ANOXIA IN THE NEWBORN RAT

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ARTS

in the Department

of

Physiology

We accept this thesis as conforming to the standard required from candidates for the degree of MASTER OF ARTS.

Members of the Department of

Physiology

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1954

ABSTRACT of thesis submitted by S. Segal in partial fulfilment of the requirements of the degree of Master of Arts in the Department of Physiology, University of British Columbia, April, 1954.

ANOXIA IN THE NEWBORN RAT

A technique was developed for studying the effect of anoxia in the newborn rat with particular reference to persistence of electrical activity in the heart. In contrast to previous investigations in this field, no drastic surgical procedures were used, and the animals were held in a relatively undisturbed state in a closed temperature regulated chamber which could be filled with a gas mixture of any desired composition. Eleven newborn rats could be placed in the chamber at the same time under the same conditions, and electrocardiographic recordings could be obtained simultaneously from four animals at a time.

Anoxia was produced by flushing and filling the chamber with tank nitrogen (99.9% N_2), and the period of persistence of electrocardiographic activity was determined taking as endpoint the last recorded electrical potential from the heart. Two hundred and thirty-two rats were used, ranging in age from three hours to eight days postnatal. The results obtained agree substantially with those reported by other workers using cruder methods. The "survival time" of electrocardiographic activity in the four day old group was only half that observed in newborn rats less than twelve hours postnatal, the difference being highly significant. However, there was no further significant change in "survival time" during the period from four to eight days.

The technique developed should prove useful in studying many problems of neonatal physiology.

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A. PROBLEM OF ANOXIA IN THE NEWBORN

According to Yandell Henderson (21), the first fifteen minutes constitute the most dangerous period of life; more lives are lost in that interval than in any subsequent month.

Of 11, 147 births at Guy's Hospital from 1946 to 1949, Gibbard (18) has calculated that for every 1,000 births, 107 died of anoxia alone. Morison (36) observed that of 397 live born infants who died in their first three days, one-half succumbed to anoxia caused by some maternal or obstetrical abnormality, with no sign of congenital anomaly, trauma, infection or any non-respiratory lesion.

In a review of the 33 year period from 1915 to 1948, Wegman (52) demonstrated the significant reduction of mortality rates from all causes in individual age groups of the human population. This trend included the under-a-year and the under-a-month categories. In contrast, there was no apparent improvement during this period in the mortality figures for newborn infants during the first twenty-four hours of life (Fig. 1).

Anoxia may occur *in utero*, resulting in stillbirth or in death during the first few hours. Interference with the baby's supply of oxygen may also occur during delivery or in the early neonatal period, and may result in irreparable injury or death. As Haldane stated more than thirty years ago:

"Anoxemia not only stops the machine but wrecks the machinery." (20). The so-called "devastation areas" of the cerebral cortex, described by Courville (8) and by Schrieber (38) stand as mute evidence of the end effect of a period of oxygen deprivation.

There are ample opportunities for interference with oxygen supply during intra-uterine life, for the gas must be transported through devious pathways and across several membrane barriers before it reaches the foetal brain.

