after temporary muscle paralysis are a common clinical occurrence. We have duplicated this clinical sequence pharmacologically in the monkey. The EMG recording technique described insures precise localization of the muscle desired, without the need for conjunctival incision or direct exposure of the muscle. Diffusion of the drug into adjacent muscles has been reduced by the use of small volumes of drug injected. The effect seems able to be reduced or nullified also by the simultaneous injection of a small amount of antitoxin into adjacent areas, a modification of the approach of Kupfer. Effective paresis has been produced in the monkey by injection of $1.0 \times 10^{-5}$ up to $1.6 \times 10^{-2}$ mg of botulinum Type A neurotoxin. Relative to the (oral) LD$_{50}$ dose in man, approximately 1 mg, this represented from 1/100,000 to 1/1,000 of the LD$_{50}$.

Clostridium botulinum produces a specific neurotoxin, which, because it appears spontaneously in the culture medium from which it can be isolated, is classified as an exotoxin. To date, six antigenically distinguishable toxines have been identified, and we have done our work exclusively with Type A toxin. These toxines are proteins with a molecular weight of approximately 900,000. Present evidence indicates that botulinum toxins acts presynaptically as an extremely powerful blocker of cholinergic transmission. Evidently toxins directly affects the mechanism by which quanta of acetylcholine are liberated from the nerve endings. This effect is not a consequence of interference with impulse conduction in the motor nerve or of inhibition of synthesis or storage of acetylcholine. Neither the nerve nor the muscles suffer impairment of electrical excitability or conductivity in the presence of complete neuromuscular blocks produced by botulinum toxin, and the changes in nerve or muscle are considered secondary atrophic consequences from loss of cholinergic transmission.

The clinical relevance of the use of botulinum neurotoxin or other drug injections into extraocular muscles awaits appropriate human trials. Such a pharmacologic approach may be used to replace or augment existing methods of surgical correction of strabismus. The weakening of overactive extraocular muscles in comitant squint and the weakening of the antagonist muscles in paralytic squint, both as treatment and in smaller doses to prevent secondary contractures, seem possible. Extension of this approach to reducing lid retraction in endocrine exophthalmos, reducing blepharospasm, and influencing skeletal muscle spasm seems entirely feasible.

A. E. Maumenee, M.D., suggested the use of botulinum toxin to us; Edward J. Schantz, Ph.D., provided the botulinum toxin and gave valuable advice in many ways; and Bert L. Tate helped in recording and electrode design.

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Key words: strabismus, paralysis, eye muscles, botulinum toxin.

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In vivo measurement of optic-disk oxygen tension. J. TERRY ERNEST.

Davies and Brink first demonstrated the feasibility of using a platinum microelectrode to electrolyze dissolved oxygen in animal tissue. The stability of the system is poor because of drift due to change in electrode performance. Thus, slow changes are difficult to analyze but transient qualitative measurements may be obtained with moderate reliability. The present study was undertaken to develop a valid method for measuring the oxygen tension of the optic disk in vivo using a platinum microelectrode.

Fifty-five adult cats, both male and female of mixed breeds, ranging in weight from 2.0 to 3.5 kilograms, were used. The animals were anesthetized with sodium pentobarbital, 30 mg. per kilogram, administered intraperitoneally. General anesthesia was maintained by supplements of sodium pentobarbital administered intravenously. Tracheostomies were performed and the animals were given artificial respiration (Respirator Model 671, Harvard Apparatus Co.) and then paralyzed and maintained in this state with a mixture of tubocurarine chloride, 0.4 mg. per milliliter, and gallamine triethiodide, 2 mg. per milliliter, administered intravenously in 0.5 ml. increments as needed.

