finches subjected to a short day regimen (6L/18D), or in those subjected to cycles of 48 hours (6L/42D) or 72 hours (6L/66D). The birds responded to these cycles as though they had received short-day, nonstimulatory treatment. In contrast, the birds subjected to cycles of 12 (6L/6D), 36 (6L/30D), and 60 hours (6L/54D) responded as though they had been subjected to long days. Their testes increased in weight and spermatogenesis began (Table 1). Spermatogenesis was not initiated in birds subjected to 24-, 48-, and 72-hour cycles except for one bird from the 72-hour cycle in which spermatogonia and a few spermatocytes were present.

A number of conclusions may be drawn from these data. First, those hypotheses that stress the necessity for a given duration of light to trigger the photoperiodic response are inapplicable to house finches. All the birds received 6 hours of light per cycle; thus, no treatment constituted a long day in the usual photoperiodic sense. Yet the birds on the 36-hour cycle showed testicular enlargement despite the fact that they were given only 6 hours of light every day and a half. As the source of energy input in a photodependent system, light is necessarily important, but in these experiments the length of the light period was not critical; nor was it the length of the dark period which initiated testicular recrudescence. The six experimental treatments differed in their durations of darkness. There is no seeming reason why, if darkness itself is important, the birds subjected to 36-hour cycles should respond while those subjected to 24- and 48-hour cycles should not.

These results indicate that there is within the bird an endogenous rhythm with a periodicity of about 24 hours. When light is given at the proper phase of this rhythm recrudescence occurs, and when light is given at the incorrect phase of the rhythm no response is elicited. This circadian rhythm may therefore serve as a biological clock to time the photoperiodic response of testis maturation in the house finch (*12*).

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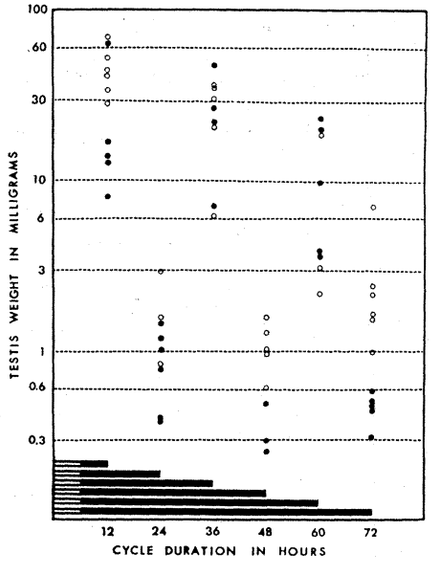

Fig. 1. Data on the weights of the testes from birds in experiment 1 (solid circles) and experiment 2 (open circles). Solid black bars represent different durations of darkness; open boxes represent standard light period of 6 hours.

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9 September 1963

Oxygen Uptake from a Reservoir of Limited Volume by the Human Cornea in vivo

Abstract. *Oxygen flux across the anterior surface of the human cornea from a closed reservoir of air-saturated, physiological, isotonic saline was measured in vivo as a function of time. The rate of oxygen consumption calculated from this flux compares favorably with estimates given in the literature, which were based on studies in which the microrespirometer technique of Warburg was used.*

The consumption of oxygen by the cornea directly from the atmosphere has been known for some time (*1*). Although the atmosphere is the major source of oxygen for the cornea, no direct measurements of the flux across the cornea-atmosphere interface have been reported previously.

Rates of oxygen uptake by the cornea of a variety of animals have been published (*2–6*) (see Table 1), but the results were obtained by methods which differ considerably from our technique. Previous workers macerated the corneas and suspended the cells in saline. The oxygen uptake of the resultant suspension was measured by the microrespirometer technique developed by Warburg. By our method, the flux of oxygen across the cornea-atmosphere boundary can be measured in vivo. This flux is known to be only a part of the total supply of oxygen to the eye, but is probably the major source for the cornea.

