Oxidative Metabolism in Cerebral Ischemia


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SUMMARY. Regional oxidative metabolism was studied in vivo. Oxygen availability was monitored from polarographic electrodes in frontal cortical and subcortical tissue. The rate of oxygen extraction in these regions was estimated at frequencies of up to 3 measurements per minute. Transient ischemia of the frontal regions was induced and the resultant decay in the oxygen trace was analyzed kinetically. The oxygen extraction slopes (OES) were steeper in grey than in white matter. They were relatively insensitive to alterations of arterial blood gas concentrations within the physiological range. The slopes were predictedly influenced by pharmacologic agents known to alter the rate of oxidative metabolism. Artifacts which may interfere with the OES measurements were considered. This method of estimating regional tissue oxygen extraction may be appropriate for studying aspects of focal CI.

KETY AND SCHMIDT† described a method for measuring the oxygen consumption rate (CMR0₂) of whole brain from the product of the cerebral blood flow (CBF) and the cerebral arteriovenous difference in oxygen content (A-V0₂). The oxygen consumption rates of smaller volumes of tissue, however, have been found to vary widely when measured in vitro and reflect the regional variations of intermediary metabolism taking place in vivo. Correlation of oxidative metabolic rates with physiological function or focal pathology would be facilitated if the rate of oxygen consumption could be determined in small volumes of brain tissue in situ.

Polarographic methods were introduced to analytical chemistry by Heyrovsky in 1920. In 1946 Davies and Brink§ utilized this approach to study oxygen availability levels in the brain. Davies, McCulloch and Roseman† suggested that the rapid change in the oxygen availability just prior to the onset of seizure activity indicated a change in the oxygen consumption rate of the tissue. Davies and Remond in 1946 measured the rates of fall in cortical oxygen tension after interrupting the afferent circulation and suggested that these rates were related to the local metabolic activity.

In the experiments reported here the oxygen extraction slope (OES) was determined by polarographic means to estimate the local rate of oxidative metabolism. The data indicate that such methods may be appropriate when studying the evolution and treatment of the cerebral ischemias.

Methods

Electrodes for simultaneous measurement of local cerebral blood flow (CBF) and local oxygen extraction slopes (OES), were made from lengths of platinum-iridium wire (250 μm) insulated with Teflon. The distal 1–2 mm were bared and cleaned with concentrated nitric and sulphuric acids. This active tip was then electroplated in platinic chloride solution with a 0.5 ma current over 30 seconds and then dipped in dilute cellulose acetate. The effect of stirring and the uniform response of the electrodes constructed in this manner were evaluated in an oxygen respiration chamber. Frozen and thawed rat liver mitochondria (10–15 mg/ml) were placed in a 40 μl respiration chamber, and the noise level of the output of the electrode was monitored at the 5mv level with adequate stirring. A noise level of 5% was considered acceptable since in tissue sampling there is no rapid stirring. The response time and sensitivity were judged by adding specific amounts of NADH or peroxide to the mitochondrial preparation. The presence of catalase in such preparations afforded a rapid release of O₂, and the time constants thereby obtained were in the 50–100 msec. range. The electrons from NADH terminate in a reduction of oxygen via the electron transport chain of mitochondria, (NADH + H+ + 1/2 O₂ → NAD+ + H₂O). Therefore, adding known amounts of NADH to a preparation of broken mitochondria should give an estimate of the reproducibility of the electrode and a calibration of the electrode would be obtained. Although absolute calibration values obtained in vitro were not directly transferable in vivo, they afforded adequate comparison so that electrode construction was standardized. Because the oxygen diffusion rate in tissue and the catalytic electrode activity in vivo are unknown, it is more accurate to describe the observed oxygen current as oxygen availability (O₉) rather than oxygen tension (P₀₂).

Twenty-two young adult cats weighing 2.8 to 4.9 kg were tranquilized with Sernylan (phencyclidine hydrochloride) 0.25 cc intramuscular, intubated and anesthetized with 75% ether. Through a midline cervical incision 2–3 cc/kg/hr. Through a midline cervical incision...
umbilical tapes were loosely placed around each common carotid artery and 0.5 cc of 1% Xylocaine were injected into the sheath around the carotid sinus. A polarographic circuit was interposed between the sensing electrodes and a stainless steel reference screw placed in the calvarium. Fine copper wire connected to the sensing electrodes was coiled and suspended over the skull, to eliminate possible displacement of the electrodes because of brain pulsation. A bias of +0.6V was employed to measure blood flow with the hydrogen dilution technique and a bias of −0.6V was used to measure the level of oxygen availability. Variations in hydrogen concentration at the active electrode tip will cause a change in current according to the half-cell oxidation reaction $H_2 \rightarrow 2H^+ + 2E^-$. In the oxygen mode a reduction reaction occurs as: $1/2O_2 + 2H^+ + 2E^- \rightarrow H_2O$. To test the completeness of ischemia produced by carotid ligation, the animal was given a 5–7% hydrogen gas intratracheally until a saturation level was recorded polarographically. The gas was then withdrawn and, as the desaturation proceeded, bilateral carotid ligation was performed. With total ischemia the washout slope changed abruptly to a horizontal line (fig. 1).

