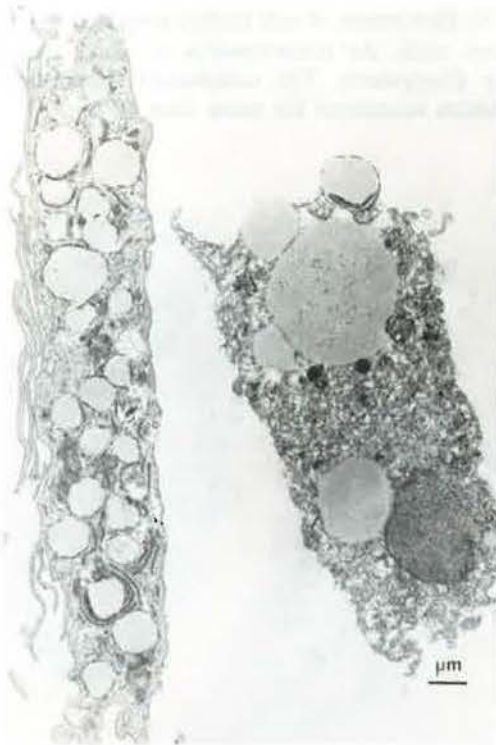
Fig. 2. – Electron micrograph of guinea-pig alveolar macrophages cultured anaerobically in gas phase for 72 h (magnification: ×15,000). Vesicle number is greater in comparison with figure 2 and the nucleus is pyknotic. Most cells have lost their plasmalemmal membranes whereas intact mitochondria are missing. Necrotic debris are visible.
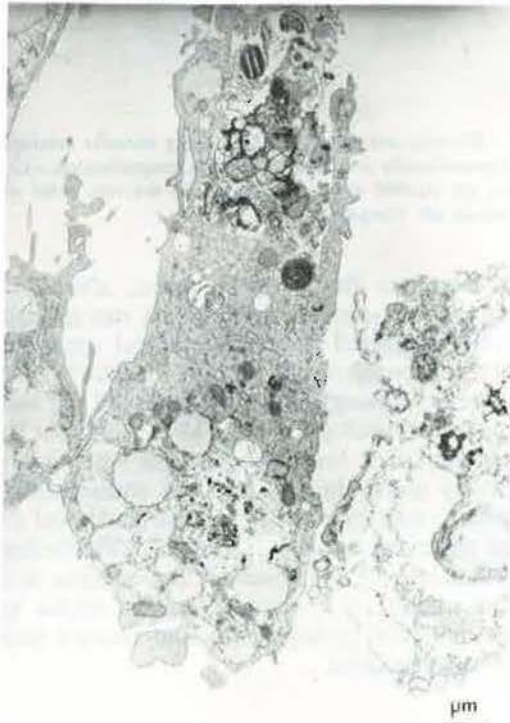

Fig. 3. – Electron micrograph of guinea-pig alveolar macrophages which have recovered in normoxia for 24 h after surviving in anaerobic environment during 72 h (gas phase culture) (magnification: ×15,000). No morphological recovery can be observed relative to figure 2.

68% increase in lactate production as early as 24 h. On the other hand, total oxygen deprivation induced disturbances of all parameters from 48 h. ATP content, considered as an indicator of cell vitality, decreased by 68% (p<0.04) after 48 h, and by 88% (p<0.02) after 72 h compared to AM cultured in air (table 2). Culture medium pyruvate level correspondingly decreased by 76% (p<0.001) after 48 h and further decreased by 85% (p<0.001) after 72 h compared to AM cultured in air (table 3), while lactate production increased nearly two-fold by 48 h and threefold by 72 h, relative to that for AM cultured in air (p<0.001, table 1). As noted in the same tables, success of recovery (24 h in normal atmosphere after surviving in anaerobic environment) was directly linked to duration of exposure to anaerobic environments. Especially for ATP content, cells cultured in anaerobiosis for 48 h retrieved a mean value of ATP that was not statistically different from that recorded in normoxia, contrary to 72 h anaerobically cultured AM. With respect to lactate production, the sole parameter that significantly varied after 24 h anaerobiosis, the recovery value (i.e. after supplementary 24 h culture under aerobic conditions) was lower than that recorded after 48 h anaerobiosis. This reduction in lactate production, in comparison with anaerobiosis, clearly indicates a return to aerobic metabolism.

