THE EFFECTS OF VARIOUS CONCENTRATIONS OF OXYGEN ON THE SPONTANEOUS MOTILITY OF THE CHICK EMBRYO

A Thesis

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by

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ABSTRACT

The first appearance of body movements in the chick embryo was seen around 3 1/2 days of development. It was thought to be non-reflexive motor activity, the result of self-generated discharges of the spinal motor neurons.

The possible influence of increased oxygen levels (42% and 84%) on early embryonic motility was studied. Fifteen minute observations were made of the chick embryo in vivo. The time spent in activity and inactivity was recorded. The mean duration of the activity periods and the mean duration of the inactivity periods, the per cent of overall activity per cycle (one activity period combined with the successive inactivity period) were the parameters considered. All observations were manually recorded by pressing the marker key of a Sanborn polygraph.

The younger embryos (days 4-6) moved towards an overall increase in activity during and after 1 hour exposures to 42% and 84% O_2.

The motility pattern of the older embryos (days 7-9) did not change during or following a 1 hour exposure to 42% O_2. However, a 1 hour exposure to 84% O_2 resulted in a significant decrease (95% level of significance) in activity after 40 minutes of exposure and 30 minutes after removal of the oxygen.

The functional appearance of the carotid body, in the 8-day embryos, might have resulted in a decrease in their spontaneous activity during exposure to 84% O_2.

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I. INTRODUCTION

This study was embarked upon to obtain a better understanding of the mechanisms that initiate and propagate embryonic motor activity. The ability of a neuron to discharge spontaneously and, as a consequence, to produce body movements has existed as a subject of doubt and controversy in the literature for many years. It was our aim to study the phenomenon of spontaneous activity by exposing early chick embryos to increased levels of oxygen.

In order to study the phenomenon of spontaneous activity, a system was required where the functional development of the motor system appeared before that of the sensory system.

Tracy (1926) studied the development of behavior in the toadfish (Opsanus tau), in which there was a time interval of 2 1/2 weeks between the onset of motility and the beginning of responsiveness to tactile stimuli. He showed that, during the earliest stages of motor activity, a bilateral motor pathway existed in the medulla and spinal cord of the toadfish, while the afferent system was mainly undifferentiated.

Tracy concluded that:

> Spontaneous movements are those which, under certain homogeneous environmental conditions, arise in a protoplasmic system when the external energy relations remain constant; they appear to be dependent on periodically recurring variations in internal energy conditions and hence tend to be rhythmic.

Coghill (1929), in his work on Ambystoma, laid down basic principles that he believed valid for vertebrate behavior in general. In his own words:

> The behavior pattern from the beginning expands throughout the growing normal animal as a perfectly integrated unit, whereas partial patterns...always remain under the supremacy of the individual as a whole.

Neither Coghill's idea of a total integrated pattern, nor Tracy's idea of areflexive motor activity was accepted; instead the opposite point of view, the theories of reflexology, bore fruit. These theories advocated behavior as originating in local reflexes that were integrated as development proceeded. Activity was the result of exogenous stimuli producing localized action that culminated eventually in an integrated pattern.

Weiss (1942) approached the stimulus-response theory with the idea that "central coordinating mechanisms originate by self-differentiation." He took a big step in postulating that a central discharge need not be the result of an afferent influx but can originate within the centres themselves.

Bullock (1961) has theorized that all activity has some spontaneous discharge associated with it. He said that "a primary patterned burst arises in a single cell." This primary burst was acted upon by a feedback mechanism, that is, sensory input, which modified it. The central timer or pacemaker was dependent on a certain steady state of conditions, such as temperature and the ionic and organic milieu of the animal.

Hamburger (1963), Hamburger and Balaban (1963), Hamburger et al. (1965), and Hamburger et al. (1966), provided evidence for the autonomous nature of the early functioning of the motor system. They demonstrated

the existence of rhythmical spontaneous motor activity in chick embryos whose sensory system was as yet non-functional.

Tracy (1926) and Hamburger (1963) have shown that early motor activity patterns were altered when they exposed developing embryos to different mixtures of carbon dioxide and oxygen. Hamburger (1964) reported that 100% O_2 would raise the activity of 8-day chick embryos above the normal line for that age. These results were supported by the idea of Bullock (1961) and others that a pacemaker existed within the central nervous system which caused neurons to fire spontaneously. This pacemaker depended on a certain steady state in the internal environment of the animal. We proposed the possibility of obtaining a deeper understanding of the mechanisms, that caused the motor action system to be turned on, by exposing early chick embryos to various oxygen concentrations. Were there cells in the developing central nervous system that were sensitive to different oxygen levels? Did these cells respond by affecting the turning on of the motor action system, and would they prolong the discharge once it had occurred?

II. REVIEW OF THE LITERATURE

During the development of the nervous system in the chick embryo, the primitive neural tube becomes segregated into two pairs of longitudinal strips called the alar plates and the basal plates. By 3 1/2 days of incubation, axons of the neuroblasts in the basal plate grow out as the ventral roots and make contact with the skeletal musculature. At this stage, the genesis of movement is seen. Axons from the neuroblasts of the neural crest grow into the alar plate to complete the formation of the sensory system. It does not become functional until 7 1/2 - 8 days of incubation.

When movements first occur in the chick embryo, there appears to be no integration of individual motor units. The association and commissural elements are unconnected with the motor neuroblasts. Early ventral and lateral twitches of the head and trunk are possibly the result of spontaneous discharges of the motor neuroblasts (Windle and Orr, 1934).

Many authors have referred to the very apparent spontaneous movements observed during the development of the chick embryo, but they have failed to explain them satisfactorily.

Viktor Hamburger, in the late fifties, initiated a series of experiments in an attempt to show a correlation between central discharges and the ontogeny of embryonic behavior. He coined and defined the term "spontaneous motility" as non-reflexive motor activity (Hamburger, 1963). He noted, furthermore, that spontaneous motility was rhythmical or cyclic in nature. The rhythm was characterized by activity and inactivity periods. The duration of these periods changed with an

increase in the age of the embryo.

Hamburger, Balaban, Oppenheim, and Wenger (1965) gave a detailed account of the activity and inactivity patterns of normal chick embryos. From day 3 1/2 of incubation, there was a steady rise in the mean duration of activity periods, from 3 seconds to a peak of 80 seconds at day 13. Concurrently, the inactivity periods decreased from a mean of 15 seconds at day 3 1/2, to a mean of 50 seconds at day 13.

Hamburger and Balaban (1963) removed parts of the cervical, brachial, or thoracic segments of the spinal cord of the 7-8 day chick embryo. They found the activity and inactivity periods of the isolated parts to correspond very closely to those of the normal embryos. Furthermore, the phases of the isolated parts were more regular than the normals, and were characterized by a higher percentage of longer activity and inactivity periods. They concluded that the brain contributed 10 to 20% to the overall activity of the embryo without altering the pattern. It emitted shorter phases that were superimposed on the longer cycles intrinsic in the cord. This appeared to support the theory that the motility of the chick embryo was a result of the self-generated discharges of the spinal motor neurons.

The sensory system of the chick is not complete until 7 1/2 to 8 days of incubation. Hamburger, Wenger, and Oppenheim (1966) completely removed the sensory system and found that the overall activity of the experimental animals approached or matched their controls. This indicated the autonomous nature of the motility of the chick embryo in the absence of sensory input.

The experiments of Hamburger and his associates appeared to substantiate a definite rhythmicity to the behavior pattern of the chick embryo. In addition, they hinted at a possible cause and effect relationship between spontaneous discharges of the anterior horn cells and motility.

Another convincing piece of evidence was given by Campbell (1940). He cut gaps in the spinal cord of the hagfish and showed that nearly all isolated segments possessed the ability to initiate waves in a cephalad and caudad direction. In the discussion of his results, Campbell spoke of observed spontaneous motility in activity and inactivity cycles, although he did not classify them as Hamburger has done.

Isolated Tissue Recordings

An area of study that is essential to understand the nature of spontaneous discharges is direct recordings from isolated tissue in culture.

Cunningham (1961a) cultured explants from the pontine flexure of the myelencephalon at the level of the middle cerebellar peduncles, and from the fifth, sixth and seventh cranial nerve nuclei of the 11-day chick embryo. He demonstrated the existence of spontaneous potentials from these explants.

After 5 hours in culture, a complex combination of relatively simple signals against a background of a repetitive signal was seen. The simple signals were 5 microvolts in magnitude and were separated by intervals of 1 1/2 seconds. The larger potentials, about 15 micro-

volts, were superimposed on the lesser signals. They were at intervals of 10 seconds with the individual members separated by 2 seconds.

Cunningham and Rylander (1961) cultured 10-day chick embryo cerebellum. They noted that the spontaneous potentials emitted from these explants occurred as a regular series of longer and smaller potentials, separated at first by a 5 second interval. The interval gradually increased to a mean of 10.1 as the culture proceeded.

Cunningham (1961b) demonstrated the existence of spontaneous potentials from the telencephalon of the 11-day chick embryo. The potentials consisted of amplitudes between 20 and 30 microvolts, separated by 16 1/2 intervals, and amplitudes between 10 and 15 microvolts lasting 7 seconds, each with a 2 minute interval.

Finally, Crain and Peterson (1963) cultured human, rat, and chick spinal cord in long term cultures. In one human preparation they observed spontaneous potentials.

In the light of the evidence just presented, we must return to the statement of Hamburger and Balaban (1963) that the brain contributed 10 to 20% to the overall activity of the chick embryo. It emitted shorter waves that were superimposed on the larger waves intrinsic in the cord. Cunningham has clearly shown the existence of rhythmical spontaneous discharges from the 10 and 11-day chick cerebrum, cerebellum, and medulla oblongata.

Myogenic or Neurogenic

There exists the possibility that early motility could result from the spontaneous discharges of the primitive myoblasts, or a combination of discharges from both neuron and myoblast.

Alconero (1965), working with chick embryos, transplanted 6 somites with the spinal cord and 6 somites without the spinal cord on to the chorioallantoic membrane of the host embryos. She found, without exception, that only somites transplanted with neural tissue contracted spontaneously. From the 33 grafts made, 20 showed spontaneous contractions. Histological investigation of the other 13 showed a lack of sufficient muscle tissue or a lack of sufficient innervated muscle tissue. The 20 grafts that exhibited spontaneous contractions were the most thoroughly innervated. The muscle tissue of both types of grafts appeared normal. Finally, the rhythm of the contractions of the innervated grafts resembled very closely the cycles noted by Hamburger, et al. (1965) for the chick embryo in vivo.

Corner and Crain (1965) explanted early frog neural tissue with presumptive axial muscle. They also cultured the chord mesoderm without any neural attachments. Only those explants which contained neural tissue exhibited spontaneous twitching. The burst activity was characterized by its rhythmical nature. Their data indicated that the activity of the muscle in their explants had been initiated by endogenous rhythmic neural activity.

After sectioning Anuran embryos at the level of the hindbrain and cervical and thoracic regions of the spinal cord, Corner (1964) showed that the pattern of spontaneous activity was being generated independently

in all three regions.

In another group of embryos with the upper cord segments removed during neurogenesis, once again, spontaneous activity occurred independently in the hindbrain and spinal cord.

Corner concluded that there was no myogenic phase of motor development. He felt that the earliest movements were neurogenic in origin, that is, the result of spontaneous neural activity which was rhythmical and continuous throughout development.