Our equipment is shown schematically in Fig. 1. A Lucite tube was cemented to a scleral contact lens made of acrylic plastic, and the material directly under the tube was cut away. An oxygen electrode covered with a polyethylene membrane (of the Clark type) (*7*, *8*) fitted tightly into the Lucite tube so as to exclude passage of liquid between the tube and the electrode. By moving the electrode assembly the volume in the reservoir could be changed as desired. The exit and inlet tubes cemented to the Lucite tube enabled the reservoir to be flushed rapidly with a solution of known oxygen tension, so that we could make a calibration while the lens was on the subject's eye (*9*).

The oxygen consumption of the electrode was negligible, as shown by the decline in oxygen tension when the membrane-covered electrode was pressed against a soft surface impermeable to oxygen (a synthetic leather cushion).

Table 1. Rates of oxygen uptake by the corneas of different animals, recalculated on the assumption that all the cornea's oxygen requirements are met by the atmosphere.

Animal	$\mu l/cm^2$ per hour	Source of original data
Ox	7.6	de Roetth (*2*)
Rabbit	8.6	Langham (*3*)
Rabbit	4.6	Robbie (*4*)
Rat	24.9	Lee and Hart (*5*)
Rat	40	Bessey and Wolbach (*6*)

These data are shown as solid squares in Fig. 2. For these data, the "reservoir volume" refers to an equivalent volume of water that contains as much oxygen in solution as is in the polyethylene membrane covering the electrode.

The fit of the chamber and the lens to the eye was made exceedingly tight. The lens was just tolerable without anesthesia. Under the conditions of steady fixation, as were maintained while the measurements were made, any exchange which took place between tears and the solution in the chamber was negligible, as demonstrated by the rapid and maintained depletion of oxygen (when the volume of the reservoir was at its minimum) the moment the eye came to rest (see lowest part of Fig. 1).

Oxygen tensions were obtained from the electrode current as measured by a Model 160 Beckman physiological gas analyzer. Data were recorded directly on a Varian potentiometric recorder or a Sanborn medical recorder. Temperature variation in the chamber, and its influence on the electrode response, was checked carefully during control runs by means of the thermistors cemented into the chamber walls and projecting into the reservoir. Temperature drift in the reservoir while the equipment was in position on the subject's eye was found to be negligible.

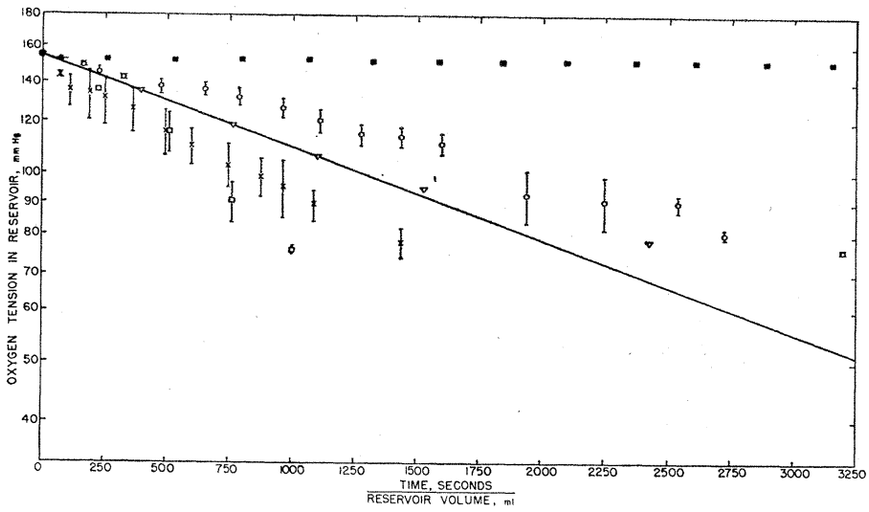

Fig. 2. Oxygen tension in the reservoir shown as a function of the parameter time divided by reservoir volume. Crosses are for 0.83 μl, reservoir volume, circles for 0.62 μl, open squares for 0.20 μl, using the electrode of Fig. 1 (top). Triangles are for a minimum clearance lens (Fig. 1, bottom) (see text). Vertical lines represent one standard deviation about the mean of several determinations at each reservoir volume. A horizontal line indicates only one determination. The solid line is the best estimate regression line. Solid squares show the response of the electrode assembly when pressed against a surface impermeable to oxygen.