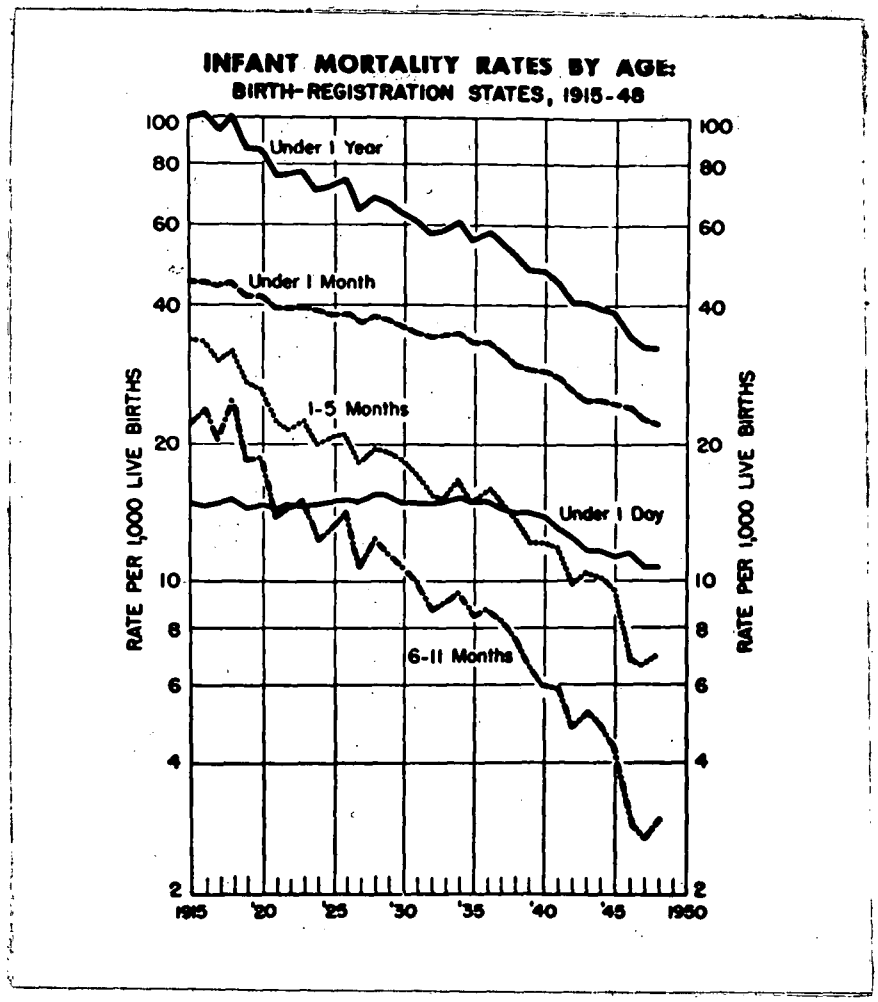

Fig. 1. Infant Mortality Rates by Age, showing apparent lack of improvement in the age group under one day (52).

To follow page 1.

Certain regions are particularly critical, especially the placenta and umbilical cord.

The oxygen supply remains precarious during the transition of birth. Once the infant is delivered and the umbilical cord is tied, pulmonary respiration must immediately take up the function recently served by the placenta. This is dependent on impulses arising in the respiratory centers in the brain—depression of these centers will often delay the onset of breathing and may result in fatal anoxia. LeLong (30) considers that anoxia is responsible for the great majority of deaths *in utero* before and during parturition and is also responsible, directly or indirectly, for a large part of the mortality and morbidity immediately after birth. Pre-natal and post-natal anoxia cannot be considered separately, for the latter is usually the result of circumstances originating during or even before birth.

B. EXPERIMENTAL STUDIES ON ANOXIA OF THE NEWBORN

1. Historical Background

Probably one of the earliest reports in this field was that of Le Gallois (29) in 1813. He observed that the newborn rabbit had an extraordinary ability to survive without oxygen, as measured by the duration of respiratory movements after submersion, decapitation, opening the thorax and extirpation of the heart. In the same century, Paul Bert (5) described an unusual persistence of respiratory movements in newborn rats and cats, when submerged in water.

In subsequent investigations by Avery and Johlin (2), by Selle and Witten (44) and by Fazekas *et al.* (14), their subjects included mice, rats, rabbits, cats, dogs and guinea pigs of various ages. Some of these were rendered anoxic by breathing nitrogen, carbon dioxide, argon, hydrogen or illuminating gas. In others, treatment consisted of ligation of the cerebral vessels, decapitation or injection of sodium cyanide. Duration of mandibular movements decreased progressively

with advancing age. In neonatal mice made anoxic by breathing oxygen-free gases (2), these gasping movements lasted three to six times as long as in adults under the same conditions. Fazekas et al. (14) suggested that such apparent prolongation of life was due to the poikilothermic cooling of the neonate, which acted as a powerful factor in reducing cerebral metabolic requirements.

2. Effect of Age

Glass et al. (18) examined the possibility of permanent injury to rabbits as a result of such prolonged anoxic episodes. Prematurely delivered foetuses at the gestational ages of twenty-nine to thirty-one days survived, on the average, forty-four to thirty-four minutes, respectively, in pure nitrogen. No permanent effects were observed after such exposure and the animals developed into apparently normal adults.

Newborn rats, left under water at 37° C. for forty minutes and then removed, recovered completely (14), and developed to apparently normal adulthood.

In the rabbit experiments (18), it was found that the respiratory movements of prematurely delivered rabbits persisted for a longer time than those of full-term newborns. Furthermore, it was shown that by delaying parturition with the use of hormonal therapy, the post-mature subjects exhibited a persistence of gasping activity that corresponded to neonates whose age equalled the period of post-maturity. Thus parturition itself was merely incidental to the relationship of age to this type of survival time. Similarly, newborn rats continued to gasp for fifty minutes during oxygen deprivation; at one day of age their limit was reduced to forty minutes, while the adult stopped after two minutes. Such a relationship in the isolated heads of mice is represented graphically in Fig. 2 from data published by Thoms and Hiestand (49).