Oxygen and Carbon Dioxide Experiments

Tracy (1926) reported that the spontaneous body movements exhibited by Opsanus larvae were altered during varying concentrations of carbon dioxide in the environment. During increased carbon dioxide levels, spontaneous activity was initially increased followed by a depression; withdrawal of carbon dioxide was followed by a decrease of movements below the normal. Tracy postulated that the conditions influencing spontaneous motility were regulated by the concentration of acid metabolites brought to the nerve centers. Local variations in the movement of tissue fluid around the centers, different distances of cells from the capillaries, and variations in the volume of blood flow were other contributing factors.

This evidence appeared to support the concept that changes in gas levels of the tissue fluids might alter the metabolites in the fluid and thus affect the metabolism of cells in different nerve centers and subsequently alter the behavior of these cells.

On exposure of 11-day chick embryos to 10% CO_2 and 90% O_2, Hamburger (1963) observed a sharp decline in their spontaneous activity. He studied the recovery process with special consideration to the frequency of activity cycles and the per cent of overall activity per unit time (15 min.). The results indicated that the carbon dioxide treatment had no affect on the turning-on mechanism of the motor action system, because the number of activity cycles per unit time remained the same (i.e., similar to normal embryos), despite a decline in the percentage of overall activity. After the gas mixture was removed, the percentage of overall activity increased. This indicated that the carbon dioxide had a reversible effect on the turning-off mechanism of the motor action system.

In attempting to explain the nature of the ontogeny and periodicity of spontaneous motility, Hamburger (1964) proposed two theories. A timing device might be built into the motor system which could be modified by the internal milieu. Alternately, the motor system was discharging continuously, but was interrupted periodically by changes in carbon dioxide or oxygen concentrations of the body fluids, changes in pH or other factors in the internal environment. To test these possibilities, he exposed 8-day chick embryos to pure oxygen and 95:5 and 90:10 mixtures of oxygen and carbon dioxide.

The embryos exposed to pure oxygen showed a marked increase in movement, but not to the point of continuous activity. Hamburger stated "that each developmental stage has a maximal capacity for activity beyond which it cannot be activated by oxygen". He did not support this

statement with any quantitative experimental results.

Embryos exposed to a 95:5 mixture of oxygen and carbon dioxide did not show any change in periodicity of movement. Exposure of embryos to a 90:10 mixture of oxygen and carbon dioxide was followed by a drop in motility to a level 50% below the normal. If these embryos were exposed to pure oxygen, recovery took place within two minutes and activity increased above the normal line. Similar results were obtained in 7 1/2 - 9 day embryos.

III. MATERIALS AND METHODS

Materials

The chick embryos used in this study were a hybrid of White Leghorn, Shaver's 228. They were obtained from the Animal Science Department of the University of Manitoba.

The eggs were allowed to develop in a dry type bacteriological incubator at a temperature of 37°C and 70% relative humidity. During primary incubation, the eggs were turned many times a day. This facilitated normal development by preventing the extraembryonic membranes from sticking to the inner shell membrane.

Upon reaching the required age, the eggs were removed from the incubator and candled to determine the position of the embryo. A small hole was made in the air space (blunt end of the egg) and another one above the embryo. The air space was shifted to lie above the embryo by removal of air from the blunt end of the egg with a small eye dropper. Then a window was carefully made above the embryo with a fine dissecting needle and forceps (Fig. 1).

Any embryo that exhibited hemorrhagic sites in the vitelline circulation or allantoic circulation was discarded. If the heart beat was arrhythmical, the embryo was not used. Finally, if the amnionic fluid appeared milky, indicating a possible puncture of the yolk sac or a bacterial infection, it was also discarded.

Prior to recording, each embryo was staged according to Hamburger and Hamilton (1951).

Finally, the embryo was placed in an observation chamber, on a cotton ring in a shallow petri dish, located on a shelf three inches from the lid (Fig. 1).

Fig. 1A. Experimental technique for studying the chick embryo in vivo.

a. Egg is candled to determine position of the embryo.

b. Air is displaced by puncturing shell in the air space and above the embryo.

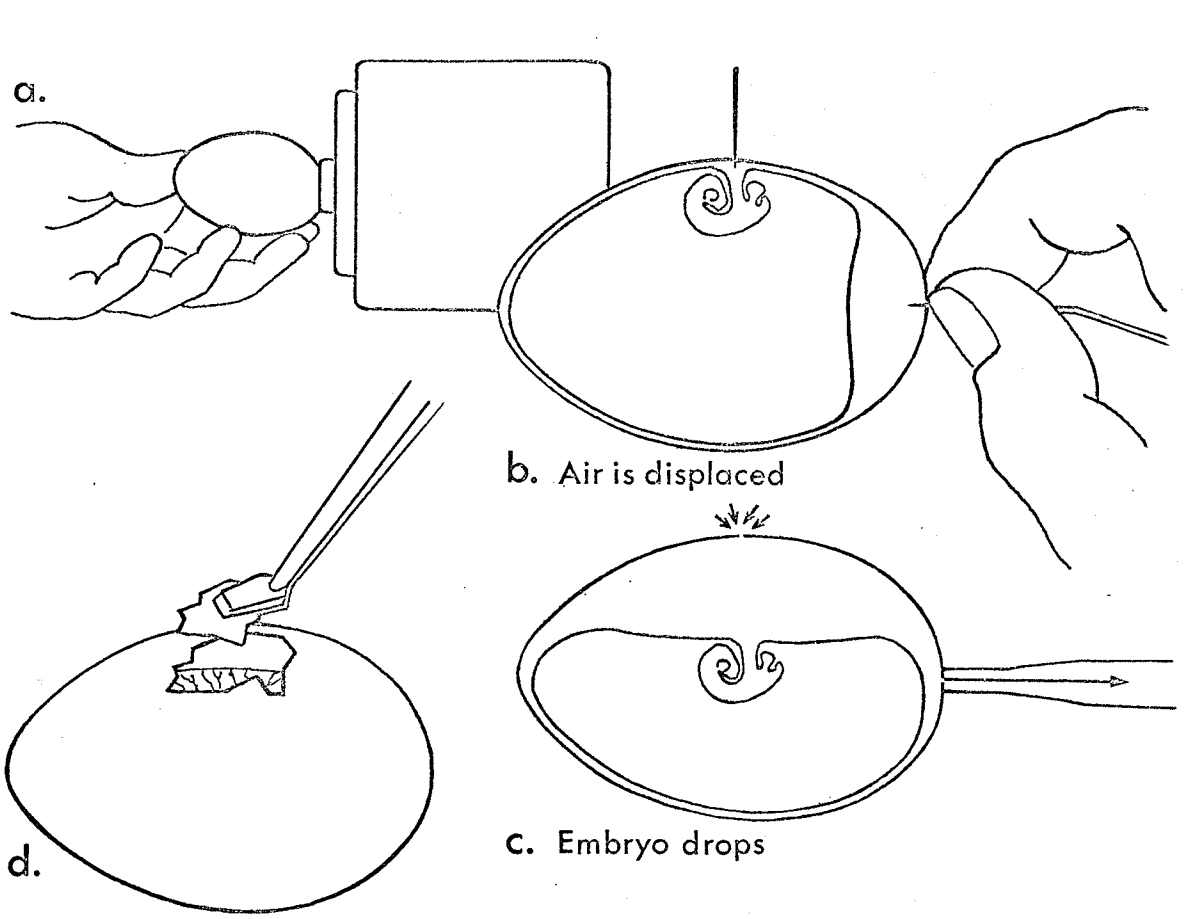
c. Embryo drops as air is removed from the air space with an eye dropper.

d. Shell is peeled away to make a window for observation.

e. Embryo is placed in the observation chamber.

View of the observation chamber is from above with the lid removed. Shaded area is the heating unit.

1A


a.
b. Air is displaced
c. Embryo drops
d.

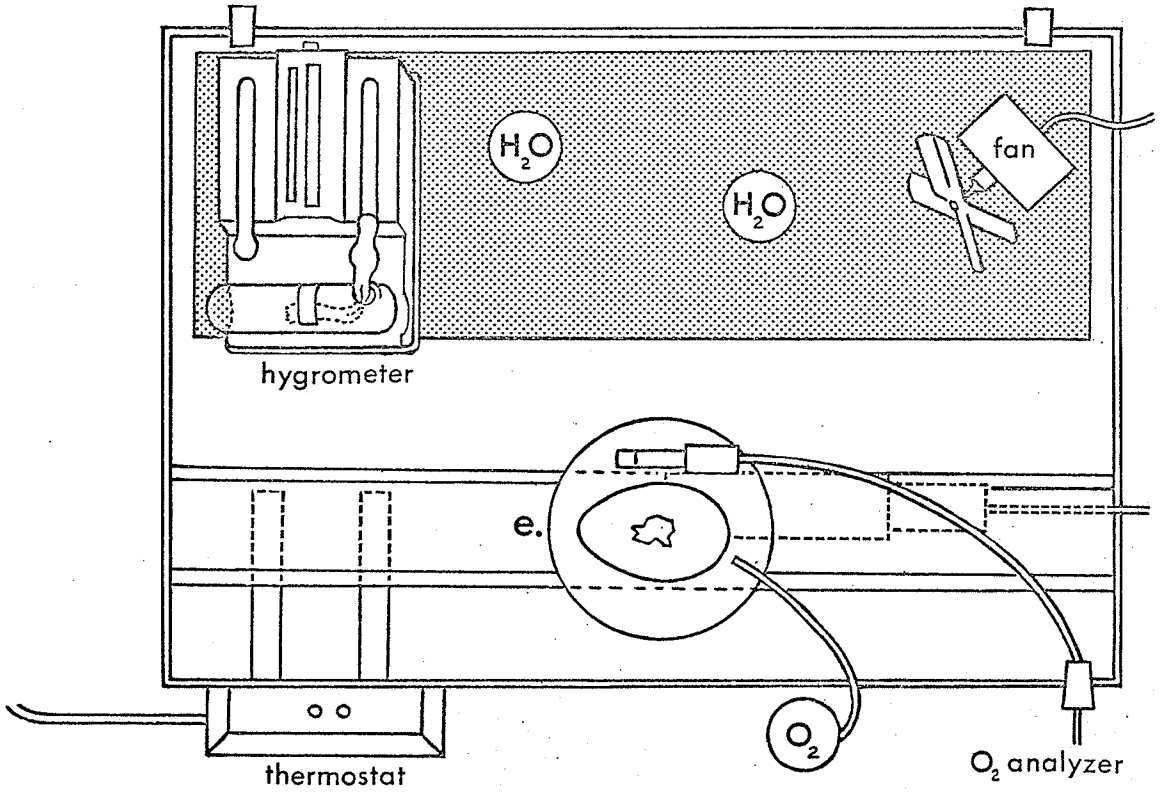
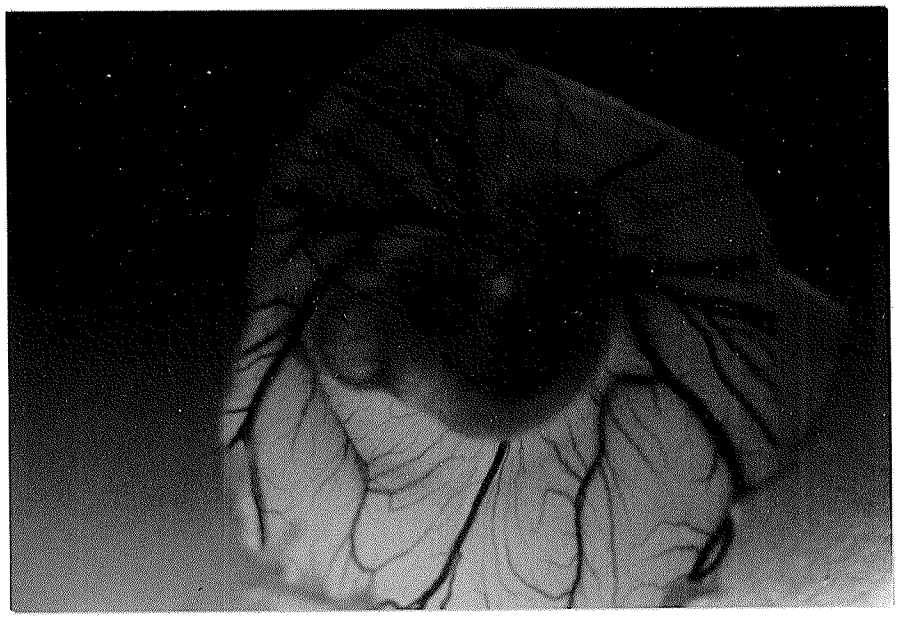

H_2O
H_2O
fan
hygrometer
e.
O_2
thermostat
O_2 analyzer

Fig. 1B. The chick embryo in vivo.