Arterial blood gas concentrations and blood pressure were determined prior to measurements of the local oxygen extraction slope (OES). Temporary carotid ligation was then performed and the rate of fall in oxygen availability to a new baseline was observed for 10-15 seconds (fig. 2). The ligature was released and, after a transient (4–8 seconds) hyperemic overshoot, the voltage level returned to baseline levels. The slope was calculated and the monoexponential nature of the decay verified using a linear regression analysis for paired voltage and time data. After steady state measurements were made, the animals were divided into groups to study the influence of physiologic and pharmacologic alterations on the oxygen extraction slopes. At the end of each experiment, the polarographic electrodes were heated with a diathermy unit to mark the location of the tips and the brains were removed. They were then fixed in formalin for 4–6 days following which coronal sections were cut and the location of the active electrode tip verified.

Variations in Arterial Blood Gases

In eight animals the arterial oxygen pressure was varied between 20–300 mm Hg by varying the oxygen content between 10–40% of the total inhaled mixture. Arterial CO$_2$ levels were maintained between 24–30 mm Hg by varying the minute volume. Following these test runs the oxygen level was maintained at 100–125 mm Hg with 25–35% O$_2$ and the arterial CO$_2$ level was varied between 25–60 mm Hg by the addition of 5% carbon dioxide to the inhaled mixture. In two animals the sequence was reversed so that the effect of hypercarbia was tested before hyperoxia.

Metrazol

To circumvent the systemic effects of Metrazol (pentylentetrazol) ventriculocisternal perfusion was performed in five animals according to Heisey, Held and Pappenheimer. Metrazol was diluted in warm (38°C) Elliots B solution to a concentration of 20 mg/ml and after steady state measurements of OES were made 5 cc were perfused into the right lateral ventricle at a rate of 1 cc/min. The outflow pressure was adjusted so that the outflow and inflow were equal (VO = Vi).

Nembutal Sodium

After steady state measurements as described above, the inhalation mixture was changed to room air. Nembutal (sodium pentobarbital) 50 mg/kg was given intravenously in five animals. The depth of anesthesia was then supplemented over the next one to two hours to a point where the corneal reflex was just barely elicited.

Hypothermia

Esophageal and rectal temperatures were maintained within 0.5° of each other other and in the range of 26°–37°C.
by a small hypothermia mattress. Serial OES measurements were made within this range in four animals.

Results
Collateral Blood Flow and Complete Transient Ischemia

Sixty-nine of 95 electrodes in 22 animals were found suitable for the study of oxygen extraction and were located in the frontal cortex and subcortex in the regions of the ec- tosylvian, suprasylvian, coronal, lateral and sigmoid gyri (fig. 3) and in the caudate and putamen. In response to carotid ligation blood flow ceased as indicated by the plateau during hydrogen washout (fig. 1). If carotid occlusion was maintained for longer than 30-35 seconds a slow washout of hydrogen often resumed again and was probably related to recruitment of collateral channels. Twenty-six of the 95 electrodes were not suitable for study of OES and in eleven of these a shallow slope persisted after ligation indicating residual flow. Twenty-three electrodes were relatively posterior in the hemisphere and probably had a persistent blood flow from either the posterior or emissary circulation.

Evaluation of the Movement Artifact

The operating microscope was set at X 40 magnification with a short focal length and the cortical surface directly observed through the burr holes prior to electrode placement. In four of the 22 animals a loss of focus during carotid ligation indicated downward movement of the cortical surface. Fixed electrodes recorded an initial positive deflection whose amplitude was 10-20% of the total deflection and 1.5-2.5 sec in duration. This was followed by a rapid negative deflection lasting 1-2 secs in duration of 3-4 mv amplitude before a baseline (fig. 4). In these animals and in an additional six animals a more stable baseline and an OES trace free of motion artifact was achieved using suspended rather than fixed electrodes.