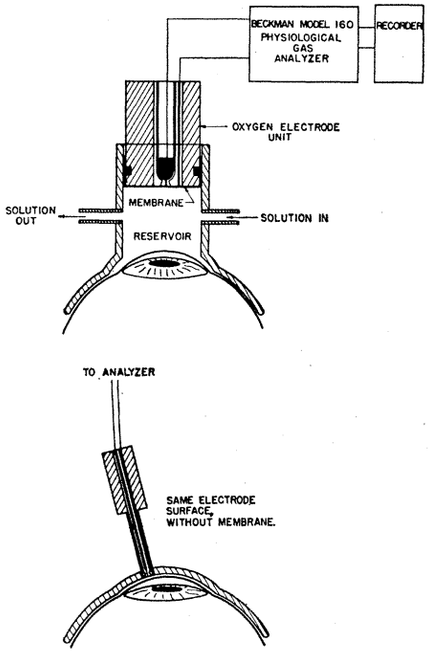

Fig. 1. Schematic diagram of the electrode and lens assemblies used to obtain data for calculating the rate of oxygen consumption by the cornea in vivo. (Top) The reservoir volume may be changed as desired by moving the electrode assembly. (Bottom) The volume of the reservoir is at its minimum—that is, the clearance is normal for a scleral contact lens; the electrode is used without a membrane in this bottom assembly.

The method of plotting used in Fig. 2 allows data for all reservoir volumes to fit the same time parameter scale. The data, which were obtained when the volume of the reservoir was at its minimum, show only that there is a semilogarithmic decline for this lens-electrode assembly. The reservoir volume could not be measured; therefore, a reservoir volume was chosen to give data points falling within the range obtained from lenses with a larger reservoir. The volume so obtained, 1.3 μl, is not unreasonable for this type of contact lens.

Consumption of oxygen by the cornea, under our experimental conditions, might have occurred in four different ways. (i) At the outer surface of the cornea, the rate being independent of oxygen concentration. This would have to lead to a linear fall of oxygen tension in the reservoir rather than to the semilogarithmic decline observed. (ii) At the outer corneal surface, the rate being proportional to oxygen concentration in the reservoir. Concentration in the reservoir would then be given by $C = C_0 \exp(-at/V)$, where a is the rate proportionality constant, t is the time, and V is the reservoir volume. (iii) Deep within the cornea, the consumption, therefore, being diffusion-controlled, but the rate of oxygen consumption by the cells being independent of oxygen concentration. Concentration in the reservoir would then be $C = C_0 \exp(-ADt/VL)$, where A and L are the area and length across which there is diffusion, and D is the diffusion coefficient of oxygen in the cornea. (iv) Deep within the cornea, the consumption, therefore, being diffusion controlled, but the rate of consumption by the cells being linearly dependent on the oxygen tension at the cells. Oxygen tension in the reservoir would then be given by

$$C = C_0 \exp -\left[\left(\frac{DA}{LV}\right) \Big/ \left(1 + \frac{DA}{La}\right)\right] t$$

Hypotheses (ii), (iii), and (iv) all lead to an exponential decrease of oxygen tension in the reservoir as a function of time divided by reservoir volume.

These four hypotheses do not include the possibility of movement of oxygen from the lens reservoir across the cornea to the anterior chamber. This oxygen flux is possible under certain conditions because the anterior chamber is reported to have an oxygen tension of 40 to 50 mm-Hg *(10)*. This flux is included in our experimental data and, therefore, we are reporting the

total oxygen flux inward across the cornea. That fraction of the oxygen flux resulting from the gradient in oxygen tension from the lens reservoir to the anterior chamber could account for 25 percent of the total observed. This would be the flux calculated from Fick's first law of diffusion for an inert cornea in which the oxygen diffusion coefficient and oxygen solubility are taken as equal to that in a 25-percent protein solution. The metabolic use of oxygen by the cornea will reduce the tendency of oxygen to move from the outer surface of the cornea to the anterior chamber. This movement of oxygen is probably much below the 25 percent of the observed flux as estimated herein for the limiting condition of an inert cornea. In view of the limited precision of our data we have chosen not to attempt a correction for this flux.