1B

The chamber measured 48 x 31.5 x 20 centimeters and was made entirely of durable plastic. The heating unit, located at the rear of the chamber, consisted of a metal box housing two light bulbs. This was connected to a dual thermostatic system which was set at a temperature range of 37-39°C. Humidity was maintained at the 70% level by placing two ounce jars, containing distilled water, on the top of the heating unit. A light on the floor of the chamber served to transilluminate the embryo. A small beaker of distilled water was placed between the egg and the light source to prevent heating of the embryo. Vents, located on the sides of the chamber, allowed for an exchange of gases as the oxygen was put in. A propeller fan was placed on the top of the heating unit and could be turned on from outside the chamber. When the oxygen was put into the chamber, the fan was turned on to aid in maximum displacement throughout the interior. The oxygen was removed by turning the fan on and opening the side vents (Figs. 2, 3, and 4).

Oxygen

Therapy oxygen, B.P. 99.5% pure, was introduced into the chamber through a humidifier. The dry, cold oxygen leaving the storage tank was humidified and warmed to room temperature as it passed through the wash bottle (Fig. 3).

The oxygen concentration in the ambient environment of the embryo, that is, in the plastic incubator, was determined on a Beckman Oxygen Analyzer (Fig. 3).

Fig. 2. A diagrammatic representation of the plastic observation chamber.

Front view.

Shaded area is the heating unit.

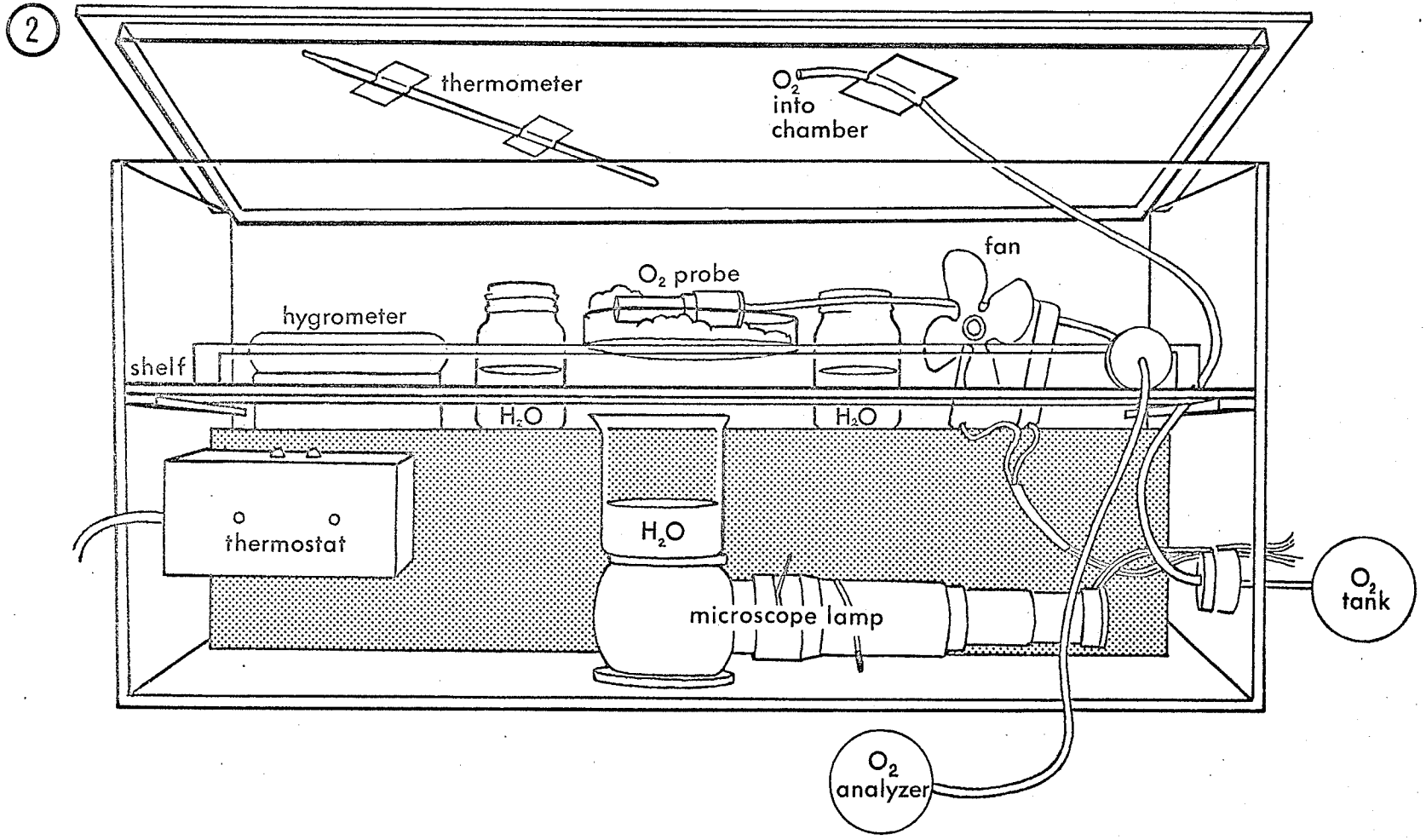
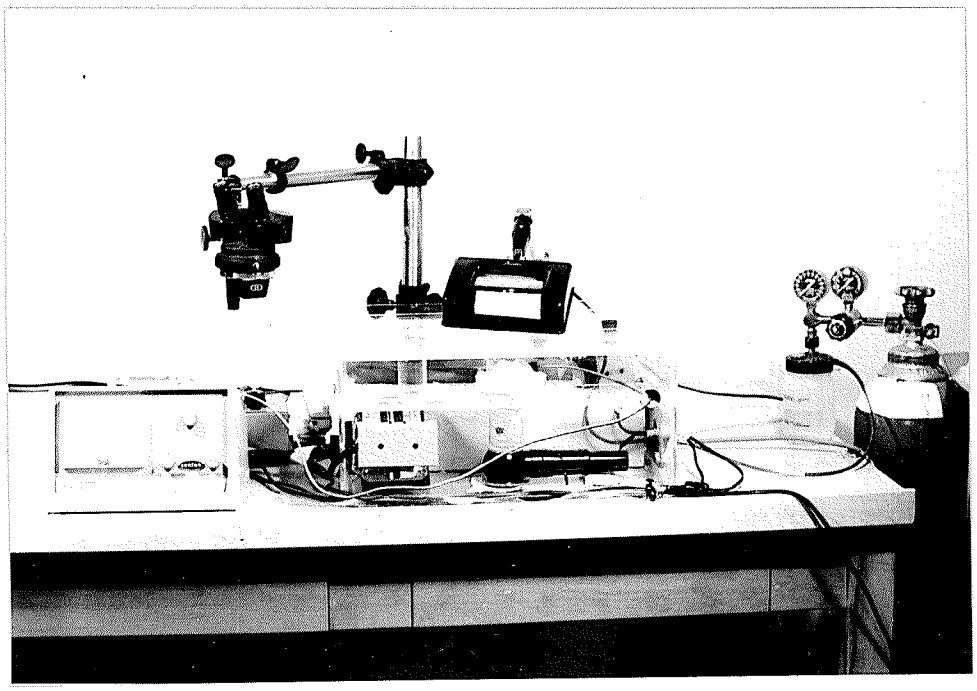
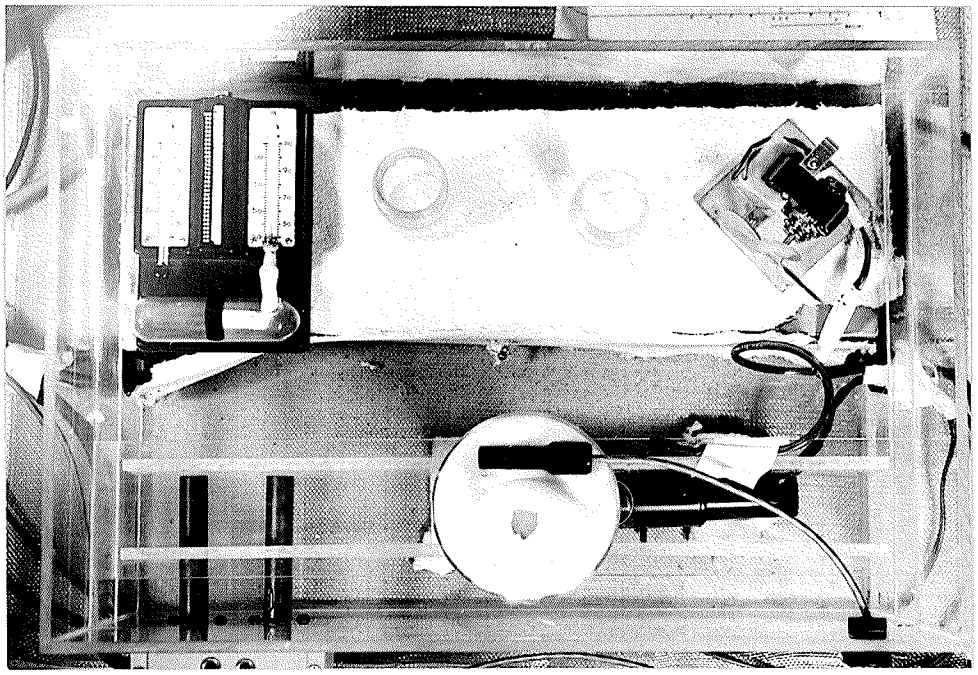

2
thermometer
O_2 into chamber
fan
O_2 probe
hygrometer
shelf
H_2O
H_2O
thermostat
H_2O
microscope lamp
O_2 tank
O_2 analyzer

Fig. 3. The experimental set-up.

Fig. 4. A top view of the observation chamber.
Lid removed.



3



4

Samples of the pO_2 in the amnionic fluid of the embryo were taken on a portable polarographic analyzer. It was possible to obtain amnionic fluid without contaminating the sample with the ambient environment. The readings indicated a definite increase in the pO_2 of the amnionic fluid after ten minutes of exposure to the gas.