The tidal PCO$_2$ was continuously monitored with an infrared absorption carbon dioxide analyzer (Medical Gas Analyzer Model LB-1, Spino...
Division, Beckman Instruments Co.) connected to the tracheal cannula and recorded on one channel of a polygraph (Polygraph Model 7, Grass Instrument Co.). The arterial blood pressure was monitored from the cannulated abdominal aorta with a pressure transducer (Model P23 De, Statham Laboratories, Inc.). The intraocular pressure was monitored and maintained at a constant level by cannulating the anterior chamber with a 19-gauge needle connected to a saline reservoir through a second-pressure transducer. The hemoglobin saturation of the blood was monitored by constantly pumping (Peristaltic Pump Model AL2E, Signamotor, Inc.) the femoral arterial blood through a cuvette transmission oximeter (Oximeter Cuvette Model XC-50B, The Waters Co.) and back into the femoral vein at 2.4 ml per minute.

The partial pressure of oxygen \( (P_o) \) of the optic disk was measured in the vitreous body 10 microns above the center of the disk with a glass-insulated platinum microelectrode (Micro-oxygen Sensor No. 721, Transidyne General Corp.). The characteristics of these probes are somewhat variable and only those microelectrodes with an impedance of 20 megohms or greater and oxygen tension-current outputs of the order of magnitude of 10^-9 amperes or less with a polarizing voltage of 0.6 were used. At this polarizing voltage there is no other reducible substance than oxygen. The electrodes are not pH or \( P_{CO_2} \) sensitive. The microelectrodes were calibrated in 0.9 per cent NaCl solution at a temperature of 38.0° C. saturated with 100 per cent \( N_2 \), 5 per cent \( O_2 \), or 10 per cent \( O_2 \). The microelectrodes were sensitive to changes in the temperature. A change of 1° C. resulted in a corresponding change of 6 per cent in the oxygen-tension measurement in the same direction.

The microelectrodes were inserted into the eyes by first cannulating the vitreous cavity with a 17-gauge needle 6 mm. posterior to the limbus controlled by a micromanipulator (Model RP-1V/R, Brinkmann Instruments). The eye was stabilized by the technique previously reported and the anterior chamber cannulated and maintained at a pressure of 25 mm. Hg. The microelectrode was inserted through the cannula into the vitreous body and its depth controlled with a hydraulic micromanipulator (Microdrive Model 607B, David Kopf Instruments). Fig. 1 is a diagram of the cannula and microelectrode inserted into the vitreous cavity. The microelectrodes were advanced under ophthalmoscope control until the tips encountered the optic disk. This was signaled by a transient fluctuation in current. The microelectrode was then withdrawn 10 microns. The positioning of the electrode in the vitreous eliminated the problem of tissue destruction. The reference anode was a Ag-AgCl wire inserted in the anterior chamber of the opposite eye.

All the parameters were recorded on the polygraph with a paper speed of 2.5 mm. per second and 5 second time marks. The optic-disk oxygen tension was recorded on one channel and the tracing was integrated on a second channel. Arterial blood samples were analyzed throughout each experiment for pH, \( P_{CO_2} \), and \( P_o \) by a blood microsystem and recorded on a digital acid-base analyzer (Models BMS 3 and PHM 72, The London Co. and Radiometer A/S).

Absolute measurements of oxygen tension with a microelectrode have poor reliability and are often not valid. Of the 55 experiments, 31 produced useful data and of these, five were selected because of the similarity of the microelectrode calibration before and after optic-disk measurements (Table I). The measurements recorded in Table I are not corrected for the fact that the temperature of the vitreous over the optic disk is approximately 1.5° C. lower than the body temperature which was used as the calibration temperature. The corrected value is thus about 9 per cent higher than that reported in Table I. The average arterial pH and blood-gas values for one awake and unexcited cat were pH 7.36 (S.D. ± 0.05), \( P_{CO_2} \) 27.5 (S.D. ± 2.7), and \( P_o \) 93.8 (S.D. ± 4.4).

A comparison was made between the response of the optic disk and of the retina to the inspiration of 100 per cent oxygen. Fig 2, A is a photograph of the record obtained when the animal breathed 100 per cent oxygen for 55 seconds and the oxygen microelectrode was 10 microns above the center of the optic disk. The optic-disk oxygen tension increased three times and was off scale. The oxygen microelectrode was then immediately moved 4 DD superior temporal to the optic disk and adjusted 10 microns above the retina. Fig. 2, B is a photograph of the record
Fig. 2. The effect of the inspiration of 100 per cent oxygen for 55 seconds on the optic-disk oxygen tension (A) and on the retinal oxygen tension 4 DD superior temporal to the disk (B).

showing that there was an increase in the retinal oxygen tension, but the increase was only a fraction of that obtained over the optic disk.

