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Influence of Oxygen Tension on Lactate Dehydrogenase and Aldolase Activities in Chang Liver Cells

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The influence of oxygen tension on the specific activities of lactate dehydrogenase (LDH) and aldolase has been studied in mammalian cells cultured *in vitro*. Cells exposed to anoxic conditions showed an increased synthesis of LDH, especially of the M sub-unit, and of aldolase. A rise in oxygen tension did not produce any change in activity of these two enzymes. The results suggest a common mechanism for the regulation of aldolase and LDH.

Oxygen tension has been reported to influence the synthesis of several enzymes in micro-organisms [1–3] and of tetrapyrrole compounds in bacteria and many animals.[1] The catalase activity has been found to respond to aeration in mammalian cells in tissue culture.[4]

Lactate dehydrogenase (LDH) in higher animals is usually composed of two kinds of sub-units,[5] which have been designated heart (H) and muscle (M) type.[6] These sub-units combine randomly to form enzymatically active tetramers. The synthesis of LDH, particularly of the M sub-unit, has been found to be regulated by the concentration of oxygen in the environment. The synthesis of the M sub-unit in chick embryos is suppressed by raised [7–9] and promoted by lowered oxygen tension,[9] a tendency that has also been observed in some established cell strains.[7,11] When explanted into tissue culture many tissues from chick and other species produce relatively more M than H sub-units, and this change can be delayed by exposure to increased oxygen tension.[8,10]

Aldolase activity increases in the ascitic fluid of Ehrlich ascites tumour suspensions kept in a nitrogen atmosphere,[12,13] and elevated LDH and aldolase activities have been observed after long-term treatment of "L" cells in monolayer cultures, also in a nitrogen atmosphere.[14] Increased aldolase activity in response to anoxia has also been found in Salk monkey heart cells.[7]

To better understand the mechanisms involved in the regulation of enzyme activity in mammalian cells the effect of oxygen tension on the LDH and aldolase activities in an established cell strain has been investigated. In the

experiments presented here the activities of these two enzymes in Chang liver cells were found to respond to changes in oxygen tension.

MATERIALS AND METHODS

Cells and tissue culture procedures. Chang liver cells were grown in suspension (spinner-culture) in Eagle's medium with the addition of 10 % of horse serum and antibiotics. The experimental cultures were derived from a stock culture whose growth was maintained by regular dilution with fresh medium twice a week over a long period. Repeated attempts to cultivate mycoplasma from the stock culture were unsuccessful. The doubling time of the cells varied between 25 and 30 h. At the start of each experiment the medium was completely changed by centrifuging the cells and resuspending them in fresh medium to a density of about 0.5×10^6 cells per millilitre. Cell samples were removed from the culture flasks under sterile conditions and the cells collected by centrifugation. The samples were then washed with a balanced salt solution to ensure complete removal of culture medium and kept frozen at −20°C until analysis.

Homogenates. After thawing an aqueous suspension of the samples of the required density was homogenized in a sonic oscillator.

Analytical procedures. The LDH activity was determined by the spectrophotometric method of Stambaugh and Post.[15]

The aldolase activity was determined by a modification of the method of Sibley and Lehninger;[16] 0.1 ml of the homogenate to be assayed was added to a test tube containing 1.50 ml of 0.067 M barbitone buffer, pH 8.6; 0.25 ml of 0.56 M hydrazine, pH 8.6, and 0.25 ml of 0.10 M fructose-1,6-diphosphate (FDP). After incubating for 1 h at 37°C the reaction was arrested by the addition of 3 ml of 10 % trichloroacetic acid. For each enzyme sample a reagent blank was prepared and incubated in the same way, except that the substrate was not added until after the reaction had been stopped with trichloroacetic acid.

After centrifuging for 5 min to separate precipitated protein a 0.5 ml aliquot of the supernatant was transferred to a test tube containing 0.5 ml of 0.75 M sodium hydroxide and the mixture was allowed to stand for 10 min at room temperature; 0.5 ml of 0.1 % 2,4-dinitrophenylhydrazine in 2 M hydrochloric acid was then added and the tubes were incubated at 37°C for 10 min. The colour was developed by the addition of 4 ml of 0.75 M sodium hydroxide and 10 min later the extinction was measured at 540 mμ. Attempts to use fructose-1-phosphate as a substrate [17] were unsuccessful.