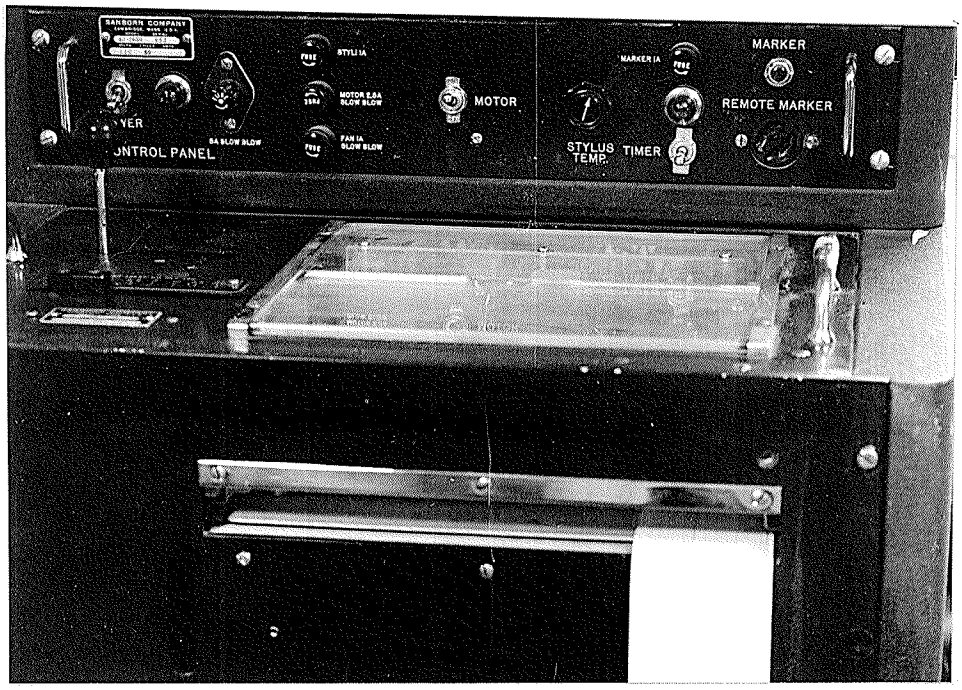
Observations

All observations were visual. A dissecting microscope (used for the younger embryos) and a magnifying lamp were suspended freely over the top of the chamber. The experimenter was seated so that he could observe the embryo through the top of the chamber and simultaneously press the marker key of a Sanborn polygraph (Fig. 5). The paper speed of the recorder was set at 1 mm/second. Every active movement of the embryo was recorded on the paper by pressing the marker key. Figure 6 is a sample strip of a 15 minute observation period. The groups of 'pips' represent activity periods and the intervals, inactivity periods. Determination of the length of the periods was made with a ruler.

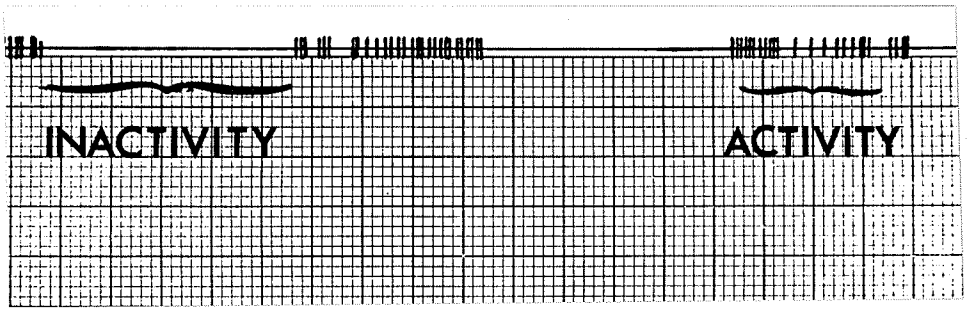
The observations were made by three observers in an attempt to increase the objectivity of the results. Only active movements, that is, activity that did not result from amnionic contractions, were recorded. Oppenheim (1966) studied the motility of chick embryos with the amnion intact and with it removed. He showed that the passive swinging of the embryo caused by amnionic contractions did not alter the pattern of spontaneous activity.

Fig. 5. The control panel of a Sanborn polygraph.

Fig. 6. A sample recording strip.


SANBORN COMPANY
MOTOR
MARKER
REMOTE MARKER
STYLUS TEMP.
TIMER

5


INACTIVITY
ACTIVITY

6

Experimental Procedure

The pattern of spontaneous activity of chick embryos, days 4-6 and days 7-9, was studied by noting the time spent in activity and inactivity during 15 minute observation periods.

The spontaneous activity of the embryo was not continuous. The interruptions varied in duration. Hamburger et al. (1965) allowed a 1 to 9 second rest period during an activity period. Thus, an inactivity period was defined as lasting 10 seconds or more. Although this lower limit of an inactivity period was arbitrary, we decided to retain it. It is important to point out that, if Hamburger's limit had been changed, or done away with entirely, the ratio of the duration of the activity to the inactivity periods would be altered, but the general trends would remain the same.

Our study consisted of two parts. In both, the embryos were exposed, placed in the observation chamber, and allowed to acclimatize for 40 minutes. This was followed by the first 15 minute recording to establish a normal base of activity for each animal.

In the preliminary experiments, each embryo was exposed to the required oxygen concentration (42%, 63%, 84%, 100%) for 4 minutes. The oxygen was removed and a second reading was taken 4 minutes after return to normal environmental conditions. This was followed by two more readings, one 30 minutes and one 60 minutes after the oxygen had been let out (Table I).

In the major study, the embryos were exposed to the required oxygen concentrations (42%, 84%) for one hour. During the exposure period, two recordings were made, one at the 10 minute mark and one at the 40 minute mark. Once again, the oxygen was removed and three more readings were taken, one 4 minutes, one 30 minutes, and one 60 minutes from the time of removal (Table I).

Control series were run for each age level, which paralleled the time sequence of the oxygen treated groups, but without exposure to oxygen (Table I).

TABLE I

TIME SEQUENCE OF EXPERIMENTS

The Preliminary Study

R1 - control recording.
four minute exposure to oxygen (42%, 63%, 84%, or 100%).
R2 - recording 4 minutes after the oxygen has been removed.
R3 - recording 30 minutes after the oxygen has been removed.
R4 - recording 60 minutes after the oxygen has been removed.

The Major Study

R1 - control recording.
one hour exposure to oxygen (42% or 84%).
R2 - recording 10 minutes after exposure to oxygen.
R3 - recording 40 minutes after exposure to oxygen.
R4 - recording 4 minutes after the oxygen has been removed.
R5 - recording 30 minutes after the oxygen has been removed.
R6 - recording 60 minutes after the oxygen has been removed.

The Control Series

R1 - control recording.
R2 - corresponds with recording taken 10 minutes after exposure to oxygen.
R3 - corresponds with recording taken 40 minutes after exposure to oxygen.
R4 - corresponds with recording taken 4 minutes after the oxygen has been removed.
R5 - corresponds with recording taken 30 minutes after the oxygen has been removed.
R6 - corresponds with recording taken 60 minutes after the oxygen has been removed.

Analysis and Statistical Treatment

Changes in the activity patterns of chick embryos exposed to various oxygen concentrations were evaluated by considering several parameters.

The values obtained from the recording strips represented the raw data, that is, the durations of the activity and inactivity phases. The total time which the embryo spent in activity during a 15 minute recording period was measured and expressed as the per cent of overall activity. The per cent of activity per cycle was also measured. A cycle represented one activity period combined with the successive inactivity period. Finally, the number of activity and inactivity periods in a 15 minute recording were tabulated as the frequency.

In the preliminary study, 5 animals for each of the oxygen levels (42%, 63%, 84%, 100%) were used. Four readings were taken that followed the activity of the embryo for approximately 1 1/2 hours. The major study involved 10 embryos for each oxygen concentration (42%, 84%). Each experiment consisted of 6 readings that were spread over 3 hours.

The first recording of each experiment established a base line of activity for the embryo and represented the control reading. This reading was compared to each successive one, that is, those taken during and after treatment with oxygen.

All tests of significance were based on the Wilcoxon matched-pairs signed-ranks test (two-tailed). A non-parametric analysis was chosen because our sample standard deviations indicated that the population which we were considering showed a wide distribution, that is,

the standard deviations were large. The signed-ranks test prevented us from placing undesirable parameters on our sample.

IV. RESULTS

Motility was seen to begin in the chick embryo around 3 1/2 days of incubation. It started as a slight bending of the head and neck region. As development proceeded, the trunk and limbs participated in a S-wave undulation of the whole body. These movements extended both in a cephalad and caudad direction. Cycles of motility consisted of activity and inactivity periods. An activity period in the younger embryos (days 4-6) consisted primarily of S-flexures of the body, or just head and neck movements from side to side.

Individual movements of the limb primordia were seen around 6 1/2 - 7 days of incubation. In the older embryos (days 7-9) slight twitches of the lower limbs and/or tail bud signalled an activity period. Within 1-5 seconds, generalized activity of all body parts capable of movement at this age took place.

Beginning around day 4 of incubation, contractions of the smooth muscle cells in the walls of the amnion caused the embryo to swing back and forth. This passive motility continued with increasing frequency and severity until day 13 of incubation (Hamburger, 1966). However, it was shown by Oppenheim (1966) to have no effect on the spontaneous activity of the developing chick.

The Control Series

A series of observations were performed that paralleled the time sequence of the oxygen experiments but without exposure of the animals to the gas. It was our aim to compare these control results with those of our experimental groups.

Inadvertently, we discovered a phenomenon that was at first inexplainable. The control groups were studied in February and the treated ones in April. Working with rigidly consistent conditions, we did not forsee any possible difference arising between the groups that could be related to the time of year the experiments were carried out. However, the February embryos showed a marked overall depression of activity. This finding may be compatable with the work of Johnson (1966), who showed a significant seasonal variation in the metabolic rate of chick embryos. He has shown that the metabolic rate (i.e., total daily oxygen consumption) was highest in the spring and lowest in the fall, with winter falling in the middle. This might explain why an energy-requiring process, that is, neuromuscular integration, expressed as spontaneous bodily movements, would lag behind in the winter months.

As the control series consisted of "slow movers", they were incomparable statistically with our experimental groups. However, they did exhibit trends that were important in a qualitative comparison of the two groups.

Embryos, days 4-6 of incubation, did not demonstrate any significant change in their motility patterns over a 3 hour period. (Table III) The older embryos (days 7-9) did show a general trend towards increased activity. (Table IV) This was expressed as a significant increase in the mean duration of activity periods at the time, in the control study, corresponding to 4 minutes out of oxygen in the experimental study. The importance of this trend will be discussed with the results of the major study.

The Preliminary Study

The preliminary study was based on the conclusions of Hamburger (1964) that 8-day embryos, exposed for 4 minutes to 100% O_2 every 1/2 hour, respond by a marked increase in activity. Embryos, exposed to a 95:5 mixture of oxygen and carbon dioxide, showed a depression of overall activity. On subsequent treatment with 100% O_2, the embryo recovered within 2 minutes and the activity increased above the normal line.

In our experiments, chick embryos, days 4-6 and days 7-9 of incubation, were exposed to raised oxygen levels (42%, 63%, 84%, 100%) for 4 minutes.

Our early results did not correspond to those of Hamburger (1964). However, to justify our criticism of his work, the following changes in our experimental design (which, initially, was similar to Hamburger's) were necessary.

1. The exposure time was raised from 4 minutes to 1 hour with two readings taken during the exposure period, one after 10 minutes, and one after 40 minutes. It was possible that the response the animal was making could have occurred during the actual exposure period. Moreover, 4 minutes may have been insufficient time to allow for diffusion of the oxygen molecules into the blood stream of the embryo.

2. As an indication of the concentration of oxygen in the immediate vicinity of the embryo, the pO_2 of the amnionic fluid was determined.

3. Ten animals per oxygen concentration were used in order to employ a 95% confidence level (p=0.05) with the Wilcoxon signed-ranks test for matched-pairs.

