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Oxygen Tension Measurements in the Intervertebral Disc

A methodological and experimental study

Arvid Ejeskär and Sten Holm

From the Department of Orthopaedic Surgery I, University of Göteborg, Sahlgrenska sjukhuset, Göteborg, Sweden

ABSTRACT

A polarographic membrane-covered electrode has been used for measurements of oxygen tension in the intervertebral disc. In vitro studies showed that the method measured partial pressures of oxygen with good reproducibility. However, in long continuous experiments, disc matrix adhering to the membrane influenced the readings negatively. The results of in vivo measurements in canine nucleus pulposus showed tensions of the order of 0.53 - 1.06 kPa (4 - 8 mm Hg). No significant variations between different disc levels were found.

INTRODUCTION

Much of the work on the aetiology of low back pain has concentrated on the behaviour of healthy and degenerated intervertebral discs. The normal and pathological appearance, as well as the mechanical behaviour of the disc, have been the subject of numerous investigations (2, 4, 9, 19, 23, 24). The biomechanical changes with age and degeneration have also been described (21). A field of speculation has been the nutrition of the disc, particularly as it has been postulated that nutritional deficiency might lead to disc degeneration (18, 20). The intervertebral disc is the largest avascular tissue in the body and one of the questions has been whether diffusion alone can ensure and adequate supply of nutrients to the cells in various parts of the disc (6, 16, 18). Quantitative diffusion studies (27, 28) have elucidated the routes of solute transport and the metabolism of the sulphate ion. Investigations on glucose-metabolizing enzymes (14) and sulphate incorporation (5, 13) in chondrocytes and articular cartilage have indicated that the cell metabolism is affected by variations in oxygen tension.

Metabolic studies on articular cartilage (8, 22) show that the chondrocytes follow a predominantly anaerobic pathway, but in a recent report (10) it was suggested that either chondrocytes can partially shift from anaerobic to aerobic metabolism, or that two different populations of chondrocytes are present: one anaerobic and a less common aerobic type. However, no experimental data are available in the literature on the oxygen exchange of the cells of the

intervertebral disc. In view of the above findings, and in order to obtain basic information on the oxygen situation, the present investigation was initiated to develop a method permitting in vivo measurement of oxygen tension (pO_2) in the intervertebral disc.

MATERIALS AND METHODS

The oxygen electrode used in this study was a polarographic silver-platinum electrode (see Fig. 1) similar to the one described by Aust and Drettner in 1972 (3). In our electrode the centrally located platinum thread had a thickness of 5 µm and the total length of the electrode was 15 centimetres.

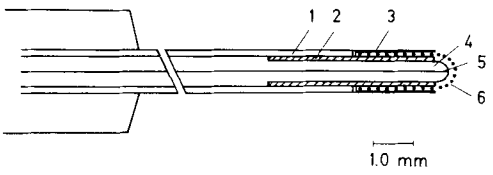

Fig 1. The polarographic electrode
1. Stainless steel cannula
2. Silver cylinder (anode)
3. Nylon cuff employed when using the teflon membrane
4. Glass capillary
5. Platinum wire
6. Membrane

The electrode was connected to a pH-meter (PHM 27b Radiometer Copenhagen) with a pO_2 monitor (PHA 927b Radiometer Copenhagen) and operated at approximately 630 mV. The sensitivity was adjusted manually on the pH-meter (10^{-11} to 10^{-12} A/mm Hg). Two different membranes and application techniques were tested: a) a 12 µm thick teflon membrane, keeping a layer of 0.9% saline between the membrane and the glass tip. The membrane was kept in place by a small nylon cuff. A special applicator was used for mounting the membrane (see Fig. 2).

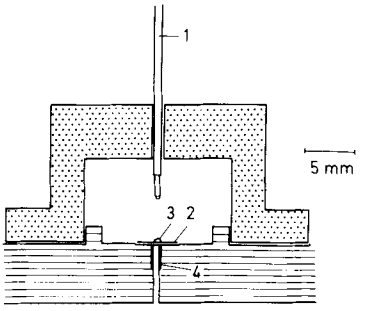

Fig. 2. The teflon membrane applicator.
1. Electrode
2. Teflon membrane
3. Saline
4. Nylon cuff

b) a membrane made of rubber-modified polystyrene in toluene. The tip of the electrode was dipped in KCl electrolyte and after drying the membrane was dip-coated onto the electrode (26). The function of both membranes was tested during calibration. To avoid disturbances of membrane function during oxygen measurements,the electrode was placed in a protective cannula, which had a solid end and an opening at the tip of the electrode.