The use of the micro-oxygen electrode for transient relative measurements is accompanied by a high degree of reliability. The inspiration of 100 per cent oxygen for 15 seconds doubled the optic-disk oxygen tension and raised the \( P_{O_2} \), to approximately 250 mm Hg, and transiently saturated the hemoglobin. When the inspiratory air was 100 per cent \( N_2 \), there was a delay in the fall of the hemoglobin saturation because of the relatively long tubing and slow pumping rate from the femoral artery to the oxygen cuvette. The optic-disk oxygen tension decreased in spite of a slight elevation in the systemic blood pres-
Table I. Optic-disk oxygen tension

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Optic disk Po&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</th>
<th>pH</th>
<th>Arterial blood</th>
<th>Po&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>12.2</td>
<td>7.41</td>
<td>20.5</td>
<td>115.7</td>
</tr>
<tr>
<td>20</td>
<td>18.8</td>
<td>7.41</td>
<td>22.6</td>
<td>98.7</td>
</tr>
<tr>
<td>22</td>
<td>10.2</td>
<td>7.42</td>
<td>24.5</td>
<td>91.7</td>
</tr>
<tr>
<td>28</td>
<td>7.4</td>
<td>7.43</td>
<td>28.2</td>
<td>108.3</td>
</tr>
<tr>
<td>31</td>
<td>6.4</td>
<td>7.38</td>
<td>27.7</td>
<td>86.5</td>
</tr>
<tr>
<td>Ave.</td>
<td>11.0</td>
<td>7.41</td>
<td>24.7</td>
<td>100.2</td>
</tr>
<tr>
<td>S.D. 2</td>
<td>4.4</td>
<td>0.01</td>
<td>2.1</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Sure. The continual inspiration of 10 per cent oxygen and 90 per cent nitrogen resulted in an initial fall in optic-disk oxygen tension followed by a return to control levels even though the oxygen saturation remained low. When the animal was returned to air breathing, the optic-disk oxygen tension overshot normal and then slowly returned to baseline levels.

Oxygen diffuses out of the red blood cells, through the plasma, capillary wall, and into the extracellular fluid. The mean extracellular fluid oxygen concentration is directly related to the mean capillary oxygen concentration when consumption by the cells remains constant and assuming the blood-flow rate and diffusion coefficient of oxygen are constant. The extracellular fluid and the vitreous do not consume oxygen and the vitreous has little protein and is probably not circulating. The electrode consumes oxygen and the oxygen current depends on the diffusion of oxygen to the platinum surface. The average optic-disk oxygen tension was of the same order of magnitude as those previously reported by Aim and Bill over the cat's retina and by Tsacopoulos and co-workers over the monkey's retina. All of these values, however, are considerably lower than those reported by Krause and Goren for the cat. The values obtained in the study herein reported for the optic-disk oxygen tension were not statistically different from those obtained over the posterior pole retina.

The response times to step changes in inspiratory gases were extremely rapid. Examination of the microelectrode responses obtained with the inspiration of 100 per cent oxygen over the optic disk versus over the retina furnished evidence that the extracellular oxygen of the peripapillary retina did not contribute to the measurements obtained by the probe 10 microns over the center of the optic disk (Figs. 2, A and B). The retinal response is less than that of the optic disk. This may be due to a constriction of the retinal vessels during 100 per cent oxygen breathing. Further evidence that the retinal circulation does not contribute to the optic-disk measurement is obtained by an examination of the latencies of the oxygen responses. The fact that they are similar suggests that a significant movement of oxygen to the optic-disk microelectrode from the surrounding retina does not occur. The large cilio-retinal vessels surrounding the cat's optic disk did not furnish oxygen to the local vitreous since changing the distance between them and the probe did not significantly affect the oxygen-tension measurements.