The Major Study

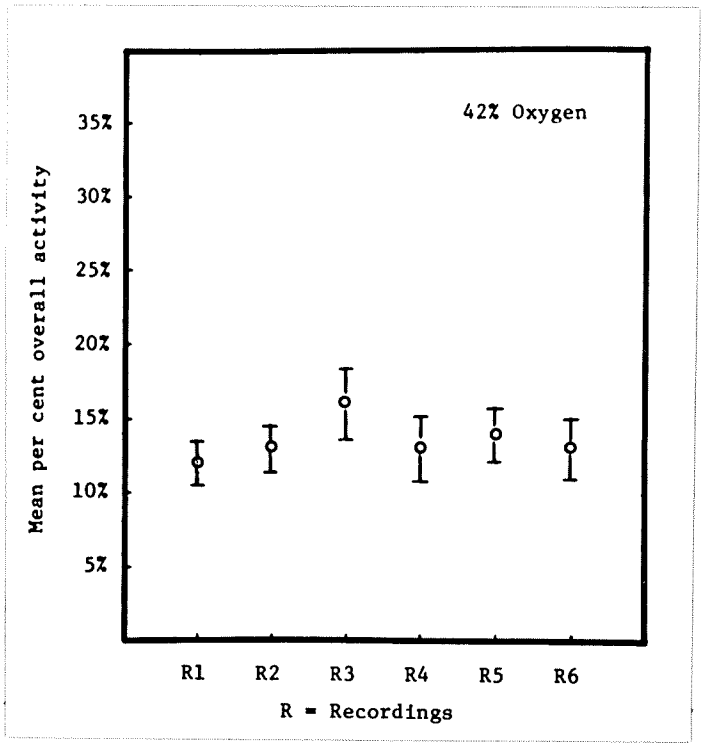
Changes in the periodic motility of chick embryos over a three hour period, during one hour of which they were exposed to 42% or 84% oxygen, were studied. Ten animals at each oxygen level were used. Embryos at days 4-6 and days 7-9 of incubation were used.

In 4-6 day embryos exposed to 42% oxygen for 1 hour, there was a significant increase in the mean per cent of overall activity after 40 minutes of exposure (Fig. 7)*. This was accompanied by a significant decrease in the mean duration of inactivity periods in readings taken at the 40 mark of exposure, and 4 minutes, 30 minutes, and 60 minutes following the removal of oxygen (Fig. 8)*. The mean duration of the activity periods for this same time sequence did, however, remain within the limits of normal variation (Fig. 9)*. Although not significant statistically, these embryos demonstrated an increase in the number of activity cycles in a 15 minute recording period. This was seen in all the six readings, but especially after 40 minutes in oxygen and 60 minutes after the oxygen was removed (Fig. 10).

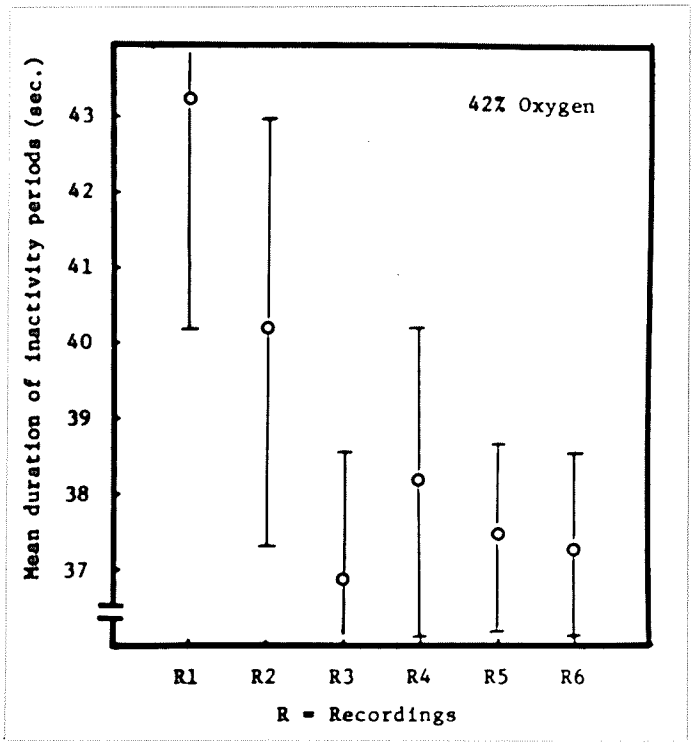
* Table V

Fig. 7. Mean per cent of overall activity of chick embryos, days 4-6, during and after a one hour exposure to 42% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.

Fig. 8. Mean duration of inactivity periods, in seconds, of chick embryos, days 4-6, during and after a one hour exposure to 42% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.


42% Oxygen
Mean per cent overall activity
35%
30%
25%
20%
15%
10%
5%
R1
R2
R3
R4
R5
R6
R = Recordings

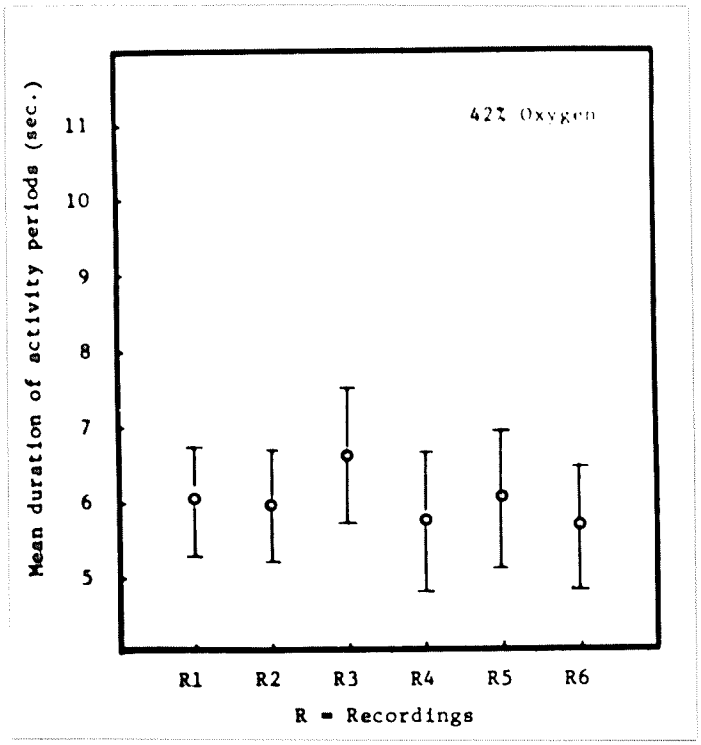
7


42% Oxygen
Mean duration of inactivity periods (sec.)
43
42
41
40
39
38
37
R1
R2
R3
R4
R5
R6
R = Recordings

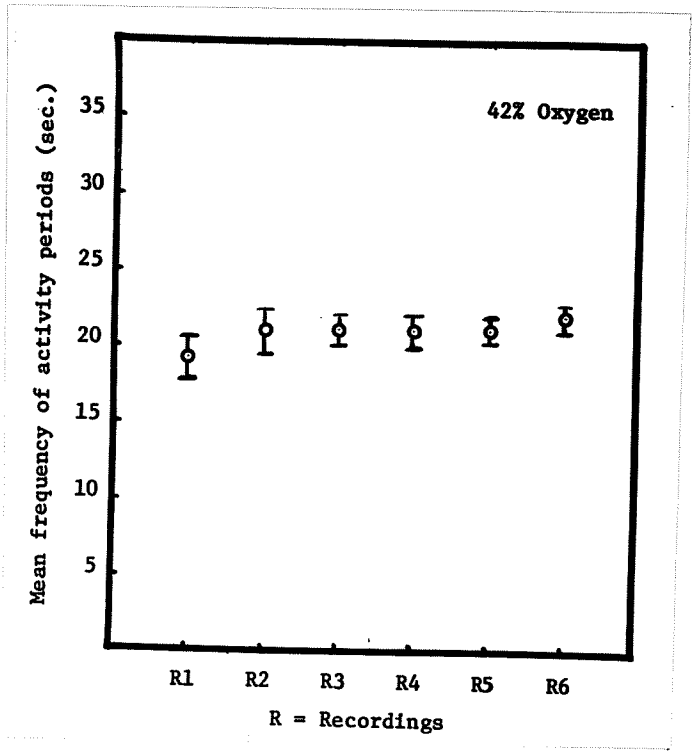
8

Fig. 9. Mean duration of activity periods of chick embryos, days 4-6, during and after a one hour exposure to 42% oxygen. The vertical lines represent the standard error of the means. For explanation of recordings (R1-R6) please refer to Table I, page 21.

Fig. 10. Mean frequency of activity periods of chick embryos, days 4-6, during and after a one hour exposure to 42% oxygen. The vertical lines represent the standard error of the means. For explanation of recordings (R1-R6) please refer to Table I, page 21.


42% Oxygen
Mean duration of activity periods (sec.)
11
10
9
8
7
6
5
R1
R2
R3
R4
R5
R6
R = Recordings

9


42% Oxygen
Mean frequency of activity periods (sec.)
35
30
25
20
15
10
5
R1
R2
R3
R4
R5
R6
R = Recordings

10

At 84% O_2, the early embryos (days 4-6) did not exhibit any significant change in activity (Fig. 11, 12)*. There was a hint of one, however, because there existed a significant decrease in the mean duration of inactivity 60 minutes following recovery from the oxygen treatment (Fig. 13)*.

The mean per cent of overall activity of the older embryos (days 7-9), after 40 minutes in 84% O_2, showed a significant depression below the normal line (Fig. 14)†. Furthermore, the mean per cent of activity per cycle decreased significantly for the same animals after 40 minutes in oxygen and 30 minutes after the oxygen (84%) was removed (Fig. 15)†. The mean duration of the activity periods and the mean duration of the inactivity periods did not alter significantly at 84% O_2 (Fig. 16, 17)†.

The results indicated a difference between the age groups (Table II). The younger embryos (days 4-6) showed a general increase in activity. This was in contrast to the control series, in which no significant changes in motility patterns were seen, during the same time interval.

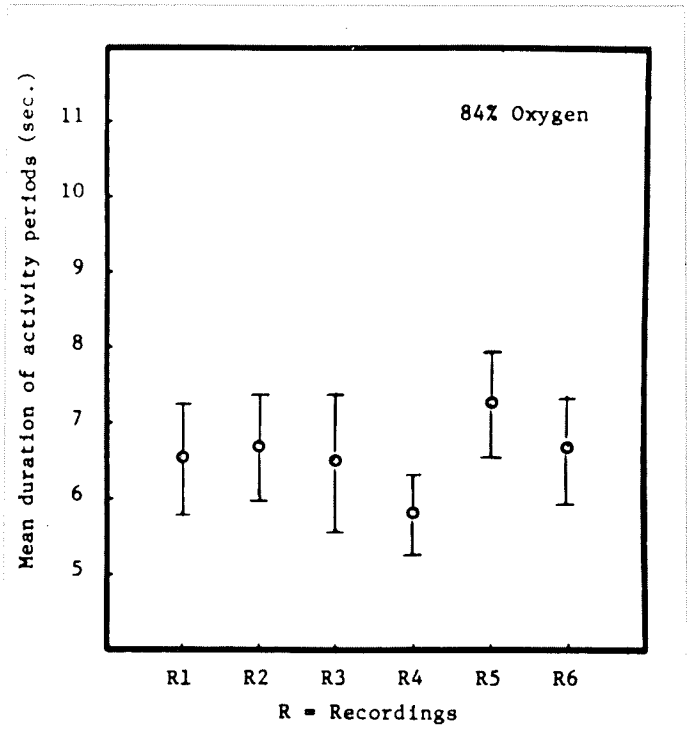
The older embryos (days 7-9) did not respond at all to a one hour exposure to 42% O_2 (Fig. 18, 19, and 20). However, a one hour exposure to 84% O_2 resulted in a general decrease in activity. This was a reciprocal response to the control series which displayed an increase in activity over the same time sequence.