3. Effect of Maturity of the Species at Birth

Newborns of several species were subjected to pure nitrogen breathing

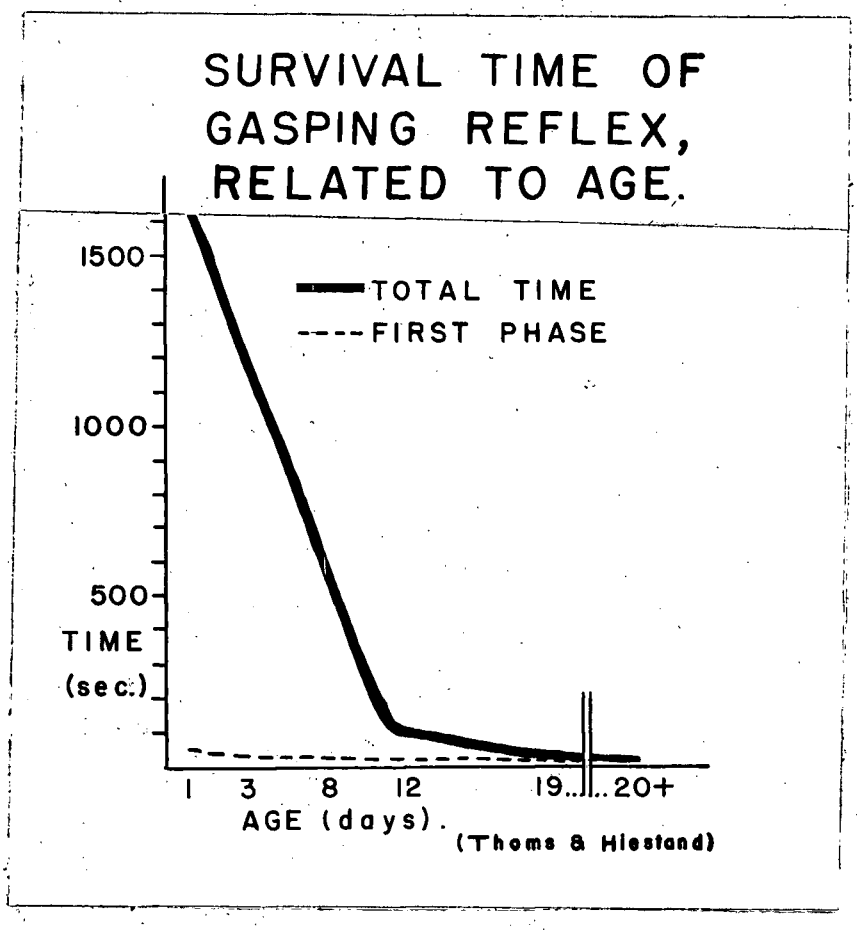

Fig. 2. Survival Time of Gasping Reflex, Related to Age, showing the decreasing duration of mandibular movement in the isolated head of the mouse, with advancing age. The survival of the first phase of gasping, which persists to adult life, is shown at an almost constant value.

To follow page 3.

at an environmental temperature of 24° C. Fazekas et al. (14) found that the persistence of their respiratory movements, shown in Table I, was related to the degree of maturity of the particular species at this age.

TABLE I

RELATIVE PERSISTENCE OF GASPING ACTIVITY IN NEWBORNS OF VARIOUS SPECIES IN PURE NITROGEN.

Species	Average persistence of Gasping movements (min.)
Newborn rats	50
Newborn cats	25
Newborn dogs	23
Newborn rabbits	17
Newborn guinea pigs	7
(Adults, any species	3)

Another comparative study by Glass et al. (18) yielded results of a similar order, although the endpoint was defined somewhat differently. One group of investigators (18) recorded the time of the last spontaneous gasp, while the other (14) fixed the end point as the time at which "movements could no longer be evoked by any stimulation".

In both of these experiments, the most prolonged gasping activity during anoxia was associated with the species that was characteristically the least mature at birth. Thus, the newborn rat had no hair or teeth. Its eyes were not yet open. It was totally dependent upon its mother and acted like a bulbospinal animal. The newborn guinea pig, on the other hand, which gasped for only seven minutes, was relatively mature, with co-ordinated locomotion, righting reflexes, temperature regulation and, therefore, with a functioning cephalad portion of the brain stem (24).