Oxygen Extraction Slopes — Steady State

Rhythmic variations in oxygen availability were seen within two hours after the electrodes were implanted. The waves were of the highest amplitude in the putamen. A frequency of 7-13 cycles/minute was noted in cortical grey matter electrodes and 4-10 cycles/minute in the centrum semiovale. Electrodes located within 3.0 mm of each other showed synchronous dipole coupling for 50-250 cycles although some were 180° out of phase. The tracings from more distant pairs of electrodes were asynchronous.

Sudden bilateral carotid ligation maintained for 15 seconds produced a decline of 79-90% in oxygen availability but in no case did the voltage value fall to zero (fig. 2). The mean time constant for the decay under steady state conditions was 1.6 seconds. After release of the ligatures there was a return and overshoot of the oxygen availability values. Within approximately 10-15 seconds of carotid release the resting level of oxygen availability (O2a) as well as the frequency and amplitude of the spontaneous oxygen waves were restored.

Two hundred sixteen measurements were performed in 22 animals under steady state conditions as defined above, at suitable electrode locations. When the arterial oxygen tension (PaO2) was maintained above 50 mm Hg, 97% of the curves from cortical and deep grey nuclei and 90% of the decay curves from white matter were composed of a single exponential function. The extraction rates were not calculated in those instances where two exponentials were found. The variation in the oxygen extraction slope in the same animal at the same location was less than 9% and the values for grey and white matter oxygen extraction slopes were expressed as ratios of voltage drop per unit time in table 1. The OES values for cortical and deep grey matter were consistently and significantly (<.01, <.001) greater than that measured in the centrum semiovale.

Variations in Arterial Blood Gases

a. Oxygen: As arterial oxygen tension (PaO2) was varied between 60-300 mm Hg no significant change was seen in

![Figure 3. Cortical and subcortical locations tested by the hydrogen dilution method. Circles represent electrode sites suitable for the study of oxygen extraction slopes. These were distributed in frontal gyri as indicated. Cross marks represent areas of persistent or reversed flow. Suitable regions in deep grey matter are not shown (see text).](http://stroke.ahajournals.org/)

![Figure 4. Relative movement of brain and electrode was indicated by a rapid positive and immediate negative deflection. These artifacts could be largely eliminated by using suspended rather than fixed electrodes.](http://stroke.ahajournals.org/)
the oxygen extraction slopes. In eight animals PaO2 values were reduced below 50 mm Hg (table 1). The effects on OES were related to both the severity and duration of hypoxia and were additive. Re-oxygenation to normal PaO2 values within 8-12 minutes of the onset of hypoxia resulted in a return of both oxygen availability levels and oxygen extraction slopes to normal (fig. 5). Re-oxygenation after more prolonged hypoxia in 3 animals was less effective in restoring both parameters (fig. 6).

b. Carbon Dioxide: Increases of Paco2 within the range of 20-55 mm Hg were associated with increases in oxygen availability but produced no consistent effect on the extraction slope (fig. 7). Further degrees of hypercapnia were associated with systemic hypotension.

Metabolic Stimulation and Inhibition

a. Metrazol: Within 20 seconds after commencing the perfusion of Metrazol an increase in OES was measurable at both grey and white matter electrodes. An increased slope was noted in all animals with a mean increase of 210% in deep grey, 170% in white matter and 190% in cortex (table 2). A significant increase in blood pressure and pulse was noted 15-20 minutes after completion of the perfusion and a tachycardia persisted for 30 minutes to one hour thereafter.

b. Sodium pentobarbital: 50 mg/kg given intravenously after control measurements had no significant effect on the blood pressure, pulse or arterial blood gas values. Within 15-20 minutes a reduction in the slope of 30-60% was seen.
The values given in Table 2 are the mean values of measurements made one-half hour after administering the drug.

c. Hypothermia: Grey and white matter oxygen extraction slopes generally decreased with a decrease in core temperature. A ten minute steady state temperature was maintained before each reading. Figure 8 is a plot of core temperature vs OES. Although OES is a continuous function of temperature there was a greater relative decrease in OES in the temperature range 26-30°C than between 31-37°C. The plot of OES vs temperature for each electrode locus showed somewhat different functions among the various brain regions (fig. 8).

Discussion

Experiments using tissue slices have shown that oxidative metabolism develops at different rates in the older and newer parts of the nervous system. In the adult, large regional differences in rates of oxidative metabolism are maintained. According to Himwich,10 the highest rate of metabolism in the adult is found in the caudate nucleus, followed by the cortex, cerebellum and brain stem respectively. These differences do not necessarily reflect the distribution of high energy substrates. Reported differences in ATP and phosphocreatine concentration11 may reflect autolytic changes resulting from a freezing delay in deeper structures of the brain. Within the cerebral cortex of the dog Gleichmann, et al.12 found a wide variation in metabolic rates for oxygen from 2.3-13.4 ml/100 g/min with a mean CMRO2 of 7.0 ml/100 g/min. Homberger et al.13 found a value of 5.9 ml/100 g/min in light anesthesia with a range of 4.2-7.6 ml/100 g/min. These are all higher than the values determined for whole brain of 3.3 ml/100 g/min in man and 3.7 ml/100 g/min in the monkey.

