OXYGEN IN THE ANIMAL ORGANISM

FRANK DICKENS

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PREFACE

JOINTLY sponsored by the International Union of Biochemistry and the International Union of Physiological Sciences the Symposium on "*Oxygen in the Animal Organism*" was held at Bedford College, London, 1st–5th September 1963. In addition to financial support from the International Unions we received generous grants from the United States Office of Naval Research, from the Physiological Society, from the Royal Society and from The British Oxygen Company.

Professor W. F. Widdas of Bedford College joined us on the Organising Committee and we owe him our sincere thanks for acting as host. We are most grateful to the Bursar of Bedford College, Miss D. G. L. Wynn Cornish, for expediting the domestic and social arrangements made for the delegates. One hundred and seventy-seven delegates attended, of whom eighty-seven were in residence in College throughout the meeting.

The present book presents the official proceedings of the Symposium. The main papers were furnished as manuscripts by the speakers nominated. Discussions were recorded on tape, but contributors to the discussion were each invited to provide a typed précis of their remarks. All but a few did so, but the transmutation of a speech on tape into the printed version was necessarily done by us in some instances. In these few cases we have taken particular care to adhere closely to the substance of the statements made by the individuals concerned.

We wish to thank Dr. N. Joels, Dr. F. S. Nashat, Mrs. J. Dunn, Miss D. Munt and quite particularly Miss Grania Fetherstonhaugh, for their devoted efforts in tape translation, proof reading and other contributions to the organisation details.

Our thanks are due to Mr. R. E. Strange and his colleagues of Pergamon Press for piloting the book to its completion.

FRANK DICKENS
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July 1964

INTRODUCTION

WALLACE O. FENN

IT SEEMS to me that the subject of oxygen is not only one that requires a multidisciplinary approach, but is a subject which has somehow expanded greatly in scope since the dawn of the Space Age. Now we look upon ourselves from a cosmic point of view and wonder more than ever how we came to be here upon this particular planet and dependent for our very life on the oxygen which was released from water by plants. Somehow, the plants learned how to capture the energy radiated from the sun in order to accomplish this intricate feat. The hydrogen molecules corresponding to our O_2 have escaped from the gravitational field of the earth leaving us with an atmosphere with a remarkably constant concentration of oxygen. This concentration is stabilized by the fact that if animals use more oxygen, they produce more CO_2. This in turn stimulates the growth of plants which use up the extra CO_2 and produce more oxygen. So one of the questions which I hope may be discussed by some of the speakers is just this cosmic or at least planetary aspect of the oxygen problem. How constant and permanent is our supply of gaseous oxygen? Why is there so little oxygen on Mars? Was the Earth once like Mars before living organisms started the remarkable transformations which have occurred since life originated? Is there a possibility that Mars could be transformed into a green and luxurious planet like the Earth by the introduction of terrestrial plants, or will its smaller gravitational field allow water and oxygen to escape to space to such a degree that our kind of life would be impossible? Could O_2 be obtained from SiO_2? Half the weight of granite is due to oxygen and a liter of granite contains enough oxygen to last a man over 2 days. Or, if O_2 is impossible, what other sources of energy are available for some other life-like development?

I am sure that most of you have given much thought to the great philosophic question of man's origin on the Earth. Is it reasonable to suppose that a man with all his spiritual qualities, his relatively great intellect, his ability to think—that this *homo sapiens* could be just the natural evolutionary result of radiant energy acting upon the particular aggregations of protons, neutrons and electrons which were present on the primordial earth? If so, it seems most likely that it must have happened elsewhere also and that some day we shall make contact with other intelligent populations. Perhaps there are other particular aggregates of elementary particles in addition to the one we call oxygen which could conveniently store large quantities of radiant

energy and then release it as needed—some aggregates other than oxygen, carbon, hydrogen and nitrogen and a few others. Therefore, as we look at oxygen we should be thinking also of alternatives. The big effort which we are now making to explore space may not give us the whole truth about these great questions but it will at least give us another point of view on many such problems and may in time give us another planet to study. So, it seems to me that the Space Age has widened our viewpoint on the oxygen questions as indeed it has in many others also.

Quite properly, however, the title of this symposium has been limited to Oxygen in the Animal Organism. This is the subject which we know most about and it is the one which is of greatest importance to man. It is also not a narrow subject but has very many facets in both physiology and biochemistry. I am sure that we shall hear of many new facts and hypotheses during this meeting and the discussions may well bring out some good creative thinking. We cannot discuss oxygen in the animal organism, however, without including all that is known about the fundamental physics and chemistry of oxygen. We shall hear about this from Doctor Griffith and others. This may tell us why oxygen is the most abundant of all elements on the earth and, after the lighter elements, hydrogen and helium, the most abundant in the solar system. I am told that there is something particularly stable about the oxygen nucleus which accounts for its cosmic abundance. This is not due to the characteristics of its orbiting electrons but to the double "magic number" of 8 protons and 8 neutrons which make up the nucleus.