* Table V

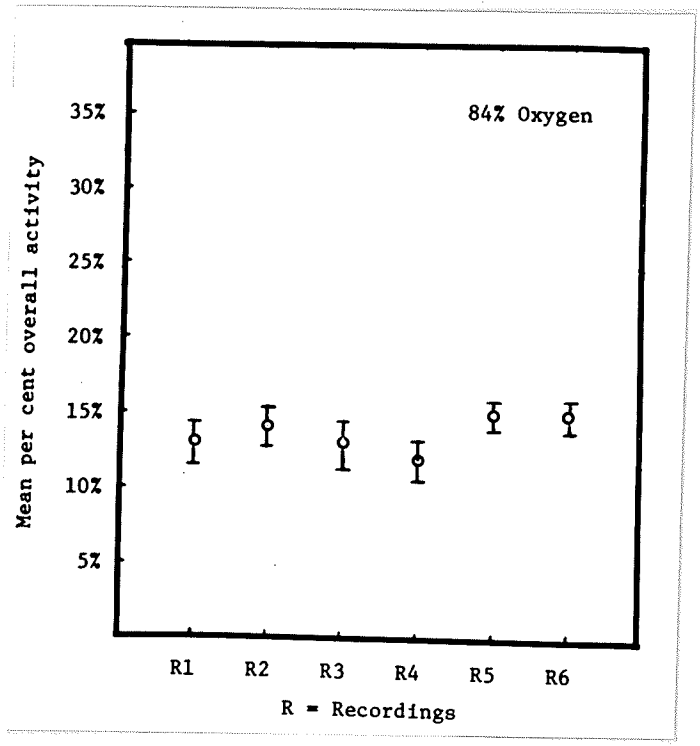
† Table VI

Fig. 11. Mean duration of activity periods, in seconds, of chick embryos, days 4-6, during and after a one hour exposure to 84% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.

Fig. 12. Mean per cent of overall activity of chick embryos, days 4-6, during and after a one hour exposure to 84% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I. page 21.


Mean duration of activity periods (sec.)
11
10
9
8
7
6
5
84% Oxygen
R1
R2
R3
R4
R5
R6
R = Recordings

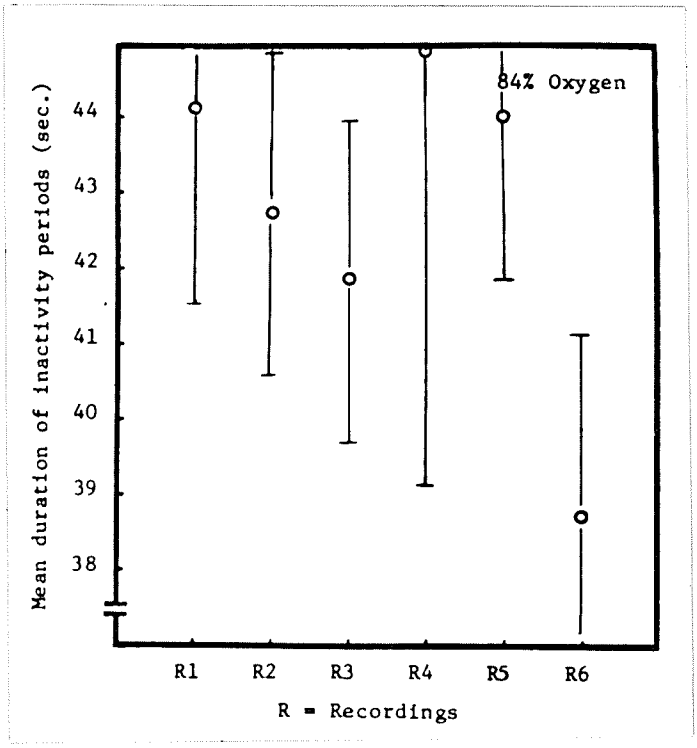
11


Mean per cent overall activity
35%
30%
25%
20%
15%
10%
5%
84% Oxygen
R1
R2
R3
R4
R5
R6
R = Recordings

12

Fig. 13. Mean duration of inactivity periods of chick embryos, days 4-6, during and after a one hour exposure to 84% oxygen.

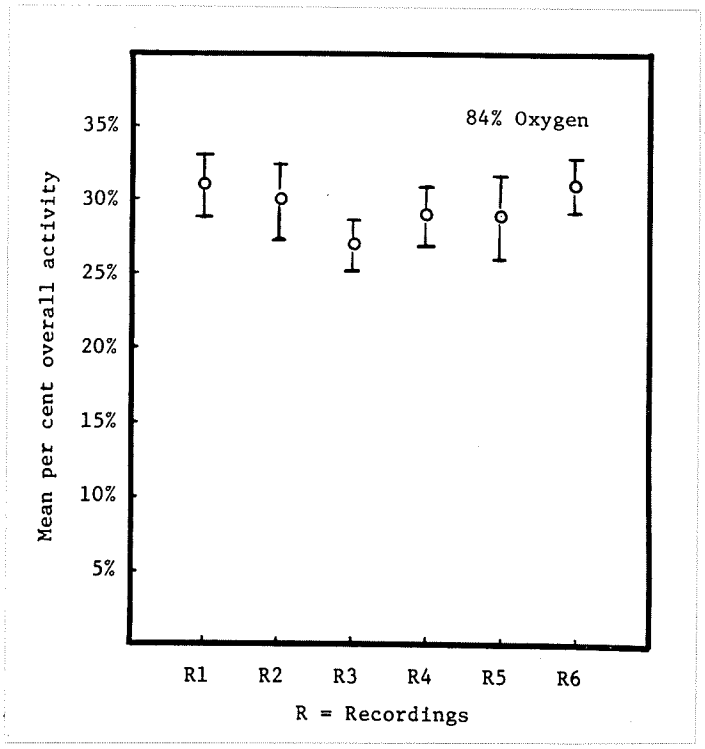
The vertical lines represent the standard error of the means. For explanation of recordings (R1-R6) please refer to Table I, page 21.


84% Oxygen
Mean duration of inactivity periods (sec.)
44
43
42
41
40
39
38
R1
R2
R3
R4
R5
R6
R = Recordings

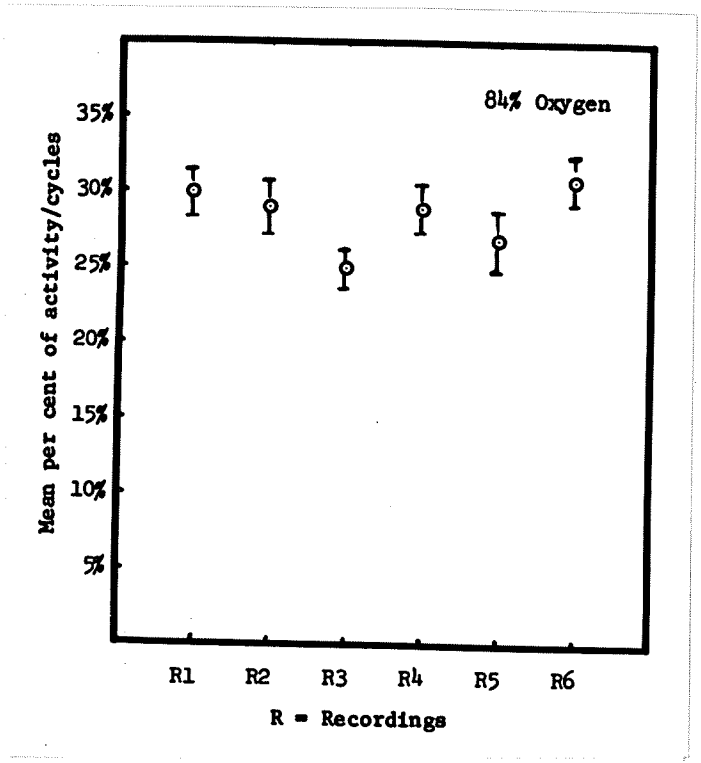
13

Fig. 14. Mean per cent of overall activity of chick embryos, days 7-9, during and after a one hour exposure to 84% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.

Fig. 15. Mean per cent of activity per cycle of chick embryos, days 7-9, during and after a one hour exposure to 84% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.


84% Oxygen
Mean per cent overall activity
35%
30%
25%
20%
15%
10%
5%
R1
R2
R3
R4
R5
R6
R = Recordings

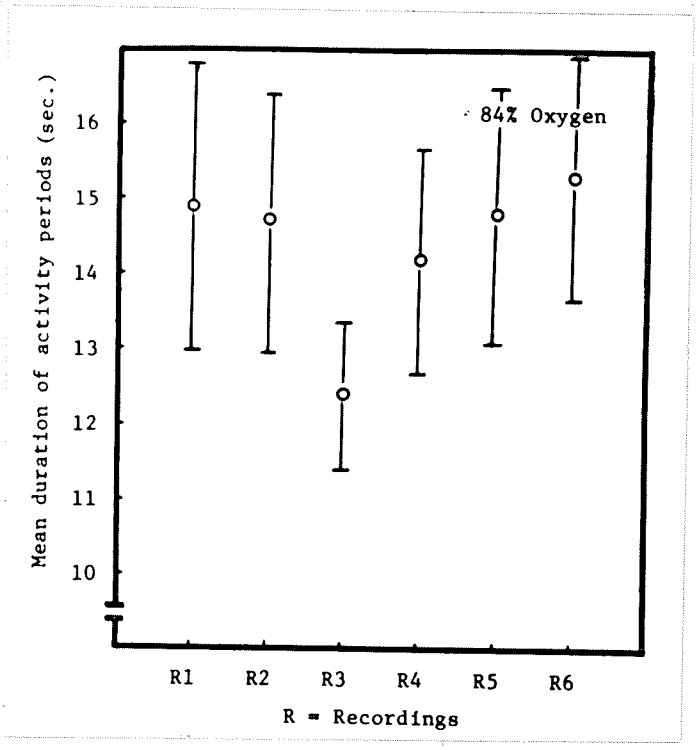
14


84% Oxygen
Mean per cent of activity/cycles
35%
30%
25%
20%
15%
10%
5%
R1
R2
R3
R4
R5
R6
R = Recordings

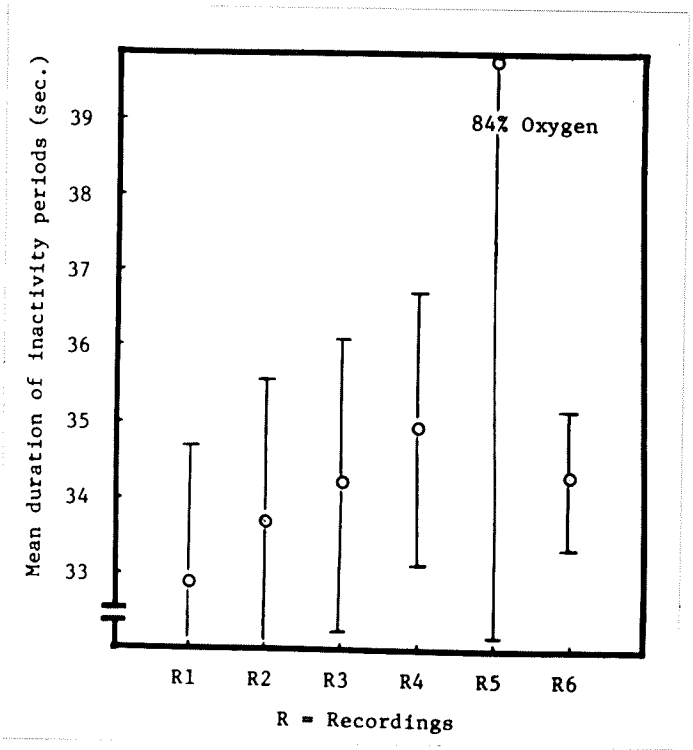
15

Fig. 16. Mean duration of activity periods of chick embryos, days 7-9, during and after a one hour exposure to 84% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.