In the study of oxidative metabolism we have sought
FIGURE 8. Cortical, deep grey and subcortical white matter oxygen extraction slopes are plotted against central body core temperature. Peripheral cortical oxygen extraction proceeds at a slightly higher rate for a given core temperature. The effect of temperature on oxygen extraction rate is more pronounced within the range 26-30°C than between 31-37°C.

FIGURE 9. Responsiveness of the oxygen electrode to changes in arterial oxygen tension is illustrated. The responses were linear although not uniform over the range of \( \text{apO}_2 \) (\( \text{PaO}_2 \)) 10-240 mm Hg.
The oxygen availability recorded at a polarographic electrode is related to the tissue pressure of oxygen, the local blood flow, the tissue temperature and the metabolic consumption rate. After sudden interruption of blood flow and oxygen supply, the available oxygen disappears largely through metabolic consumption. Its rate of loss by simple physical diffusion away from the active electrode tip should not change appreciably in the same preparation before and after an experimental perturbation. Thus, the relative rates of oxygen extraction by small tissue volumes should be measurable in vivo using this system which is sensitive to rapid changes in oxygen tension. Since the partial pressures, diffusion constants and electrode calibration are unknown factors, and since the volume and weight of the tissue recorded from were not determined in these experiments, the measurements are referred to as oxygen extraction slopes rather than oxygen consumption rates.

The relationship between the oxygen extraction slope and the local oxygen consumption rate of the tissue is empirically reasonable. Its mathematical justification may be derived utilizing Krogh’s tissue cylinder model. In this model brain tissue was functionally divided into cylindrical areas surrounding a capillary. The radius of such a tissue cylinder has been estimated by Horstmann to be 15 times that of its central capillary. An expression for the average oxygen tension at a point in the tissue cylinder was given by Kety as:

\[ \text{PO}_{2} = \text{Pa} \left( \frac{1}{R} \left( \frac{X}{2} - \frac{X^{2}}{4} \right) \right) \]

Where \( \text{PO}_{2} \) is the oxygen tension at a point in tissue, \( \text{Pa} \) is the oxygen tension in the capillary, \( X \) is the distance from that point to the nearest capillary, \( 2R \) is the intercapillary distance, \( R \) is the capillary radius, \( D \) is the diffusion coefficient of oxygen in tissue, and \( C \) is the metabolic rate of oxygen consumption by the tissue. The large gradient between the \( \text{Po} \) and \( \text{Px} \) is the necessary driving force for oxygen diffusion.

The metabolic rates of oxygen consumption in discrete volumes of brain tissue can be related to the rate of change of tissue gas pressures (\( \text{P}_{x} \)) as capillary gas pressure (\( \text{P}_{a} \)) approaches zero. By re-arranging equation [1] and solving for \( C \), the metabolic rate in tissue we have:

\[ C = \frac{D(\text{P}_{x} - \text{P}_{a})}{1/(3R^{3} - R^{2}) - \frac{R(NR/2)}{2(R^{2} - R)}} \]

Assuming that the oxygen diffusion rate and the capillary distances are stable throughout the short measurement period their expressions may be approximated by a constant \( k \), so that:

\[ C = K (\text{P}_{x} - \text{P}_{o}) \]

The metabolic consumption rate of oxygen at the point in tissue under consideration may then be related to the time derivative of the difference in capillary and tissue oxygen tension.

At \( t = 0 \) the blood supply is cut off and the tissue, almost instantly, develops a local uniform supply of oxygen represented by the partial pressure \( \text{P}_{x} \). Metabolic consumption of oxygen continues either at a uniform rate \( C \), or at a rate proportional to oxygen concentration, i.e., \( C (\text{P}_{x}/\text{P}_{a}) \).

In the first instance:

\[ \frac{d\text{P}}{dt} = C \text{ and } \text{P} = \text{P}_{x}e^{Ct/\text{P}_{a}} \]

While if the rate is dependent on the concentration of substrate:

\[ \frac{d\text{P}}{dt} = -C (\text{P}/\text{P}_{a}) \text{ and } \text{P} = \text{P}_{a}e^{-Ct/\text{P}_{a}} \]

The oxygen extraction slopes were independent of oxygen concentration at normal and high concentrations of arterial oxygen and 97% of these slopes were monoeXponential decays. Below 50 mm Hg a decrease in the extraction slope was seen. Only 80% of the curves in this hypoxic range were monoeXponential while 20% were bi-exponential suggesting that an unsaturation of the oxygen receptor sites may be a rate limiting factor and at these tissue levels, oxygen extraction rate may also be limited by its concentration in the brain.