Our symposium will be opened by Dr. Roughton with a discussion of recent developments in the transport of oxygen. There is no greater authority on this subject. I first met Dr. Roughton just about 40 years ago when I visited the physiology laboratory in Cambridge. He was carrying a fresh supply of blood upstairs to his laboratory in order to measure the rate of reaction of oxygen with hemoglobin by the superb technique which he and Hartridge devised. The oxidized and reduced ingredients flowed rapidly down a tube as they reacted while spectroscopic analysis could be made at intervals along the tube. Dr. Roughton has been working on the O_2 transport problem ever since and has contributed enormously to the subject. He very kindly wrote a superb chapter on O_2 and CO_2 transport for the Respiration Section of the *Handbook of Physiology* which Hermann Rahn and I are editing for the American Physiological Society. Thus we shall introduce the symposium quite properly at the point where oxygen is introduced into the organism.

The oxygen supply of the newborn is a subject which has been very much in the public eye recently in the U.S.A. on account of the Kennedy baby who died of hyaline membrane disease. When the baby was put into high pressures of oxygen it was obvious that the case must really be desperate. Perhaps some of those attending this symposium who are concerned with infant respiratory physiology like Dr. Dawes or Dr. Gross and Dr. Mestyán will

have some suggestions for further study of this baffling problem. Perhaps it is the surfactant experts who will supply the answer, but it is not unlikely that the solution when discovered will be somehow related to the subject of this conference.

The transport of oxygen is of course only a means to an end and the final result is the maintenance of an adequate oxygen tension in the tissues and more particularly in the mitochondria. This is where the biochemist takes over from the somewhat bewildered physiologist. Fortunately, according to Dr. Chance, all the physiologist has to do is to deliver the oxygen to the mitochondria at a tension of 1 mm or more and the biochemist is quite satisfied. More than this will not affect the rate of electron transport. So there is a clean-cut dividing line between the two disciplines at 1 mm Hg of P_{O_2}. Before the physiologist leaves off, Dr. Forster will doubtless have an important part to play in discussing the diffusion of oxygen from the capillaries to the mitochondria and he may well outline for us the distribution of the partial pressure of oxygen through this difficult region. We shall hear more of the events of the respiratory chain tomorrow from Drs. Longmuir, Cater and others.

It has always seemed to me that the oxygen transport system could profitably be likened to the diffusion of gas through two convectional and two diffusion layers (Fenn, 1953). The first layer is convectional and represents the transport of O_2 through the airway by breathing movements. The second layer is solid and represents the alveolar membrane, through which O_2 must pass by diffusion. The third layer, representing the blood, is fluid and is circulated by a pump at a certain rate. The fourth layer represents the diffusion barrier of the tissues and it is solid like the second. Through every cross-section of such a system, the total flux of O_2 and CO_2 is the same. Each layer has its own resistance and the partial pressure difference across each layer is proportional to the resistance. Thus, we can apply Ohm's law to the flux of oxygen, the flow being always equal to the product of two quantities, the potential difference and the reciprocal resistance, conductivity or diffusing capacity. Of course the resistance across the two convectional layers, representing blood and airway, is a sort of virtual resistance which becomes zero when the circulation or breathing is infinitely rapid. With finite rates of blood flow, there is a considerable difference in the mean partial pressures of oxygen which exist in the pulmonary and the systemic capillaries. Indeed, this difference between some 95 mm in the lungs and perhaps a mean of 40 mm in the tissue capillaries represents a sort of reserve partial pressure which can be utilized for diffusion during time of high oxygen consumption by merely increasing the cardiac output. With this concept for steady state conditions, one can write a series of equations in each of which the rate of oxygen consumption or the current of oxygen is equated to the product of two factors, a gradient of partial pressure and a conductivity or diffusion

capacity or reciprocal resistance. Thus, the oxygen consumed is equal to the product of a diffusion capacity and a partial pressure gradient for both diffusion barriers in the lung and in the tissues. There is also the familiar Fick equation for the transport in the blood, and a similar equation can be written for the air flow in the lung, the reciprocal resistance being the alveolar ventilation, and the potential gradient being the difference between the ambient oxygen pressure and the P_{O_2} in the alveoli. In the steady state, a similar fifth equation can be written to take care of the heat loss in the skin, for this must be equal to the heat equivalent of the oxygen consumed. Here the potential is the temperature difference between the core temperature and the skin and the capacity factor or reciprocal resistance is the cutaneous blood flow. All these equations must be balanced if a high rate of oxygen consumption is to be maintained in a steady state. It is impressive to consider what an intricate problem it is for the mathematical wisdom of the body to see that all these equations are balanced at once. Moreover, this concept emphasizes the fact, not often stated explicitly, that the total partial pressure of oxygen in the ambient air must be divided between the series of resistances existing between the mitochondria and the air and that no part of this can really be used twice. The mean pressure in the pulmonary capillaries can never be the same as the mean pressure in the tissue capillaries unless the circulation rate is increased to infinity. The limiting factor in oxygen transport is usually partial pressure, not volume of oxygen.