Calibration:

Calibrations of the electrode were performed in test-tubes containing saline, one equilibrated with pure nitrogen and the others with different oxygen concentrations. The tubes were kept at constant temperature in a thermostated waterbath with stirring. The electrode was first placed in the nitrogen-saturated saline and the zero of the pO_2 scale was adjusted. After 5 minutes, or when steady state was reached (calibration time), the electrode was transferred to the oxygen-saturated saline and the sensitivity was corrected according to the barometric pressure.

Experimental tests of the electrode

A. Response time:

The response time for the electrode was tested when transferring the electrode from the nitrogen-saturated saline to saline equilibrated with oxygen and vice versa.

B. Linearity:

Although the electrode should be linear, a simple test of this characteristic was included. After calibration, measurements were made in test tubes containing saline saturated with a mixture of nitrogen and 0.9%, 1.9%, 2.5%, 4.8% and 10.3% oxygen respectively. Five experiments were performed at each tension level and the membranes were changed between each run.

C. Temperature dependence:

The influence of temperature was tested as follows:
After calibration at 37°C, measurements were made in testtubes containing saline equilibrated with either 1.9% or 5.0% oxygen and placed in a water bath at 37°C. Similar test tubes were kept at room temperature and results were obtained at the two tension levels. The electrode was kept for 5 minutes in each tube and afterwards recalibrated. This was repeated five times at both tension levels and the averages of the results with each membrane were taken as a measure of temperature dependence.

D. Stability:

The stability of the electrode was tested by repeated calibrations during one hour, immersing the electrode each time for a period of 5 minutes in each test tube. Deviations from the original values were registered after 5, 10, 20, 30 and 60 minutes. Ten such experiments were performed and the membranes were changed between each experiment.

E. Pressure dependence:

The electrode was inserted into a rubber tube filled with saline equilibrated with 5.0% oxygen, and then placed in a pressure chamber (see Fig. 3). The pO_2 of the saline solution was measured initially, after which increasing pressure was applied to the rubber tube. Readings of pO_2 were done at 202.6, 303.9, 405.2, 506,5 and 607.8 kPa.

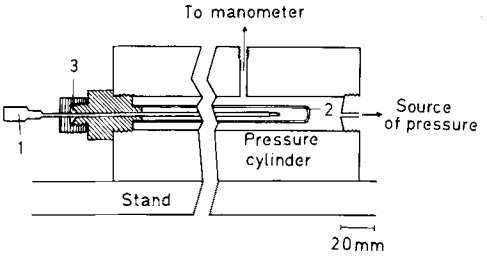

Fig. 3. Apparatus for testing the pressure dependence.
1. Electrode
2. Rubber tube filled with saline
3. Cone of silastic

F. Effects of pH variations:

Measurements were made in saline solutions equilibrated with oxygen and the following tension levels were used: 0%, 2.5%, 4.1% and 10.3% with pH variations ranging from 6.0 to 8.0.

G. Elimination of adhering matrix:

In a set of test tubes several nuclei pulposi were pooled and placed in a water bath at 37°C. The electrode was introduced with either of the two membranes into the mixed nuclei gel. After 5, 10, 20, 30 and 60 minutes, the electrode was rinsed with one of the following solutions: saline, papain (Merck, Germany) or trypsin (Merck, Germany) (all at 37°C) and then rinsed with 0.9% saline and recalibrated. Changes in calibration time and deviations in calibration were noted.

H. Introduction into an intervertebral disc:

Postmortem specimens of spines were used. The tough outer part of the disc was punctured ventrally with an outer cannula which had been designed to permit measurements at predetermined levels. The electrode was then introduced into the centre of the disc via the outer cannula and calibration before and after the procedure was compared and the differences were recorded.

In series 1 and 2 the teflon membrane was used, whereas in series 3 and 4 the rubber-modified polystyrene membrane was used.

Series 1: 36 introductions into canine discs.
Series 2: 30 introductions into human discs.
Series 3: 11 introductions into canine discs.
Series 4: 25 introductions into human discs.