Fig. 17. Mean duration of inactivity periods of chick embryos, days 7-9, during and after a one hour exposure to 84% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.


Mean duration of activity periods (sec.)
16
15
14
13
12
11
10
84% Oxygen
R1
R2
R3
R4
R5
R6
R = Recordings

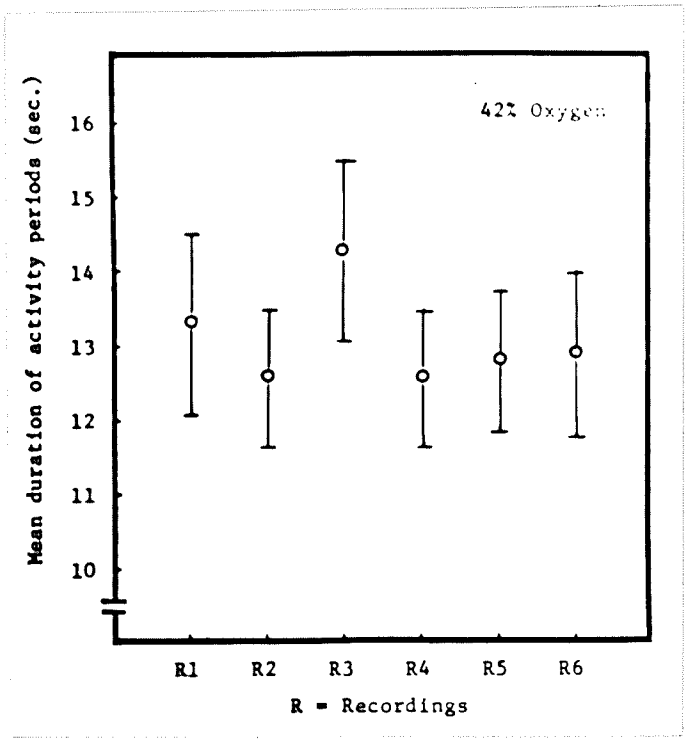
16


Mean duration of inactivity periods (sec.)
39
38
37
36
35
34
33
84% Oxygen
R1
R2
R3
R4
R5
R6
R = Recordings

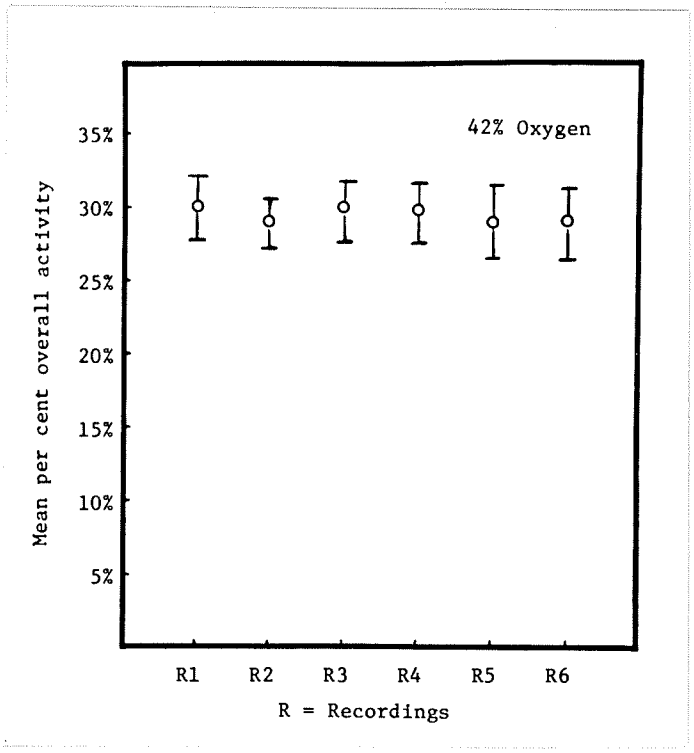
17

Fig. 18. Mean duration of activity periods of chick embryos, days 7-9, during and after a one hour exposure to 42% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.

Fig. 19. Mean per cent of overall activity of chick embryos, days 7-9, during and after a one hour exposure to 42% oxygen.
The vertical lines represent the standard error of the means.
For explanation of recordings (R1-R6) please refer to Table I, page 21.


42% Oxygen
Mean duration of activity periods (sec.)
16
15
14
13
12
11
10
R1
R2
R3
R4
R5
R6
R = Recordings

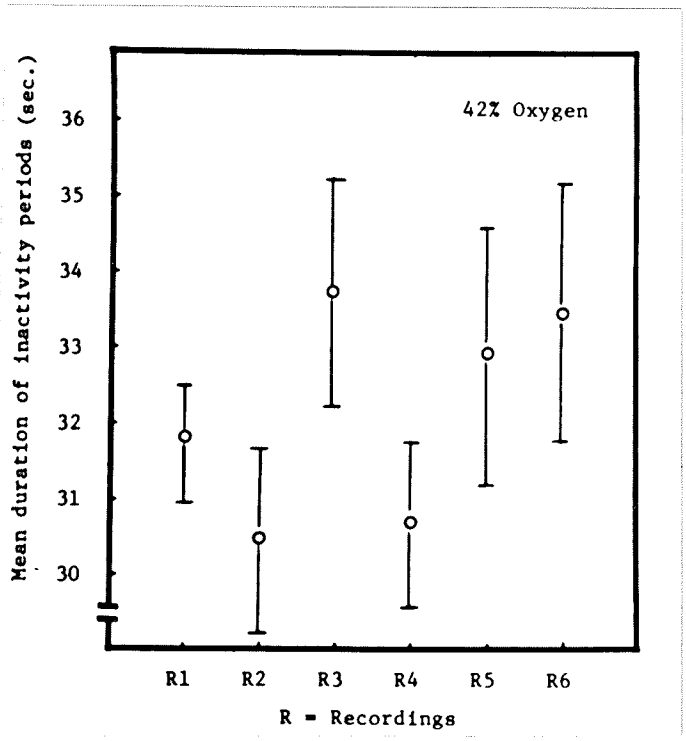
18


42% Oxygen
Mean per cent overall activity
35%
30%
25%
20%
15%
10%
5%
R1
R2
R3
R4
R5
R6
R = Recordings

19

Fig. 20. Mean duration of inactivity periods of chick embryos, days 7-9, during and after a one hour exposure to 42% oxygen.

The vertical lines represent the standard error of the means. For explanation of recordings (R1-R6) please refer to Table I, page 21.


Mean duration of inactivity periods (sec.)
36
35
34
33
32
31
30
42% Oxygen
R1
R2
R3
R4
R5
R6
R = Recordings

(20)

TABLE II

SIGNIFICANT CHANGES IN THE MOTILITY OF CHICK EMBRYOS

DAYS 4-6 AND 7-9

EXPOSED TO 42% AND 84% OXYGEN FOR ONE HOUR

Day	10 minutes in oxygen		40 minutes in oxygen		4 minutes after oxygen		30 minutes after oxygen		60 minutes after oxygen	
	42%	84%	42%	84%	42%	84%	42%	84%	42%	84%
4-6			+		⊕		⊕		⊕	⊕
7-9				−				−		

\+ - significant increase in activity.

⊕ - significant decrease in inactivity.

− - significant decrease in activity.

All tests of significance (95% level) were based on the Wilcoxon matched-pairs signed-ranks test.

V. DISCUSSION AND SUMMARY

According to our results, the younger and the older embryos respond differently to increased oxygen levels in the ambient environment.

The 4-6 day embryos exhibit an overall increase in activity, after 40 minutes of exposure, during a 1 hour treatment with 42% O_2. This trend continues up to 1 hour after removal of the oxygen (42% or 84%).

The 7-9 day embryos, on the other hand, demonstrate an overall depression of activity after 40 minutes of exposure to, and 30 minutes after removal of 84% O_2. The older embryos do not respond to a 1 hour treatment with 42% O_2.

There is no response at all after 10 minutes in oxygen (42% or 84%) at either age level.

Our conclusions are incongruous with those of Hamburger. He reports an increase in activity for 8-day embryos while we find a significant decrease in activity for the same age. He exposed 8-day chick embryos to 100% O_2 for 3 to 4 minutes, every 1/2 hour, and studied their motility patterns (Hamburger, 1964). He does not mention any control reading, taken prior to exposure, or how many recordings he made following the exposure period. According to our results, at least 10 minutes of exposure is required to raise the pO_2 of the amnionic fluid significantly. The increase in activity of Hamburger's 8-day embryos is similar to the pattern observed in our control series. We suggest that a 4 minute exposure is insufficient to allow the oxygen molecules to diffuse into the blood stream of the chick.

Although further experiments are required to obtain a more conclusive explanation for the trends exhibited, the following possibilities are suggested.

The sensory system of the chick embryo does not become functional until 7 1/2 - 8 days of development. Prior to this time, changes in the pO_2 of the circulation may affect the motor neuroblasts directly and cause them to prolong their discharges. At the age corresponding to the functional appearance of the sensory system, the affect of oxygen on the motor neuroblasts appears to be inhibitory to the point where the duration of their discharges is reduced.

We feel that the simultaneous appearance of the sensory system and the decline in activity at 84% O_2, in the older embryos, is more than coincidence. The motor neuroblasts appear to be affected by an intervening sensory mechanism which reacts to ionic imbalances in the blood stream.

An increase in the concentration of oxygen in the ambient environment will result in an increased pO_2 of the embryo's circulation. There is a possibility that a developing chemoreceptor system exists in the 7-9 day embryo. Its first response to different gas concentrations may be coupled with the functional development of the sensory system. As the chemoreceptor system is triggered, it may influence the activity of certain brain centers. Hamburger and Balaban (1963) believe that the brain contributes 10 to 20% to the overall activity of the embryo. If this percentage contribution to spontaneous activity is inhibited due to the action of the chemoreceptors, the level of spontaneous motility

will be reduced. It is important to note that the decrease in activity of the 7-9 day embryos, in our experiments, is 10 to 16% from the control.

The carotid body is believed to be responsible for sampling the blood gas concentrations and relaying the message to the respiratory center in the medulla. According to Adams (1958), the carotid body in the chick embryo does not become functional until just before hatching. Our experiments were conducted in the belief that they might amplify the conclusions reached by Hamburger (1964).

After all the data was tabulated, a further search through the literature brought forth a recent article by Murillo-Ferrol (1967), who shows that neural elements do exist in the carotid body of 8-day chick embryos. Innervation is from the nodosum ganglion of the vagus, the glossopharyngeal nerve, and the recurrent laryngeal nerve. Vascularization of the carotid body also occurs by the 8th day of development. The arterial supply is from the joint carotid artery. The venous drainage is by a small vein into a larger vein that anastomoses with a periesophageal plexus and the anterior cardinal vein or the internal jugular vein. The parenchyma of the carotid body also reaches a high degree of differentiation by 8 days.

With innervation complete and the cellular elements established, the carotid body is in a functional state from the 8th day of development. In the light of this new evidence, a further interpretation of our results become possible.