One of these equations which must be balanced is the one which regulates the ventilation and this involves the whole program of the nervous and chemical control of respiration which was discussed so thoroughly at the Haldane Symposium two years ago in Oxford. Indeed, the most impressive fact about muscular exercise is the remarkably constancy of the ratio of alveolar ventilation and the rate of oxygen consumption. For this reason, it is an appropriate subject for discussion at this conference.

My own somewhat heretical view of this subject is that we have been led a bit astray by the hope that the ventilation rate could be explained quite simply by summating certain reflexes and certain chemical stimuli. In particular, the stimulating effect of CO_2 on the respiration has been exhaustively studied. When the increment in P_{CO_2} in muscular exercise proved to be an inadequate stimulus, we were encouraged to look for other stimuli to supplement this one. We looked earnestly for other chemical compounds which deviated from normal during exercise which could provide a continuing stimulus to the breathing. Instead, we found that CO_2, oxygen and pH all remained for the most part much too constant to serve in this capacity. It seems to me that we should instead have been looking for the quantities which remained constant in spite of the exercise, for these are the quantities which are being actively regulated.

Dr. Dejours (1963) has shown by his studies that there is a neural component to the regulation of ventilation as well as a humoral component. Hesser and Bjurstedt in Stockholm (personal communications) have nicely confirmed this by showing that the humoral factors continuously monitored in the arterial blood will account quantitatively for about half the observed ventilation but the neural component must account for the remainder. Kao (1963) has separated these two components by cross-circulation experiments. In his preparation the blood from the exercising legs of one so-called neural dog goes to the humoral dog and increases the ventilation. The neural dog also increases its ventilation as a result of afferent impulses from the legs. Studying the increases of ventilation quantitatively, he concludes that the neural component would probably have accounted for all the observed ventilation of an intact dog if the CO_2 had been available to fill up the lungs with CO_2 to the normal level. Actually, the neural dog increased his ventilation and produced a fall in alveolar P_{CO_2} which inhibited the response. Defares (personal communication) has interpreted this experiment as indicating that during exercise, the afferent impulses from the exercising muscles reset the control mechanism at a higher level so that the resulting ventilation will just take care of the amount of extra CO_2 which is, so to speak, expected from the perceived severity of the exercise. Anyway you look at it, however, there is a neural component and it must be translated with appropriate precision into an amount of ventilation which just meets the needs. This inevitably means that the respiratory center has to learn to interpret the afferent impulses in terms of liters per minute of ventilation. Control of ventilation becomes in the last analysis therefore a learned response. As such, it resembles to a degree any other sort of bodily behaviour and as such it is very difficult and perhaps impossible to quantitate by precise equations. This learned behaviour is of course supplemented or superimposed upon certain inherent, innate and more or less invariable responses to chemical stimuli like the response to CO_2. If, however, the ventilation is properly adjusted by the neural component, if the respiratory center guesses the demand with sufficient accuracy, there will be no deviation of the arterial P_{CO_2} from normal and there will be no need of a CO_2 or an oxygen stimulus. The CO_2 response therefore becomes a sort of emergency reaction and not a necessary or usual component of the exercise response. Perhaps this view is not fundamentally different from the usual view but it does emphasize, perhaps unhappily, the learned-behaviour aspect of the control mechanism. This is an unconscious learning by the vegetative or visceral control centers in the hypothalamus and elsewhere. Control of ventilation in exercise therefore becomes only a part of the whole neural pattern of muscular exercise. The primary control mechanism is neural, according to this hypothesis, but a certain increase in CO_2 tension is tolerated in order to avoid the excessive ventilatory effort required to keep it at a normal level.