I. Intradiscal oxygen tension in vitro:

Canine spines were taken, deep-frozen, thawed and refrozen for at least 24 hours and rethawed to ensure that the cells were dead.

a) The discs were cut out leaving a tiny layer of the vertebral body on each side. These segments were immersed in a 0.05% solution of sodium azide in 0.9% saline with a small amount of heparin added in an open beaker. This was stored at 4°C, and after varying intervals specimens were removed and warmed in saline at 37°C in a water-bath. The electrode was introduced via the outer cannula into the disc and readings of oxygen tension were taken until steady state was reached.

b) Another set of tests were performed with the disc in a 100% humidity chamber exposed to air, with one of the vertebral endplates trimmed away. The electrode in its protective cannula was placed in the central part of the nucleus at varying depths from 0.5 to 2.0 mm from the air-exposed surface and continuous measurements were made until steady state was reached or for 3.5 hours.

In vivo measurements

20 adult labrador dogs were used. The animals were anaesthetized with pentothal (Abbott, Italy) (30 mg/kg body weight) and ventilated in an Engstroem respirator. Blood gases and blood pressure were controlled during the experiment.

Laparotomy was performed and the ventral aspects of the lumbar and lower thoracic discs were freed by minimal dissection and under careful haemostasis. The electrode was introduced into the central part of the nucleus pulposus and the oxygen tension was registered until steady state was reached. Althogether, 63 intradiscal measurements were included in this study.

RESULTS

A. Response time:

With a well-fitting teflon membrane, 90% of full excursion was reached within 15-20 seconds and 100% after at most 3 minutes. The dip-coated membrane of the rubber-modified polystyrene material reached 95-100% of full excursion within 15-30 seconds, depending on the membrane thickness.

B.+C. Linearity and temperature dependence:

The maximum deviation was ±2.1%. The results are shown in Fig. 4. Temperature dependence varied between different electrodes, ranging from 2.0% to 3.2% per degree centigrade.

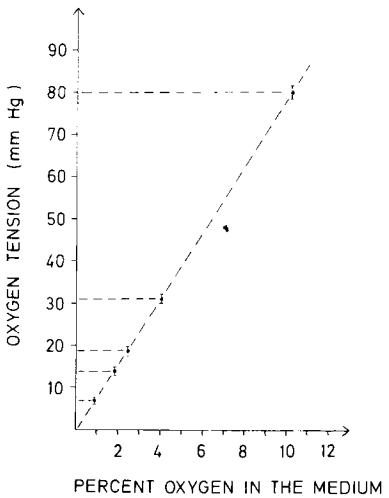

Fig. 4. Diagram showing the linearity of the electrode response. The bars represent S.D.

D. Stability:

The results of the stability test are presented in Table 1. The deviation increased with time and the average deviation in 5.0% oxygen after one hour was ±7.1% when the teflon membrane was used. With the polystyrene membrane the deviation after one hour was ±3.3%.

Table 1. Results of stability tests of the electrode using different membranes.

Calibration Tension (kPa)	Membrane	± Deviation (kPa) Time (min) 5	10	20	30	60
0	Teflon	.050	.053	.053	.053	.093
0	Polystyrene	.025	.030	.030	.035	.068
5.067	Teflon	.041	.053	.092	.200	1.333
5.067	Polystyrene	.038	.050	.063	.120	.820

E.+ F. Pressure dependence and effects of pH variation:

No influence of increasing pressure up to 607.8 kPa could be found. The variations in pH did not seem to influence the oxygen tension measurements.

G. Elimination of adhering matrix:

The adhering matrix caused increased calibration times and deviations in calibration which were not eliminated by rinsing in saline only or immersion in papain solution for 5 minutes. Trypsin, however, reduced the effects of both these negative factors (see Fig. 5). The polystyrene membrane showed less disturbance compared to the teflon membrane.

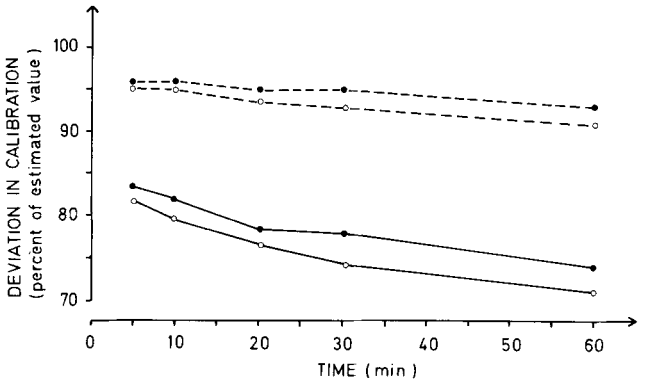

Fig. 5. Deviation in calibration after measurements in pooled samples of nuclei pulposi and the effect of rinsing.
● = polystyrene membrane
o = teflon membrane
----- rinsed in trypsin
——— rinsed in saline

H. Introduction into an intervertebral disc:

Series 1: In six cases membrane function was lost (deviation in calibration of more than 1.333 kPa (10 mm Hg) or instability). The remaining 30 specimens showed an average deviation of ±0.280 kPa or ±6.2%.