Table 1. – Effects of anaerobiosis on time course of lactate production from guinea-pig alveolar macrophages surviving in gas phase

Time h	Lactate production $\mu g \cdot 10^{-6}$ cells		
	Normoxia	Anaerobiosis	Recovery
24	336±110	565*±114	650**±78
48	403±76	757*±33	1191**±201
72	461±72	1351*±121	1466**±169

Data are mean±SD for six experiments. *: p<0.001 compared to normoxia; **: p<0.001 compared to normoxia and p<0.01 compared to anaerobiosis.

Table 2. – Effects of anaerobiosis on time course of ATP content in guinea-pig alveolar macrophages surviving in gas phase

Time h	ATP content $\mu g \cdot 10^{-6}$ cells		
	Normoxia	Anaerobiosis	Recovery
24	2.32±1.14	2.23±1.11	1.98±1.25
48	2.22±1.59	0.72*±0.42	1.31†±0.54
72	2.38±1.19	0.29**±0.22	0.41††±0.32

Data are mean±SD for six experiments. *: p<0.04 compared to normoxia; ** :p<0.02 compared to normoxia; †: nonsignificant compared to normoxia and p<0.02 compared to anaerobiosis; ††: p<0.03 compared to normoxia and nonsignificant compared to anaerobiosis; ATP: adenosine triphosphate.

Table 3. – Effects of anaerobiosis on time course of culture medium level of pyruvate for guinea-pig alveolar macrophages surviving in gas phase

Time h	Pyruvate level $\mu g \cdot 10^{-6}$ cells		
	Normoxia	Anaerobiosis	Recovery
24	12.1±1.3	12.8±1.3	10.4**±0.5
48	10.8±1.3	2.6*±0.8	6.2†±1.1
72	10.5±1.7	1.6*±0.7	3.5†±0.1

Data are mean±SD for six experiments. *: p<0.001 compared to normoxia; **: p<0.02 compared to normoxia and p<0.01 compared to anaerobiosis; †: p<0.01 compared to normoxia and p<0.02 compared to anaerobiosis.

Table 4. – Influence of anaerobiosis on ^{3}H-2-deoxy-D-glucose uptake by guinea-pig alveolar macrophages surviving in gas phase during 24 h

Environmental conditions	n*	Transport rate cmp per min$\cdot 10^{-6}$ cells
Normoxia	6	76.4±17.2
Anaerobiosis	6	171.8±23.5**

Results are mean±SD. *: number of experiments performed in triplicate; **: p<0.001 compared to normoxia.

Effects on glucose uptake

This investigation has been undertaken to demonstrate the shift in energy metabolism in cells exposed to the oxygen-free milieu, as well as the membrane integrity regarding glucose transport at the very time when ATP content was unchanged relative to normoxia. Twenty four hour anaerobiosis markedly stimulated deoxyglucose transport in the AM. Table 4 shows that tritiated glucose analogue uptake was increased by 125% (p<0.001) compared to control AM cultured in air, under our experimental conditions. This observation is in accordance with previous findings pertaining to other cell types [19, 20] and corroborates concomitant increase in lactate production which shows the metabolic deviation towards anaerobic pathways.