Oxygen is of course a very toxic substance as well as one essential for life. There are many intricate biochemical defense mechanisms which have been built up to protect against it. Probably it is fair to say that we still do not know exactly what the mechanism of oxygen toxicity may be although it seems to me that the data point clearly to some destruction or inhibition of enzymes. Presumably this occurs through some reaction with free radicals because there seems to be no other way in which oxygen could act. Some of our biochemical friends will perhaps tell us just which enzymes are destroyed, by what reaction and will perhaps tell us whether the reaction is reversible and if so, how long it takes to reverse and how to predict the kinetics of the process. Also, they should explain how this can take place without any measurable increase in the consumption of oxygen. If there are some irreversible oxidation products produced in this way which might shorten life, that would also be a key point.

Information of this sort would help to support the theory of Dr. Gerschman (1954) that there is something in common between the mechanisms of radiation injury and oxygen poisoning, presumably by summating of free radicals. I always felt that the most direct evidence for her theory was the experiment in which mice in a high oxygen atmosphere were simultaneously radiated. Under such conditions they succumbed to oxygen more quickly—in 30 instead of 45 min for example. There are of course many studies of the effects of oxygen on radiation injury but this is the only experiment I can recall on the effects of radiation on acute oxygen poisoning. Just this summer we have been repeating this important experiment therefore on *Drosophila* with the aid of Dr. J. J. Thomas and Dr. R. E. Baxter. Dr. Gerschman will be pleased to know that the results are at least in part confirmatory of her experiment. It requires a very large dose of radiation—30,000–75,000 r given in one large dose. Moreover, the effect is almost exclusively observable in males with only a small effect in females. The males die in 20 min instead of 45 if exposed to radiation as well as 100 psi of O_2. A dose of 20,000 r seems to be ineffective and the effect is much diminished if the radiation is given during the hour preceeding the oxygen treatment rather than simultaneously. It should be noted that even this large radiation dose does not appear to have any immediate effect on the flies but it does shorten their life span about 30 per cent. Dr. Gerschman would say that the free radicals formed by radiation summed with those from the metabolism of oxygen to produce this effect. Certainly this seems to be the most obvious interpretation although it is probably not the only possible one. Even this effect is not necessarily specific. Perhaps we just overwhelm the flies with a massive non-specific stress when they are nearly dead anyway.

This leads me to say a few words about some experiments I have been conducting during the past year on the rate of recovery from exposures to high oxygen pressures. This seems to me to have been a somewhat neglected

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aspect of oxygen studies in the past. It is important because the higher the rate of recovery the less the toxicity of oxygen. It is also important in planning the best regime for inhaled gases during deep diving operations. Lambertsen (1955) has proposed alternating pure oxygen and nitrogen–oxygen mixtures for this purpose. His schedule was based on the assumption that the rate of recovery from oxygen poisoning is rapid. It is not very well known, however, how rapid it is or whether the process is logarithmic or linear with time.

For these experiments I have used *Drosophila* because fruit flies are cheap and easy to raise. These flies live as long in 10 per cent oxygen as they do in air but 37 per cent oxygen definitely shortens their life span nearly 50 per cent. Females live longer than males and the frequency distribution for both sexes is very much skewed toward the longer times. One atmosphere of oxygen affects particularly the flies that live longest and produces a much narrower and a very symmetrical distribution curve. The life span is diminished 10 per cent by as little as 6 hr exposure to pure oxygen every day. This shows that in 18 hr flies do not fully recover from the effects of a 6 hr exposure to 1 atm pure oxygen. If the flies are exposed 16 hr per day, the life span is shortened 15 per cent. In other words, recovery in 8 hr from 16 hr of oxygen is nearly as good as the recovery in 18 hr from 6 hr of O_2.

When exposed to 100 psi of oxygen for 40 min, few if any flies show any immediate symptoms but the toxic effects may last for several days and can be detected easily by challenging them again with 100 psi of O_2 and measuring the time until they collapse or have the equivalent of convulsions in mammals. In this way it can be shown that after 24 hr they have recovered from 50 per cent of the effects of the preliminary oxygen treatment. In some experiments of this sort, however, recovery by this criterion seems to be by no means complete, even in 4 days.

In other experiments flies have been exposed for 2 hr to 100 psi, one group by continuous exposure and the other group intermittently with four $\frac{1}{2}$-hr periods separated by recovery periods in air of a few minutes or several hours duration between successive oxygen exposure. Recovery is better with 1 hr of recovery than with only a few minutes, but after several hours, the recovery does not appear to be progressively better as one might expect. We have not yet been able to obtain therefore a nice logarithmic recovery curve to agree with predictions.

I might add, however, that the pressure vs. exposure-time curves for flies and for paramecia are nicely explained by the type of equation that Blair had used, first for electrical excitation of nerve and later for radiation effects. According to this equation, some substance X accumulates during oxygen exposure at a rate proportional to time and pressure and simultaneously decays at a rate proportional to its own concentration. At a certain threshold concentration of X the flies succumb and below a certain "rheobasic" con-