4. Effect of Temperature.

Himwich et al. (25) varied the ambient temperature in which one-day-old rats were exposed to an atmosphere of pure nitrogen. When temperature was increased from 24° C. to 34° C., the duration of gasping activity was reduced from fifty minutes to twenty-one minutes. Adult rats did not appear to be affected in this manner by temperature change, presumably because of the homeothermic nature of the mature animal.

Assuming that glycogenolysis was the means by which energy was released under anaerobic conditions, Dixon (10) suggested that elevation of temperature probably resulted in an increase in the demand for substrate in the brain, but that this increase exceeded any acceleration of the rate of cerebral glycogenolysis.

Miller (35) exposed guinea pigs less than one day old to an atmosphere of 5% CO_2 and 95% N_2, and observed that animals at room temperature were all dead after four and a half minutes, while most of those which had been cooled survived this period of anoxia.

5. Effect of Carbohydrate availability on Survival

Himwich (26, 27) observed that administration of insulin to newborn rats prior to exposure to anoxia reduced to half the period during which gasping activity persisted. On the other hand, prior injection of glucose to rats eight to ten days old significantly increased the period of gasping activity. Selle (43) obtained similar results with respect to the persistence of gasping movements of the mandible following decapitation. He also found that injection of glucose along with insulin nullified the deleterious effect of the latter. Hiestand et al. (23) used the same technique of observing movements of the mandible in the decapitate head, and found that administration of epinephrine and pituitary extracts increased the survival period, possibly because of their hyperglycemic effect, while a period

of fasting (twenty-two hours) reduced survival.

6. Effects of Nutritional Status, Hydration, and other effects.

Hiestand et al. (22, 23) have reported that starvation reduced the resistance to hypoxia in direct relation to the duration of the period of inanition, confirming earlier reports of Selle (41). However, they observed no such effect of dehydration, and the period of gasping was actually maximal in mice which had lost twenty per cent. of their body weight due to water loss while restricted to a dry diet.

Selle (41, 42) has reported differences in survival related to differences in individual litters. Britton and Kline (7) found the adult female rat more resistant to anoxia than the male, but they were unable to demonstrate such a difference due to sex at the neonatal level.

7. Observation of the Gasping Pattern in the Isolated Head.

In these experiments, decapitation was performed with a razor just caudad to the fore-limbs, leaving the chemoreceptors and medullary centers intact. Mandibular movements in the isolated head were used as an indication of active gasping related to activity of the respiratory center.

TABLE II

DURATION AND PATTERNS OF GASPING IN HEADS OF NEONATAL MICE BREATHING PURE NITROGEN.

Age (days)	No. of Mice	Duration of First Series (sec.)	± S.E.	Total Survival Time, both series. (sec.)	± S.E.
1	8	32.2	2.2	1652.1	135.4
3	6	19.0	1.5	1459.2	101.3
8	10	19.5	2.5	574.3	127.7
12	11	13.3	1.1	71.8	10.3
19	13	18.6	1.8	19.9	1.8
20+	49	16.05	0.25	16.05	0.25

Data from Thoms and Hiestand (49) are summarized in Table II. In the isolated heads of white mice, the survival time of mandibular movements was related inversely to age. The curve was relatively steep for the first eight to twelve days reaching the adult value at the age of nineteen days and then levelling off.

The gasps occurred characteristically in two distinct volleys punctuated by a period of inactivity. In this report, the first series of volleys lasted from a quarter to a half a minute. The second series persisted up to about half an hour at the age of one day but decreased with age to become barely discernible in the adult mouse.

Hiestand et al. (23) have related this pattern of two periods of gasping separated by an apneic pause, to the different levels of integration of the respiratory centers. The primitive center, being more rugged, might be expected to survive longer under anoxia. They suggested, therefore, that the prolonged second series of gasping was probably due to activity of the primitive center. As evidence for their theory, they pointed out that these gasps were of a slow, rhythmic nature, and definitely apneustic in character. The gasps in the first series, however, were more rapid and less apneustic, and were probably caused by the superimposed regulation of the higher centers.

Using the heads of rats younger than six weeks, Selle and Witten (45) obtained results comparable with those of Thoms and Hiestand (49). The first series of gasps ceased after less than a minute. After an interval of thirty to fifty seconds, the second burst of gasping movements occurred and lasted from twenty to forty minutes, the period diminishing with increasing age, and disappearing entirely at the age of six weeks. Moreover, the effect of temperature, already described, affected the duration of the second series of mandibular movements.