Series 2: These introductions resulted in loss of function in two instances and a mean deviation of ±0.253 kPa or ±5.1% in the remaining 28 experiments.

Series 3: Two introductions gave disturbed function. The average deviation in calibration was ±0.140 kPa or ±4.4%.

Series 4: There was no loss of membrane function in any of these experiments and the mean deviation was ±0.093 kPa or ±4.1%.

I. Intradiscal oxygen tension in vitro:

a) The oxygen tension in the disc increased slowly with storage time, reaching tension levels corresponding to atmospheric air after 6 to 8 days.

b) During the time of the experiment (3.5 hours) equilibrium was reached at depths down to 1.0 mm, whereas in the deeper zones equilibrium was not quite achieved (see Fig. 6).

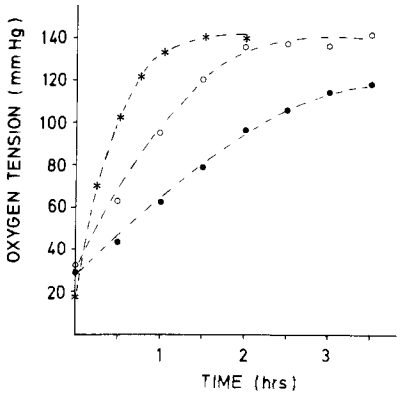

Fig. 6. Results of continuous measurements in discs, with one of the vertebral endplates removed. The electrode was placed in the nucleus pulposus at varying depths below the air-exposed surface.
* = 0.5 mm
o = 1.0 mm
● = 2.0 mm

In-vivo measurements

The oxygen tension values were consistently low, ranging from almost zero to 2.13 kPa (16 mm Hg), with a mean value of 0.92 ± 0.41 kPa (6.9 ± 3.1 mm Hg). No significant differences were found between the disc levels (see Fig. 7). The mean value for equilibrium time, i.e. the time taken to reach steady state, was 19 minutes, in spite of the fact that in a majority of the experiments it was less than 10 minutes.

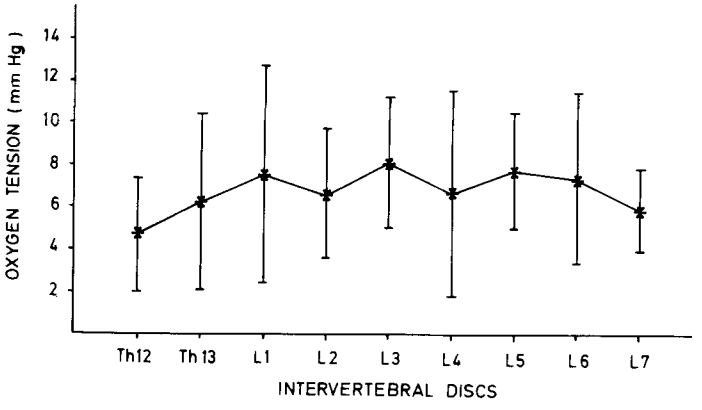

Fig. 7. Results of in vivo measurements in canine nucleus pulposus at various disc levels. The bars represent S.D.

DISCUSSION

The type of electrode used in this study has been used for pO_2 measurements in fluids, especially blood (7, 11) in maxillary sinus atmosphere (3) and in corpus vitreum (1) but not in other tissues. The reason for this has been that a probe of this size disturbs the circulation in the capillaries and therefore interferes with the normal supply of oxygen to the point of the measurements (25). The adult intervertebral disc, however, is avascular and this argument therefore did not apply in this study.

From a theoretical point of view, our electrode was working as a recessed electrode as it was dependent on the fact that tissue material completely filled the small cavity around the tip of the electrode. This also meant that the material around the measuring point was partly cut off from the surrounding tissue and the supply of oxygen might therefore be reduced.
Aust and Drettner (3) stated that one of the difficulties in handling the electrode was to get a well-fitting membrane. This statement was confirmed in this study. A faulty membrane gave instability and rapid fluctuations or slowly decreasing or increasing recordings.