The best estimate for the initial rate (at an oxygen tension of 155 mm-Hg), taken from the data in Fig. 2, is 6.3 μl/hour. On a per unit area basis, taking 1.3 cm^2 as the area of the human cornea, this is 4.8 μl/cm^2 per hour. At an oxygen tension of 100 mm-Hg the rate is 3.1 μl/cm^2 per hour; at 50 mm-Hg it is 1.5 μl/cm^2 per hour. The extremes of the initial rate caused by the scatter of the data in Fig. 2 are 3.2 μl/cm^2 per hour and 7.2 μl/cm^2 per hour.

Table 1 shows representative oxygen uptake rates given in the literature for several animal corneas as measured by the Warburg respirometer. They have been recalculated here to give an oxygen flux across the cornea surface based on the assumption that all of the cornea's oxygen demand is met from the atmosphere. Our results for the human cornea compare favorably with those for rabbit and bovine corneas (*5, 7*).

At the rates we have observed it is evident that the cornea would, in the absence of tear circulation, very quickly exhaust the limited supply of oxygen, as for example, under a scleral contact lens having a minimum reservoir volume of only a few microliters. Without replenishment through tear circulation, the cornea would be forced to draw on other sources of oxygen or undergo loss of transparency through slowing of normal metabolic processes. Smelser (*11*) has reported loss of cornea transparency often as early as two hours after insertion of such contact lenses. We have confirmed, by means of our recordings of oxygen tension versus time with minimum-clearance lenses, what has long been known clinically, that movements of the eye, when allowed, can continuously replenish the oxygen supply under such a lens (*12*).

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17 July 1963

Heparin Bonding on Colloidal Graphite Surfaces

Abstract. *Experiments on clotting, both in vitro and in vivo, showed that a colloidal graphite surface, when rinsed with a cationic, surface-active agent, was capable of bonding heparin. The resistance of this graphite-heparin surface to the formation of clots was far greater than plastic or silicone surfaces in comparable studies.*

Artificial valves in animal hearts have not been very successful. More than 95 percent of the animals have succumbed within 1 month after valve replacement, and the overwhelming cause of failure has been the formation of thrombi on the valves, with subsequent disruption of function. Because of the severity of this problem, especially in the canine heart, long-term experimental evaluation of artificial valves for human use has been virtually impossible. Fortunately, thrombus formation on prosthetic valves placed in the human has not been as serious a problem, but it is still a very significant complicating factor and frequently necessitates prolonged administration of anticoagulants.

In an attempt to reduce clot formation on artificial valves, a number of plastic materials and coatings were previously evaluated in this laboratory for their relative abilities to resist the formation of clots (*1*). Of all the materials tested in this earlier study, a new type of intravascular coating substance, colloidal graphite, appeared to give the best results. It was thought that this property of graphite was related to several factors. First, as demonstrated by microscopic studies, the graphite coating provided an extremely smooth surface, filling in small defects on a polished plastic surface. Secondly, the inertness of the carbon in colloidal graphite was considered to be of importance in inhibiting clot formation. Additional properties of graphite, considered at that time to be of questionable importance in preventing thrombus formation, included nonwettability, good lubricity and conductivity, and a negative Zeta potential. More recent data from this laboratory suggest that the most important anticoagulant property of graphite is its apparent ability to bond heparin to its surface. This factor was active in the previous study but was not appreciated by us.

The coagulation of canine blood was tested in vitro and in vivo. Test tubes measuring 9 mm in diameter were used, 1 ml of blood being placed in each one. Glass, polycarbonate (Lexan), silicone-coated, and graphite-coated tubes (*2*) were used. Three tubes of each type were prepared for each study. The first was untreated, the second was filled with heparin and then thoroughly rinsed (*3*), and the third tube was filled with a cationic, surface-active agent (*4*), then heparin, and then thoroughly rinsed (*3*). The time taken for the blood to coagulate in each tube was noted, and some of the results are shown in Table 1.

The coagulation of blood in vivo