The first series has been identified with aerobic metabolism, and the second with anaerobiosis. (24).

Selle (42, 43) has found that insulin or glucose, administered to the neonatal rat prior to decapitation, exerted practically no effect upon the first, or aerobic series of gasping movements: these agents affected only the second, or anaerobic phase. When the dose of insulin was sufficiently large, there was complete disappearance of this second phase. Likewise, the effects of pituitary and adrenal hormone administration, discussed in connection with availability of carbohydrate, were confined to the anaerobic gasping time. None of these agents had any apparent effect on the total gasping time in the adult animal, which does not show this second anaerobic period of gasping.

The injection of Iodacetate, an enzyme toxin that blocks the anaerobic glycogenolytic process, did not affect the first phase but eliminated the second gasping period, the pattern resembling that of the insulin-treated neonate or the untreated adult, (23, 42). On the other hand, sodium cyanide, which is known to inactivate the cytochrome oxidase system and thus prevent oxygen utilization by the tissues, appeared to block the first aerobic phase but had no effect upon the second phase (42).

8. Metabolic Aspect of Anoxia in the Neonate.

Studies with specific enzyme toxins, as reviewed in Table III, have shown that the metabolic processes responsible for this added resistance to anoxia involved the established patterns of carbohydrate metabolism (Fig. 3). The salient factor in the neonate, appears to be increased activity in the glucose-to-lactate portion of the metabolic pathway for carbohydrate.

Himwich (24) accounted for this specific difference on the basis of enzymes and the variation of their respective concentrations with developmental changes from neonatal to adult life.

Apparently, with this localized increase in activity, the neonate utilizes less oxygen and more glucose, and produces more lactic acid, even under fully aerobic conditions (4, 19, 50, 51, 53). In anoxia, further increase in the rates of carbohydrate consumption and lactate production have been demonstrated (11, 12, 13, 26, 53). Within the neonatal period, the effect of age on resistance to anoxia was reflected in progressive reduction in this high rate of lactic acid production (26).

According to Himwich (24), survival of the central nervous system, considered to be the area most vulnerable to the effects of anoxia (6), depends upon the metabolism of glycogen stores in the brain. Brinkman (6) however, related the anaerobiosis rather than brain storage, to blood glucose levels. Consequently, there has been speculation as to the possible function of the abundant glycogen deposits in the lungs of foetuses and neonates, as reported recently by LeLong and Laumonier (31).