The nitrogen content in the homogenates was determined by a micro-Kjeldahl method. The glucose content in the medium was assayed with a glucose oxidase reagent (Kabi, Sweden) and the lactate was determined by the method of Horn and Bruns.[18]

Experimental procedures. Unless otherwise mentioned, anoxia was produced by exposing the cells to an atmosphere of 95 % nitrogen and 5 % carbon dioxide for 24 h. For the rest of the culture period the cells were aerated with air containing 5 % carbon dioxide. Exposure to 95 % nitrogen for the whole culture period caused a slight decrease in cell number towards the end of the period due to anoxia.

Treatment with 95 % oxygen and 5 % carbon dioxide was carried out in the same way; namely, exposure to 95 % oxygen for the first 24 h and to 95 % air for the rest of the culture period. Cells exposed to 95 % oxygen for more than 24 h were usually in an extremely poor condition at the end of the experimental period owing to the toxic effect of prolonged treatment with elevated oxygen tension.

The control cultures were kept in an atmosphere of 95 % of air and 5 % of carbon dioxide throughout the culture period.

RESULTS

Cells exposed to an atmosphere of 95 % nitrogen and 5 % carbon dioxide for 24 h showed an increase in their specific LDH and aldolase activities (Figs. 1 and 2). This began during the anoxic period and continued for at least 24 h

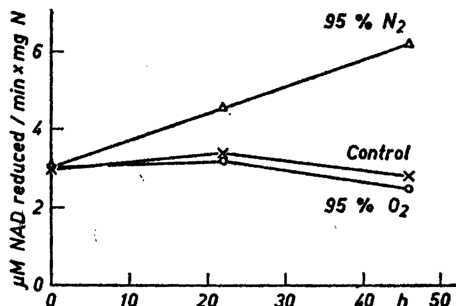

Fig. 1. LDH activity in cells exposed to 95 % N_2 + 5 % CO_2 and 95 % O_2 + 5 % CO_2 during the first 24 h of the culture period. The control culture was aerated with 95 % air + 5 % CO_2. The activity was determined with a final L-lactate concentration of 250 mM by the method of Stambaugh and Post.

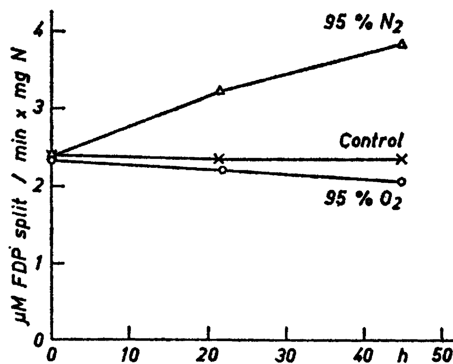

Fig. 2. Aldolase activity in cells treated as described in the legend of Fig. 1.

after the incubation in 95 % nitrogen had been discontinued. Determination of the activities of each type of LDH sub-unit by Stambaugh and Post's method [15] showed that the increase in LDH activity was due mainly to more marked synthesis of the M sub-units, whereas the synthesis of the H sub-units showed a relatively small increase (Fig. 3). As the sub-units seem to combine randomly the greater amount of M sub-units caused a shift in the LDH isozyme pattern as has been reported earlier.[11]

Essentially the same changes resulted from culturing the cells in tightly stoppered flasks as described previously,[11] or by exposing the cells to 95 % nitrogen for the whole culture period, but in these cases the activities at the end of the period were slightly lower, presumably owing to harmful effects of prolonged anoxia. The enzyme activities after about 24 h were approximately the same, irrespective of the method used to induce anoxia.

An increase in oxygen tension (95 % oxygen and 5 % carbon dioxide) produced no significant changes in the activities of the two enzymes within 24 h (Figs. 1 and 2), though at the end of the culture period there was often a slight decrease. On the other hand, if the exposure to 95 % oxygen was prolonged for more than 24 h the aldolase activity decreased rapidly towards the end of the culture period. The LDH activity decreased more slowly. Prolonged exposure to raised oxygen tension also increased the uptake of glucose to values exceeding those of the anoxic cells, and a corresponding amount of lactate being produced.