Re-oxygenation within 10 minutes after the onset of hypoxia produced a return of the extraction slopes to normal. The ischemic damage produced by more prolonged (>10 minutes) hypoxia resulted in a permanent decrease in OES rates. The relationship between the critical threshold and the level at which diffusion becomes rate limiting needs to be further studied. Calculations of Opitz and Schneider of intracellular oxygen tension based on the cylindrical model of diffusion of oxygen in tissue give a value of 8–13 mm Hg as the critical intracellular oxygen tension in the brain. Chance et al. measured the NADH redox state of rat cerebral cortical tissue extract using fluorometric analysis and determined the critical intracellular oxygen tension. This was the oxygen tension at which mitochondria are no longer capable of energy coupling as demonstrated by their ability to maintain calcium gradients. This critical oxygen threshold determined in vitro is much lower than that determined polarographically. It is apparent, however, from oxygen diffusion theory, that considerable concentration gradients are required between the extracellular and mitochondrial compartments to maintain these relatively low values in the mitochondria.

Since dynamic blood flow changes bear such a close relationship to oxygen availability the possible influence of flow on oxygen extraction was studied. Flow was manipulated by altering the arterial \( \text{P}_{co2} \). In a separate series of experiments in cats, the relationship of arterial \( \text{P}_{co2} \) to regional cerebral blood flow was established using the hydrogen dilution method, and was described by the regression equation:

\[ y = 7.4 + 1.8x \]
Although an increase in the level of oxygen availability was seen consistently with hypercapnia, there was no significant influence on the OES.

The OES values, however, changed in a predictable direction in response to pharmacologic and physiologic agents known to have potent effects on metabolism. Metrazol increased the rate of oxygen extraction and in some experiments caused a fourfold increase in steepness of the slope. While a dose response curve was not established, a suggestive relationship was apparent.

Barbiturates decrease the rate of oxidative metabolism. In a study of the kinetics of cortical metabolism Rosenthal and LaManna demonstrated an inhibition of reduction of NADH which has the most negative mid-potential of the electron transport system and is most sensitive to change in oxygen concentrations. It is still unclear whether this action is secondary to a direct membrane effect; however, a decrease in oxygen consumption was clearly reflected in a decreased rate of oxygen extraction irrespective of the level of oxygen availability.

Field, et al. studied the effect on the temperature of the oxygen consumption rate of excised brain tissue. They showed that the graph of the logarithm of oxygen consumption is a function of temperature in degrees centigrade and was linear over the range 10°C to 37.5°C. They also derived a van't Hoff temperature coefficient (Q10) for this temperature range of 2.13. Rosomoff and Holaday were able to show a parallel decrease of cerebral blood flow and oxygen consumption with decreases in temperature, while arteriovenous oxygen differences remained almost constant. Adams and Pevehouse and Woodhall, et al. have also stated that CMRO2 is a linear function of temperature. Bering plotted the CMRO2 as a function of body temperature and noted a non-linear relationship. When the In CMRO2 was plotted as a function of the reciprocal of the absolute temperature, a linear relationship was found which could be expressed as an Arrhenius equation, indicating the chemical or metabolic nature of the temperature sensitive process.

There is now abundant evidence for the preservation of many electrophysiologic functions after complete and even prolonged ischemic insults. In barbiturate anesthetized cats, Hossmann demonstrated maintenance of direct cortical responses as well as EEG after complete occlusion of all afferent blood supply to the brain for periods of up to one hour. The ability of the energy transduction system to provide adequate high energy bonds for such recovery may be related to the degree of coupling of oxidation and phosphorylation. The energy deficit seen after stroke is more likely associated with an uncoupling of oxidative phosphorylation, therefore, the rate of oxygen consumption or the oxygen extraction slope alone does not reflect the energy capacities of the mitochondrial machinery. This polarographic approach, however, will allow for an assessment of the rate of oxygen extraction in small brain regions. Determinations of the cortical redox state using surface fluorometry will be reported subsequently, and by combining these techniques it is anticipated that the nutritional adequacy of the local oxygen extraction rates can then be assessed.

References

10. Hossmann HE: Brain metabolism and cerebral disorders. Baltimore, Md, Williams & Wilkins, 1951

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