Studies in cats indicate that there is a definite "tonic" carotid chemoreceptor discharge at normal levels of arterial pO_2 (Lambertsen, 1961). These discharges are being relayed to medullary respiratory neurons, which are part of the diffuse reticular formation. An impulse reaching this area from the chemoreceptors could conceivably spread throughout the reticular formation, giving rise to a simultaneous burst of impulses. These might pass, possibly by way of a reticulo-spinal pathway, to various motor nuclei at lower levels, such as the anterior horn cells, some of which innervate the muscles of respiration.

Further experiments with cats (Lambertsen, 1961) demonstrate that increasing anoxia produces a proportional increase in chemoreceptor activity. The converse is also true. Raised levels of pO_2 are followed by a reduction in the impulses travelling from the chemoreceptors to higher centers.

The contribution of the brain to overall spontaneous activity in chick embryos has been reviewed again by Decker and Hamburger (1968). They indicate that different regions of the brain contribute to overall spontaneous motility at different stages of development. In the 9-day embryo, additional discharges from the medulla appear to enhance the autonomous discharges originating in the cord. We propose that the additional impulses result from the "tonic" discharge of the now functional carotid body.

It is suggested that partial inactivation of the chemoreceptors of the carotid body, due to exposure to 84% O_2, may inhibit the influence of the medulla on the spontaneous activity of our 7-9 day chick embryos.

Dejours et al. (1957) show that, in human subjects, the respiratory activity is reduced after a brief exposure to excess oxygen. Similar results have been obtained by Watt et al. (1943), by using unanesthetized dogs with their chemoreceptors intact.

In unanesthetized dogs, with their chemoreceptors denervated, there is an increase in respiratory activity during and after exposure to 100% O_2 (Watt et al., 1943). This suggests a direct influence of increased oxygen on the anterior horn cells causing them to either fire more frequently or to prolong their discharges. This may explain why our younger embryos, prior to the functional development of the carotid body, exhibit an increase in activity after exposure to excess oxygen concentrations.

We still agree with the theory that embryonic motility is the result of spontaneous discharges within the central nervous system. In our experiments, we have shown that they may be influenced by a chemical stimulus such as oxygen. Prior to the development of the sensory system, oxygen has a direct effect on the nerve cells. After day 8 of incubation, the response of the neurons to oxygen is modified by a regulatory, chemosensitive system. This does not appear, however, to alter the basic rhythmicity of the motor action system. This is supported by the fact that, despite a variation in the duration of activity under the influence of excess oxygen, no significant change in the frequency of the activity periods is seen.

Summary

1. The effects of increased levels of oxygen on the spontaneous activity of chick embryos, days 4-6 and days 7-9 of incubation, were studied.

2. In a control series of observations that were performed in the winter (February), an overall depression of spontaneous activity was noted when comparing them to the oxygen treated groups studied in the spring (April). This phenomenon might have been related to a decrease in the metabolic rate (i.e., total daily oxygen consumption) of the chick embryo during the winter months.

3. In 4-6 day embryos, exposed to 42% O_2 for 1 hour, there was a significant increase in the mean per cent of overall activity after 40 minutes of exposure. Concurrently, there was a significant decrease in the mean duration of inactivity periods in readings taken at the 40 minute mark of exposure, and 4 minutes, 30 minutes, and 60 minutes following the removal of the oxygen. In 4-6 day embryos, exposed to 84% O_2 for 1 hour, there was a significant decrease in the mean duration of inactivity periods 60 minutes following removal of the oxygen.

4. The response of the older embryos (days 7-9) to a 1 hour treatment with 42% O_2 did not vary significantly from the controls.

5. The mean per cent of overall activity of the 7-9 day embryos, after 40 minutes of a 1 hour exposure to 84% O_2, showed a significant depression below the normal line. The mean per cent of activity per cycle decreased significantly for the same animals after 40 minutes in oxygen and 30 minutes after the oxygen was removed.

6. The 4-6 day embryos responded to increased levels of oxygen by an overall increase in spontaneous activity. In contrast, the 7-9 day embryos responded to increased oxygen levels by an overall decrease in spontaneous activity.

7. It was suggested that the carotid body of the chick embryo might become functional in correspondence with the functional appearance of the sensory system (7 1/2 - 8 days of development). Furthermore, partial inactivation of the chemoreceptors of the carotid body, due to exposure to 84% O_2, might inhibit the influence of the medulla on the spontaneous activity of the 7-9 day chick embryos.

TABLE III

MOTILITY OF CONTROL CHICK EMBRYOS

DAYS 4-6 OF INCUBATION

	Control	Corresponds with ten minutes in oxygen	Corresponds with forty minutes in oxygen	Corresponds with four minutes after oxygen	Corresponds with thirty minutes after oxygen	Corresponds with sixty minutes after oxygen
$\bar{A}$	5.04 (±2.52)	5.14 (±2.11)	5.71 (±2.60)	5.35 (±2.70)	5.07 (±2.22)	5.66 (±2.71)
$\bar{I}A$	54.89 (±32.85)	53.23 (±20.15)	47.71 (±10.04)	45.96 (±10.95)	48.64 (±12.22)	46.84 (±11.88)
$\%\bar{O}A$	9 (±5.41)	10 (±4.45)	11 (±5.15)	11 (±4.53)	10 (±5.15)	11 (±4.49)
$\%A/\bar{C}$	12 (±6.34)	11 (±5.53)	12 (±5.28)	13 (±5.85)	11 (±5.01)	12 (±4.52)

$\bar{A}$ - mean duration of activity periods in seconds.
$\bar{I}A$ - mean duration of inactivity periods in seconds.
$\%\bar{O}A$ - mean per cent of overall activity.
$\%A/\bar{C}$ - mean per cent of activity per cycle.

Figures in parenthesis are the standard deviation.

TABLE IV

MOTILITY OF CONTROL CHICK EMBRYOS

DAYS 7-9 OF INCUBATION

	Control	Corresponds with ten minutes in oxygen	Corresponds with forty minutes in oxygen	Corresponds with four minutes after oxygen	Corresponds with thirty minutes after oxygen	Corresponds with sixty minutes after oxygen
$\bar{A}$	10.46 (±1.29)	11.81 (±2.35)	11.55 (±2.23)	13.25*(±3.41)	12.13 (±2.87)	11.46 (±3.06)
$\bar{IA}$	32.27 (±3.49)	35.89 (±7.55)	33.72 (±6.72)	37.56*(±3.94)	36.77*(±5.35)	35.42 (±5.14)
$\%\bar{OA}$	25 (±2.45)	26 (±4.72)	26 (±4.82)	26 (±5.59)	25 (±5.58)	25 (±5.15)
$\%A/\bar{C}$	26 (±2.30)	27 (±3.66)	27 (±3.13)	28 (±4.11)	27 (±3.71)	26 (±3.81)

$\bar{A}$ - mean duration of activity periods in seconds.
$\bar{IA}$ - mean duration of inactivity periods in seconds.
$\%\bar{OA}$ - mean per cent of overall activity.
$\%A/\bar{C}$ - mean per cent of activity per cycle.

Figures in parenthesis are the standard deviation.

*Significant at the 95% level (p=0.05) using the Wilcoxon matched-pairs signed-ranks test.

TABLE V

MOTILITY OF EXPERIMENTAL CHICK EMBRYOS

DAYS 4-6

DURING AND AFTER ONE HOUR EXPOSURE TO 42% AND 84% OXYGEN

	Oxygen concentration (hour exposure)	Control (before oxygen exposure)	Ten minutes in oxygen	Forty minutes in oxygen	Four minutes after oxygen	Thirty minutes after oxygen	Sixty minutes after oxygen
$\bar{A}$	42%	6.02 (±2.29)	5.93 (±2.35)	6.61 (±2.84)	5.84 (±2.91)	6.06 (±2.83)	5.73 (±2.60)
$\bar{I}A$	42%	43.28 (±9.55)	40.18 (±8.95)	36.84*(±5.52)	38.10*(±6.35)	37.52*(±3.78)	37.36*(±3.98)
$\bar{A}$	84%	6.58 (±2.28)	6.75 (±2.28)	6.50 (±2.73)	5.80 (±1.41)	7.31 (±2.06)	6.67 (±2.22)
$\bar{I}A$	84%	44.12 (±7.79)	42.71 (±6.58)	41.83 (±6.71)	45.27 (±18.03)	43.96 (±6.64)	38.76*(±7.49)
$\%\bar{O}A$	42%	12 (±4.25)	13 (±5.20)	16* (±6.80)	13 (±6.70)	14 (±5.90)	13 (±6.31)
$\%A\bar{/}C$	42%	14 (±4.98)	14 (±4.86)	15 (±6.38)	15 (±6.33)	15 (±6.49)	14 (±6.32)
$\%\bar{O}A$	84%	13 (±4.21)	14 (±4.18)	13 (±4.65)	12 (±4.00)	15 (±2.98)	15 (±3.18)
$\%A\bar{/}C$	84%	16 (±4.85	16 (±4.49)	15 (±5.47)	15 (±4.08)	16 (±3.50)	17 (±3.06)

$\bar{A}$ - mean duration of activity periods in seconds.
$\bar{I}A$ - mean duration of inactivity periods in seconds.
$\%\bar{O}A$ - mean per cent of overall activity.
$\%A\bar{/}C$ - mean per cent of activity per cycle.

Figures in parenthesis are the standard deviations.

*Significant at the 95% level (p=0.05) using the Wilcoxon matched-pairs signed-ranks test.

TABLE VI

MOTILITY OF EXPERIMENTAL CHICK EMBRYOS

DAYS 7-9

DURING AND AFTER ONE HOUR EXPOSURE TO 42% AND 84% OXYGEN

	Oxygen concentration (hour exposure)	Control (before oxygen exposure)	Ten minutes in oxygen	Forty minutes in oxygen	Four minutes after oxygen	Thirty minutes after oxygen	Sixty minutes after oxygen
$\bar{A}$	42%	13.27 (±3.74)	12.59 (±2.79)	14.29 (±3.78)	12.64 (±2.92)	12.78 (±2.94)	12.90 (±3.43)
$\bar{I}A$	42%	31.80 (±2.33)	30.46 (±3.89)	33.76 (±4.71)	30.68 (±3.37)	32.95 (±5.38)	33.45 (±5.22)
$\bar{A}$	84%	14.93 (±5.88)	14.67 (±5.38)	12.41 (±3.08)	14.19 (±4.64)	14.79 (±5.42)	15.34 (±4.99)
$\bar{I}A$	84%	32.85 (±5.57)	33.70 (±6.16)	34.21 (±5.98)	34.97 (±5.72)	40.55 (±25.62)	34.29 (±2.98)
$\%\bar{O}A$	42%	30 (±6.71)	29 (±4.97)	30 (±6.45)	30 (±5.87)	29 (±6.99)	29 (±6.58)
$\%A\bar{/}C$	42%	30 (±4.80)	29 (±3.98)	29 (±4.95)	29 (±5.25)	28 (±5.04)	28 (±5.23)
$\%\bar{O}A$	84%	31 (±6.38)	30 (±7.50)	27* (±5.48)	29 (±6.395)	29 (±9.17)	31 (±5.77)
$\%A\bar{/}C$	84%	30 (±4.82)	29 (±5.73)	25* (±3.88)	29 (±4.81)	27* (±6.20)	31 (±5.17)

$\bar{A}$ - mean duration of activity periods in seconds.
$\bar{I}A$ - mean duration of inactivity periods in seconds.
$\%\bar{O}A$ - mean per cent of overall activity.
$\%A\bar{/}C$ - mean per cent of activity per cycle.

Figures in parenthesis are the standard deviations.

*Significant at the 95% level (p=0.05) using the Wilcoxon matched-pairs signed-ranks test.

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