The cultures exposed to 95 % nitrogen or raised oxygen tension for 24 h showed a much smaller increase in cell number than the controls (Fig. 4). Prolonged anoxia or prolonged exposure to 95 % oxygen completely arrested the division of cells and the cultures, especially those exposed to an elevated

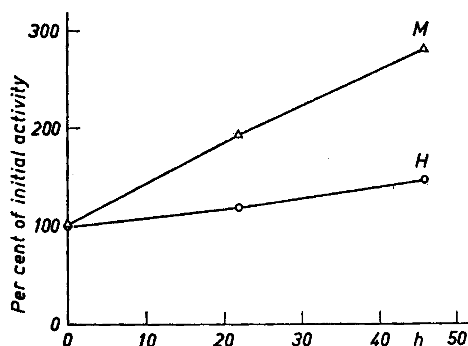

Fig. 3. Increase in LDH activity of the H and M sub-units in anoxic cells determined by the method of Stambaugh and Post. The cells were exposed to an anoxic atmosphere for the first 24 h of the culture period. In the control culture, aerated with 95 % air + 5 % CO_2, the activity of the LDH sub-units remained fairly stable throughout the culture period.

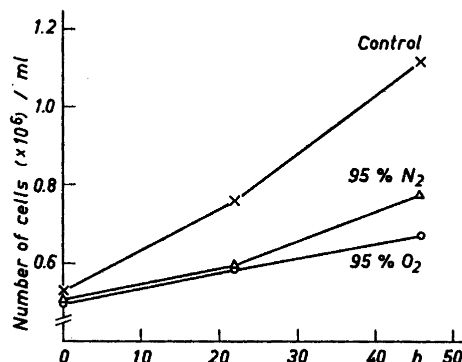

Fig. 4. Growth in cultures treated as described in the legend of Fig. 1.

oxygen tension, showed a high percentage of dead cells towards the end of the period.

The increase in the LDH and aldolase activities that occurred under anoxic conditions was prevented by the addition of puromycin (2.5 μg/ml). This suggests that the increase in the enzyme activities is associated with protein synthesis.

Attempts to produce a change in the proportion of H and M types of sub-unit by adding citric acid cycle intermediates [10] to the medium or by increasing the concentration of glucose from 1 to 10 g/litre were unsuccessful.

DISCUSSION

The observation that a low oxygen tension increased the synthesis of LDH and aldolase whereas a high tension had an inhibitory action is consistent with the results reported in respect of other cell strains after exposure to an anoxic atmosphere.[7,12-14] The regulation of LDH by oxygen mainly seems to involve the synthesis of M sub-units but there seems also to be some increase in the synthesis of the H sub-unit under anoxic conditions; this suggests that the regulation of both types of sub-units is in some way connected with the oxygen tension. But the fact that the changes in the LDH and aldolase activities under anoxic conditions are small compared with the drastic changes that occur during embryonic development suggests that the oxygen tension is not the only factor involved.

Similar changes of LDH sub-unit composition have been reported to occur in some tissues of intact chick embryos when the eggs were exposed to high [8,9]

and to low [9] oxygen tensions. Lowering of the oxygen tension (15 %) tends to increase the ratio of M to H sub-units, whereas an increase in oxygen tension has the opposite tendency. In the chick embryos, however, these changes seem to occur without any alteration of LDH activity with respect to weight;[9] this suggests that an increase in the synthesis of, *e.g.*, M sub-units must be accompanied by a corresponding decrease in the synthesis of H sub-units. In this respect the chick embryos behave differently from tissue-cultured cells, where the activity increases.

Changes in the hydrogen ion concentration of the culture medium did not affect the proportion of H and M sub-units[11] but they influenced the rate of glycolysis. Addition of citric acid cycle intermediates to the medium or an increase in glucose concentration did not affect the proportion of LDH sub-units and attempts to change the LDH composition with metabolic inhibitors have been unsuccessful.[11] The fact that the addition of different metabolites or inhibitors does not affect the LDH composition suggests that the influences of oxygen on the synthesis of this enzyme is a more direct one. The exact mechanism remains obscure, however.

The closely similar appearance of the changes in LDH and aldolase activity (Figs. 1 and 2) suggests that the regulation of the synthesis of these two enzymes is governed by a common oxygen-sensitive mechanism, which is perhaps involved in the regulation of a number of metabolic processes in the cell.

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