The optic-disk oxygen tension is time-linked to the blood-oxygen tension. However, the desaturation of hemoglobin is faster than the diffusion of oxygen molecules to the microelectrode. A rapid recovery of the optic-disk oxygen tension suggests the presence of compensatory regulatory mechanisms.

The sensitivity of the visual system to hypoxia is well known. Further, decreases in the oxygen tension of the arterial blood result in corresponding decreases in the oxygen tension in the vitreous over the retina. In the experiment herein described, however, while the inspiration of 10 per cent oxygen resulted in an immediate decrease in optic-disk oxygen tension, there was a recovery to normal levels after approximately 30 seconds. This return to normal oxygen tensions is most likely due to an increase in blood flow similar to that observed in other tissues during hypoxia. The overshoot of the optic-disk oxygen tension with the return to air breathing appears to be similar although in an opposite direction to the response to hypoxia. The results appear to represent a local regulatory mechanism responding to changes in blood-oxygen tension.
Pilocarpine hydrolysis: clinical significance. PHILIP P. ELLIS AND KATHERINE LITTLEJOHN.

Prior investigations in this laboratory have demonstrated the presence of a pilocarpine-hydrolyzing enzyme in human and rabbit sera and ocular tissues. The enzyme was also demonstrated in secondary aqueous humor of humans and rabbits at lower levels than in the respective sera; more was demonstrated in primary aqueous humor. The following studies were undertaken to investigate the clinical significance of the serum enzyme, and more specifically to answer these questions: (1) Does pilocarpine administration alter serum enzyme levels? (2) Does the serum level of the pilocarpine-hydrolyzing enzyme influence the success of pilocarpine therapy in open-angle glaucoma, i.e., are patients with high-enzyme levels less susceptible to pilocarpine control? (3) Is it possible to inhibit the pilocarpine-hydrolyzing enzyme and thereby increase the hypotensive action of pilocarpine. The laboratory methods used in these studies have been previously reported and only deviations from those methods will be noted here. In the animal experiments, New Zealand albino rabbits weighing between 2.7 and 4.0 kilograms were used.

To determine the effect of chronic topical pilocarpine therapy on serum enzyme levels, 2 per cent pilocarpine in 0.5 per cent hydroxypropyl methylcellulose (Isopto Carpine) was applied to the eyes of six animals. Application regimen consisted of two drops in each eye, three times a day, five days a week for fourteen weeks. Hydroxypropyl methylcellulose, 0.5 per cent (Isopto Tears), was applied for the same period to four control animals. Weekly blood samples were drawn by cardiac puncture and serum was analyzed for three weeks prior to the beginning of drug administration to establish baseline serum-enzyme levels. An additional seven blood samples were drawn at intervals of one to two weeks from each animal during the fourteen week period of drug administration.

Serum analysis varied slightly from the previously described procedure. Pilocarpine (2 x 10^-5 M) was mixed with 0.1 ml. of rabbit serum, incubated 10 minutes, and residual pilocarpine determined. Serum from control animals hydrolyzed 215.78 ± 30.22 (S.D.) ng of pilocarpine per minute per milliliter of serum prior to administration. Serum from treated animals hydrolyzed 218.35 ± 19.36 ng and 233.63 ± 10.66 ng of pilocarpine per minute per milliliter of serum, respectively. The differences are not significant (p > 0.05) and indicate that topical pilocarpine administration had no effect on the serum enzyme levels during the treatment period.

To determine the effect of acute pilocarpine administration, nine rabbits were injected with large doses of pilocarpine. Through trials it was determined that the animals did not survive if more than one-eighth of the lethal intravenous dose in rabbits (21.87 mg. per kilogram per day) was administered subcutaneously for three consecutive days. The daily dose was given in two injections at seven-hour intervals. This regimen produced increased salivation and diarrhea in all animals; death occurred in five animals before blood samples could be drawn on the fourth day. In the four surviving animals 0.2 ml. of serum hydrolyzed 215.29 ± 35.13 μg of pilocarpine per