The polystyrene membrane attached with the dip-coating technique was much easier to handle and its function was more stable compared to the teflon membrane. Furthermore, it was easier to clean the electrode tip without disturbances. The stability of both types of memebranes seems to be sufficiently good for measurements during short periods, but might impose problems in long continuous experiments (3 hours or more).

However, when the polystyrene membrane was used the stability was considerably better than when the teflon membrane was employed. This effect might be due to the problems of attaching the latter type of membrane. The response time was not as short as Aust and Drettner reported but in agreement with the findings of Mapleson et al (12), who found that the response time varied from one membrane to another. However, the purpose of this investigation was to measure the oxygen tension which in fact cannot be expected to vary much when the gas tensions in the blood are kept constant. The response time of the order found was therefore not a major problem.

The fact that the temperature dependence obtained was considerably higher (2-2.5 times) than was found by Aust and Drettner (3) might be due to the modification of the electrode. It is known that the normal human intradiscal pressure is higher than atmospheric pressure (17). In the canine discs, however, the situation is still unknown. According to our experience of disc punctures in vivo, the pressure in a normal nonchondro-dystrophoid canine intervertebral disc also seems to be higher than atmospheric pressure. These findings warranted the inclusion of a pressure test in the investigation. These changes in the external environment did not seem to influence the tension

measurements.

It was quite obvious that the matrix of the disc interfered with oxygen tension measurements. The deviation in calibration increased markedly with time. In order to obtain acceptable calibrations after intradiscal introductions, a carefully performed rinsing procedure was necessary. The actual adherence mechanism of the matrix, however, was beyond the scope of this investigation.

To be able to perform measurements in an intervertebral disc a robust electrode that can withstand penetration of the very tough outer layers of the annulus fibrosus is required. In the experimental situation it must also resist the mechanical forces during introduction into the disc produced by the resistant collagen fibre network and the irregularities in the endplates, which were neither flat nor parallel to each other.

This mechanical stress on the electrode was probably an important reason for loss of membrane function. Introduction into human discs caused less loss of membrane function than when canine discs were used. The anatomy of the canine spine was, however, such that penetration into the nucleus pulposus was more difficult compared to human discs, mainly because of less space between the vertebral bodies.

Oxygen tension measurements in discs stored to reach equilibrium showed a very slow increase of pO_2 with time. One reason may be blockage by coagulating blood in the capillaries underneath the vertebral endplate, preventing the oxygen from reaching the centre of the disc within the expected time. Taking this into consideration, the measured times were long and some other blocking factor was probably present. In those cases where the electrode was introduced into the nucleus pulposus below the air-exposed surface (with one of the vertebral endplates removed), oxygen equilibrium was reached down to 1.0 mm but in deeper layers the results obtained did not quite coincide with expected values. Maroudas (15) found for articular cartilage an equilibrium time (90% of final equilibrium) of less than 3 hours for a tissue thickness of 2.0 mm (with one side exposed). In long continuous measurements matrix adherence might have been the main explanation for the discrepancy between the theoretical and the experimental values. Another possible disturbing factor in this particular experimental set-up might be changes in the outermost layer of air-exposed tissue surface.

Our results indicate that a steep gradient in pO_2 must be present in the intervertebral disc. The periphery of the annulus is in contact with the arterial blood pool, which has a tension of roughly 13.33 kPa (100 mm Hg). This means that even slight differences in depth of measurement will markedly influence the result.

The variability of the measurements in vivo in canine discs, where diffusion distances to the centre of the nucleus are of the order of 8-10 mm from the

annular edge, was therefore dependent upon the measuring site rather than being an expression of an inherent error of the electrode system. The present in vivo pO_2 measurements were obtained in the central part of the nucleus pulposus, 1.5 mm to 2.0 mm from the vertebral endplate. Theoretical calculations based upon Fick´s law of diffusion (J. Urban, personal communication) give values of almost zero in the most central part of the nucleus. Accordingly, the oxygen tensions registered are in good agreement with those predicted when only taking into account diffusion from the blood pool below the hyaline cartilage endplate, neglecting the amount entering through the surrounding annulus periphery.

Although the oxygen tension values in vivo (of the order of 0.53-1.06 kPa) were low they nevertheless seemed to be physiologically relevant, taking into account the relatively low cell density and long transport distances from the oxygen supply. The significance of the tension values cannot, however, be adequately interpreted without knowledge of the oxygen requirements of the disc tissue.

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Address for reprints:

Arvid Ejeskär, M. D.
Department of Orthopaedic Surgery
Sahlgrenska sjukhuset
S-413 45 Göteborg
Sweden