An experiment, designed to further examine cell membrane integrity, was conducted in triplicate with the glucose transport inhibitor cytochalasin B. Figure 4 illustrates the ability of cytochalasin B to inhibit essentially all deoxyglucose transport (more than 95%) either in aerobic or in anaerobic environments. These inhibitory effects in such different conditions suggested that, in both cases, the same transport mechanism was affected and, consequently, anaerobiosis specifically acted on it.
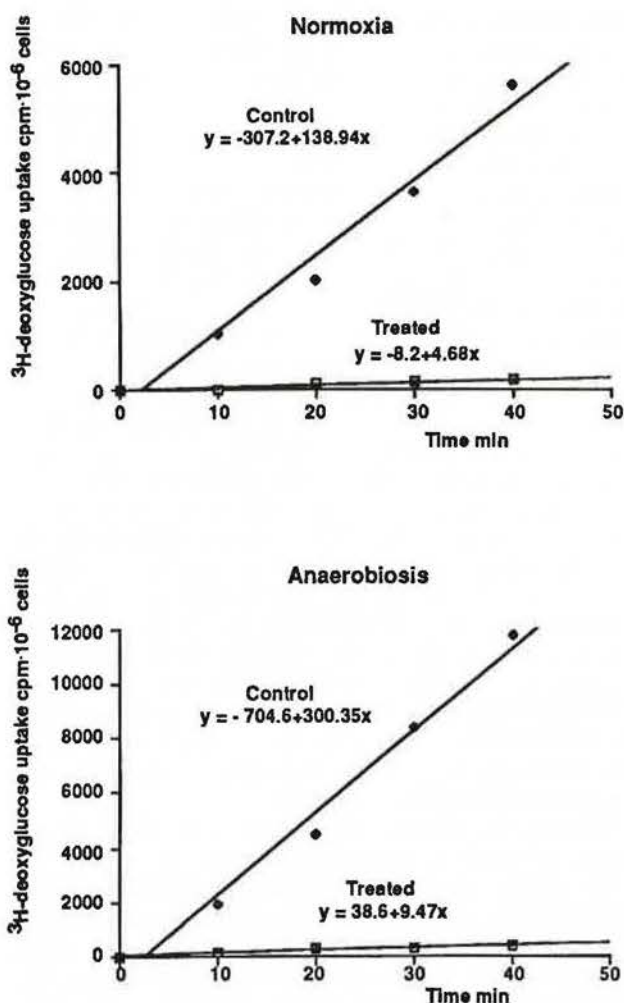

Fig. 4. – Influence of cytochalasin B (5 $\mu g \cdot ml^{-1}$) on ^{3}H-2-deoxy-D-glucose uptake by guinea-pig alveolar macrophages surviving in gas phase during 24 h. Values are results of one experiment performed in triplicate.

Discussion

Prior experiments carried out in our laboratory had shown that AM surviving in gas phase could easily adapt to difficult hypoxia conditions such as 5% O_2 or 1% O_2 environments during 48 h or 72 h [21]. These findings seemed to be rather surprising for such aerobic cells practically residing in direct contact with free oxygen in the alveoli. The present studies have demonstrated that AM can, only in part, endure total anoxia. Indeed, strict anaerobic conditions for 48 h brought about a significant decrease in energy charge, as well as a morphological degeneration, whereas such environment during 72 h led to cell death. However, those cell injuries caused by anaerobiosis were reversible if duration of trial was limited, as indicated by return of the various metabolic parameters to control values when cells were exposed to air again for 24 h.

Modification of metabolic characteristics can readily explain the relative resistance of AM to anaerobic conditions. Cessation of mitochondrial respiratory activity in absence of O_2 resulted in an anaerobic glyco-

lysis stimulation which was reflected in the increases in lactate production and glucose uptake at 24 h. It can be noted that the latter may be the result of a specific effect of anaerobiosis upon glucose transport and/or be a consequence of cell membrane injury mediated *via* cell metabolic disturbances. However, the second assumption was not supported by experiments involving inhibition of deoxyglucose uptake by cytochalasin B. This agent has proved to specifically inhibit the deoxyglucose (and glucose) transport undoubtedly by a membrane mechanism [16, 17, 22–27]. The fact that anaerobic stimulation of this glucose transport associated with the cell membrane was inhibited (by more than 95%) in the presence of cytochalasin B has been considered as an indication of membrane integrity. According to FARBER [28], disturbances in membrane function in general and in the plasma membrane in particular characterize the loss of reversibility in ischaemic injury. Thus, defects in cell membranes are regarded as an early feature of irreversible, ischaemic cell injury. Therefore, the finding that, after 24 h in anaerobiosis, the AM membrane appeared to be intact as it normally reacted to cytochalasin B, relative to normoxia, is fairly consistent with AM abilities to maintain their intracellular ATP store at this time and to recover aerobic metabolism when exposed to air again, as shown by decrease in their lactate production and normal morphological appearance.

Despite the adaptive response by AM, energy deficit occurred at 48 h since anaerobic glycolysis produces very much less ATP compared to respiratory activity and, thereby, cannot completely replace the dependence of cells upon oxidative metabolism. The fatal course in the current studies, as noted by morphological degeneration together with loss of cell volume regulation and decreased cellular ATP content, has been partially interpreted as result of intracellular acidosis (induced by excessive stimulation of glycolysis) which has been found to inhibit enzymatic systems [29]. Such a phenomenon could therefore limit cell adaptability. In this respect, it is noteworthy that, under our experimental conditions, the high concentrations of lactate in the culture medium associated with anaerobic situation probably play an important role in the time course of these events by potentiating the pH effects of intracellular acidosis. In other respects, it has been established that a membrane mechanism, not yet entirely clear and involving a massive free fatty acid release, generates irreversible changes during the acute phase of ischaemic injury in well-differentiated tissues such as heart or brain [30, 31]. A sequence of events mediating ischaemic cell death has been proposed by FARBER [28] in a study of the pathogenesis of membrane injury and the resulting coagulative necrosis in ischaemia. Accordingly, the effects of anoxia on phospholipid metabolism and, therefore, on cell integrity do not appear as a direct result of the loss of cellular stores of ATP. Actually, as oxygen is also being utilized by a number of oxygenases such as cytoplasmic fatty acid desaturases, the sensitivity to oxygen deprivation should relate to both its function in energy metabolism and the

synthesis of polyunsaturated fatty acids needed to maintain membrane phospholipids. This biochemical assumption is in good agreement with our morphological findings.

The effects of anaerobiosis on energy metabolism of AM surviving in liquid phase for 96 h have been reported previously by BUTTERICK *et al.* [6]. These investigators established a shift in energy metabolism similar to the one that we observed, without loss of cell viability or alteration in total cell cross-sectional surface area. This main difference compared to our findings appears to be related to the experimental conditions we have employe; *i.e.* the gas phase culture of AM. Quite well adapted to aerobic metabolism in such conditions, these cells seemed to be more sensitive to the actual effects of O_2 depletion. Cell energetics adaptation observed in the previous work can be partially explained by an alteration of macrophage behaviour in response to the liquid phase conditions as higher productions of lactate have been found in AM cultured in liquid phase in comparison with gas phase, regardless of environmental conditions (unpublished data). This transformation might have been facilitated by medium replacement in the experimental procedure taken as reference. Thus, the glucose indispensable for anaerobic glycolysis was correctly supplied and overproduction of lactate was eliminated, resulting in the absence of extracellular pH effects due to this substrate.

Ultimately, the study of main energy metabolic parameters and morphology of AM exposed to anaerobic conditions in gas phase provides an experimental model of anoxia. Unlike the method of Butterick, the current method is a true model of cellular ischaemia, exhibiting moderate glucose supply and overproduction of lactate as encountered in pathological situations. As the effects of this experimental ischaemia were reversible if anaerobiosis was limited to 24 h or even 48 h, it has been successfully used as pharmacological indicator for investigating the ability of various chemicals to protect against hypoxia. Moreover, similarities observed *in vitro* between respiratory activities of alveolar cells and brain cells [3] enabled us to utilize cultured AM for the study of antianoxic drugs endowed with possible cerebral protective properties, such as vincamine [12, 21]. This alkaloid actually revealed an interesting action as it was capable of maintaining cell metabolic activity for a longer period of time after the beginning of an anoxic trial when compared to the control. Other agents are being investigated.

In conclusion, AM appear to be a convenient model for studying ischaemic cell injury as they require large amounts of oxygen to provide the energy to support their specialized functions. In reality, the effects of anoxia and ischaemia are most significant in those tissues which share this metabolic characteristic. By means of gas phase culture, it is possible to experiment with alveolar cells under conditions absolutely favourable to their aerobic metabolism. Furthermore, the fact that AM are able to tolerate anaerobiosis during a certain time and exhibit reversible damage until 48 h avoids an annoying all-or-none response. Thus, the approach

of anoxia at the cell level should be perfectly adaptable to elucidation of mechanisms of metabolic activity of antianoxic drugs.

References

1. Blusse A, van Oud A, van Furth R. – Origin, kinetics, and characteristics of pulmonary macrophages in the normal steady state. *J Exp Med*, 1979, 149, 1504–1518.
2. Nichols BA, Bainton DF. – *In*: Mononuclear phagocytes in immunity, infection and pathology. R. van Furth ed. Blackwell Scientific Publications, Oxford, 1976.,
3. Oren R, Farnham AE, Saito K, Milofsky E, Karnovsky ML. – Metabolic patterns in three types of phagocytizing cells. *J Cell Biol*, 1963, 17, 487–501.
4. Simon LM, Axline SG, Horn BR, Robin ED. – Adaptations of energy metabolism in the cultivated macrophage. *J Exp Med*, 1973, 138, 1413–1425.
5. Simon LM, Robin ED, Phillips JR, Acevedo J, Axline SG, Theodore J. – Enzymatic basis for bioenergetic differences of alveolar *versus* peritoneal macrophages and enzyme regulation by molecular O_2. *J Clin Invest*, 1977, 59, 443–448.
6. Butterick CJ, Williams DA, Boxer LA, Jersild RA Jr, Mantich NM, Higgins C, Baehner RL. – Changes in energy metabolism, structure and function in alveolar macrophages under anaerobic conditions. *Br J Haematol*, 1981, 48, 523–532.
7. Sides GD, Mantich NM, Boxer LA, Baehner RL. – Transformation of energy charge and phosphofructokinase activity in alveolar macrophages. *J Reticuloendothel Soc*, 1981, 29 (5), 407–412.
8. Voisin C, Aerts C, Tonnel AB, Houdret JL, Ramon P. – Mise en survie en phase gazeuse et reconstitution *in vitro* du micro-environnement naturel des macrophages alvéolaires. *Path Biol*, 1975, 23, 453–459.
9. Voisin C, Aerts C, Jakubczak E, Tonnel AB. – La culture cellulaire en phase gazeuse. Un nouveau modèle expérimental d'étude "*in vitro*" des activités des macrophages alvéolaires. *Bull Eur Physiopathol Respir*, 1977, 13, 69–82.
10. Myrvik GN, Leake ES, Fariss B. – Study on pulmonary macrophages from the normal rabbit. A technique to procure them in a high state of purity. *J Immunol*, 1961, 86, 128–132.
11. Voisin C, Aerts C, Tonnel AB. – La survie *in vitro* des macrophages alvéolaires humains en phase gazeuse. Application à l'étude de la cytotoxicité de l'oxygène. *INSERM*, 1979, 84, 169–176.
12. Paluszezak D. – Etude pharmacologique de substances à visée protectrice cérébrale dérivées de la β-carboline: mise au point d'un modèle d'hypoxie sur macrophages alvéolaires. Thèse de Doctorat de Troisième Cycle, 1985, Faculté de Pharmacie, Lille.
13. Anderson KL, Fung DYC. – Anaerobic methods, techniques and principles for food bacteriology: a review. *J Food Protection*, 1983, 46 (9), 811–822.
14. Jakubczak E, Mazingue C, Leclerc H. – Etude critique des méthodes d'extraction de l'ATP chez les bactéries en vue du son dosage par la méthode de bioluminescence. *Regard sur la biochimie*, 1978, 2, 14.
15. MacElroy WD, Seliger HH. – The chemistry of light emission. *Adv Enzymol*, 1963, 25, 119.
16. Gee LJB, Khandwala AS, Bell RW. – Hexose transport in the alveolar macrophage: kinetics and pharmacologic features. *J Reticuloendothel Soc*, 1974, 15 (5), 394–405.
17. Khandwala AS, Gee LJB. – Factors in glucose oxidation by alveolar macrophages: glucose transport and glycogenolysis. *Chest*, 1975, 67, 60s–63s.
18. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M. – The (^{14}C) deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anesthetized albino rat. *J Neurochem*, 1977, 28, 897–916.
19. Morgan HE, Henderson MJ, Regen DM, Park CR. – Regulation of glucose uptake in muscles. *J Biol Chem*, 1961, 236 (2), 253–261.
20. Whitfield CF, Morgan HE. – Effect of anoxia on sugar transport in avian erythrocytes. *Biochem Biophys Acta*, 1973, 307, 181–196.
21. Cazin M, Paluszezak D, Poulain V, Dine T, Bianchi A, Cazin JC, Aerts C. – Use of alveolar macrophages in antianoxic drug studies. *Meth Find Exp Clin Pharmacol*, 1988, 10 (4), 231–237.
22. Brown SS, Spudich JA. – Cytochalasin inhibits the rate of elongation of actin filaments fragments. *J Cell Biol*, 1979, 83, 657–662.
23. Estensen RD, Plagemann PGW. – Cytochalasin B: inhibition of glucose and glucosamine transport. *Proc Nat Ac Sci USA*, 1972, 69, 1430–1434.
24. Kimmich GA, Randles J. – Energics of sugar transport by isolated intestinal epithelial cells: effects of cytochalasin B. *Am Physiol Soc C*, 1979, 56–63.
25. Kletzien RF, Perdue JD. – The inhibition of sugar transport in chick embryo fibroblasts by cytochalasin B. Evidence for a membrane-specific effect. *J Biol Chem*, 1973, 248, 711–719.
26. Shin Lin, Shang Lin D, Flanagan MD. – Specificity of the effects of cytochalasin B on transport and motile processes. *Proc Nat Ac Sci USA*, 1978, 75, 329–333.
27. Zigmond SH, Hirsch JH. – Cytochalasin B: inhibition of D-2-deoxyglucose transport into leukocytes and fibroblasts. *Science*, 1972, 176, 1432–1434.
28. Farber JL. – Membrane injury and calcium homeostasis in the pathogenesis of coagulative necrosis. *Lab Invest*, 1982, 47 (2), 114–123.
29. Robin ED. – Of men and mitochondria: coping with hypoxic dysoxia. The 1980 J. Burns Amberson Lecture. *Am Rev Respir Dis*, 1980, 122, 517–531.
30. Jennings RB, Ganote CE, Reimer KA. – Ischemic tissue injury. *Am J Pathol*, 1975, 81, 179–198.
31. Baron JC. – Phénomènes physiopathologiques au cours de l'ischémie focale aiguë du cerveau. *Rev Méd*, 1983, 38, 1853–1863.

Effets de l'anaérobiose sur la morphologie et le métabolisme énergétique des macrophages alvéolaires maintenus en survie en phase gazeuse. M. Cazin, D. Paluszezak, A. Bianchi, J.C. Cazin, C. Aerts, C. Voisin.

RÉSUMÉ : Des macrophages alvéolaires de cobaye ont été maintenus en survie en anaérobiose durant 24, 48 et 72 h, suivant un procédé de culture en phase gazeuse assurant un contact direct entre les cellules et l'atmosphère environnante. Dans ces conditions, la teneur cellulaire en adénosine triphosphate (ATP), expression de la vitalité des celulles, a respectivement chuté de 68 et 88% à 48 et 72 h. Des augmentations significatives de la production de lactate (68% à

24 h) et de la consommation de glucose (125% à 24 h), preuves d'une activité glycolytique accrue, ont été observées avant cette chute du contenu en ATP et une diminution parallèle de la concentration de pyruvate dans le milieu de culture (respectivement 76 et 85% à 48 et 72 h). A l'issue des 72 h, une lyse cellulaire importante a été objectivée par une analyse morphologique en microscopie électronique et les taux extrêmement faibles d'ATP. En replaçant les cellules hypoxiques dans une atmosphère normale pendant 24 h, les auteurs ont montré que la récupération était possible lorsque la durée de l'anaérobiose n'excédait pas 48 h. A la différence des données bibliographiques, les résultats de ces expériences démontrent que la résistance des macrophages alvéolaires à l'anaérobiose est limitée. Cette approche originale des effets de l'anaérobiose à l'échelon cellulaire apparaît adaptée à l'étude des activités et, en particulier, des mécanismes d'action des substances anti-anoxiques. L'analyse de médications à visée protectrice cérébrale a même été envisagée, compte tenu des similitudes observées *in vitro* entre le métabolisme énergétique des cellules cérébrales et celui des macrophages alvéolaires.

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