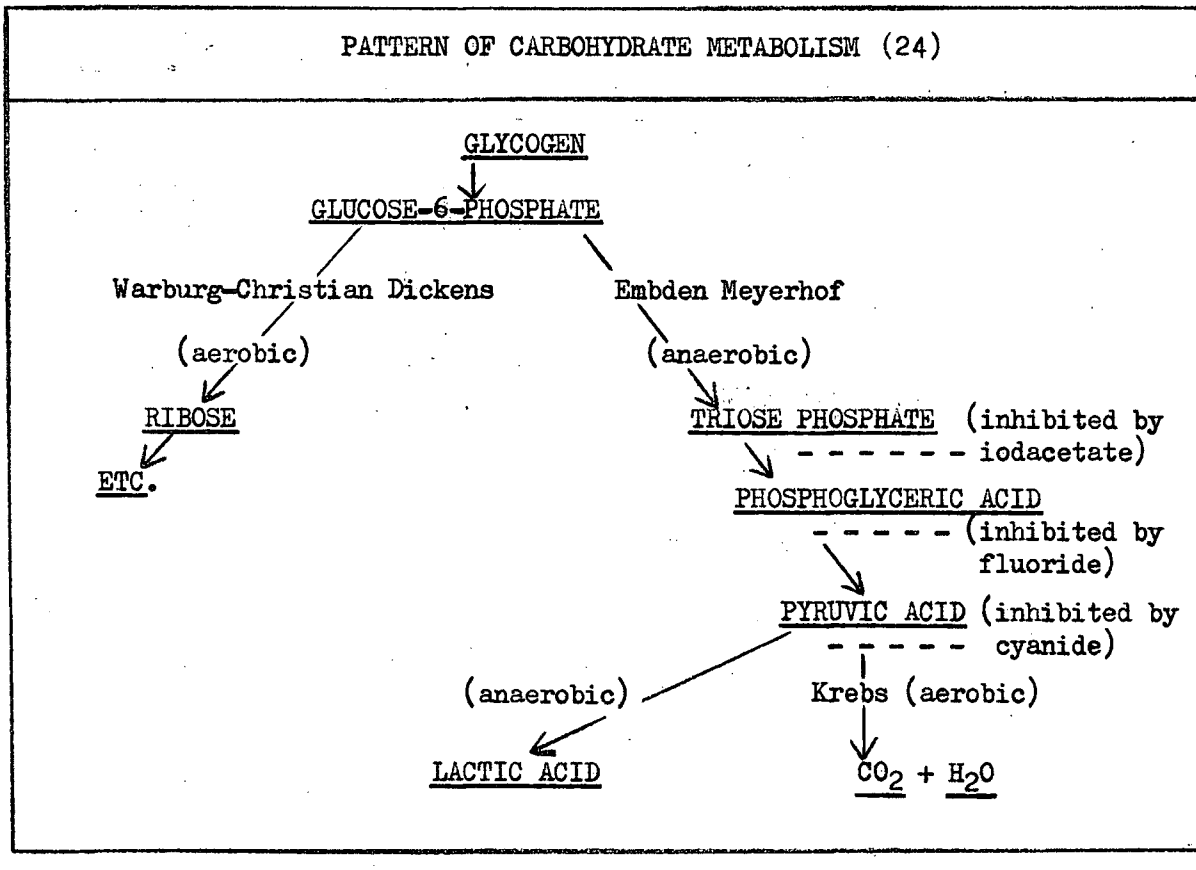

FIGURE 3.
PATTERN OF CARBOHYDRATE METABOLISM (24)

TABLE III

SYNOPSIS OF DATA OBTAINED IN REVIEW OF EXPERIMENTS WITH SPECIFIC ENZYME POISONS ON NEONATAL RATS BREATHING AIR AND PURE NITROGEN, RESPECTIVELY (14, 26, 28, 34).

Toxin injected	None		CN	Fluoride		Iodoacetate	
Gas respired	Air	N_2	Air	Air	N_2	Air	N_2
Metabolic cycles permitted by gas and toxin combined:							
Warburg-Christian-Dickens	+	0	0	+	0	+	0
Embden Meyerhof	+	+	+	$\frac{1}{2}$ ±	$\frac{1}{2}$ ±	$\frac{1}{4}$ ±	$\frac{1}{4}$ ±
Krebs Citric Acid	+	0	0				
End product postulated	CO_2 + H_2O	Lactate	Lactate	Phos. glyc.	Phos. glyc.	Triose ph.	Triose ph.
Lactate accumulation	+	+++	+++	?	?	+	+
Duration of gasping (min.)	Continues	50	50	50	16	50	3

LEGEND: Cycles: + complete cycle; $\frac{1}{2}$ ± cycle arrested part way; $\frac{1}{4}$ ± cycle shortened considerably; 0 cycle inhibited completely.

Product: Phos. glyc.; phosphoglyceric acid; Triose ph.; triose phosphate.

Lactate: + normal concentration; +++ greatly increased concentration.

These authors made use of some of the available metabolic evidence to explain the successful revival of a baby who had been delivered by Caesarean section fifteen to twenty minutes after maternal death. They suggested that this tolerance of anoxia in the human neonate may be related to:

(a) the undeveloped state of the cerebral cortex, leading to a decreased metabolic rate and to decreased vulnerability;

(b) lowering of body temperature, which would decrease the cerebral metabolic rate and its vulnerability;

(c) capacity for anaerobiosis, as shown in the rat experiments.

9. Methods Used in Experimental Studies: an Evaluation

a) Respiratory Movement

In the published reports dealing with resistance of the neonate to anoxia, the time of the last gasp has been the usual endpoint. In the intact animal, there have been two types of observations: (i) the last spontaneous gasp and (ii) the last gasp in response to a stimulus.

Considering the last spontaneous gasp, the data obtained must have lacked a reasonable degree of precision, since the gasping movements in advanced anoxia occurred after progressively prolonged and irregular intervals, each gasp being separated from the previous one by as many as three to five or more minutes (39). An observer attempting to watch a whole litter of eight to fifteen subjects at one time, might miss the very last one or two quick gasps of any one animal while looking at another and would record the so-called survival time with an error of ten minutes or more. Unintended disturbance of the animal may also stimulate a gasp, so that it is difficult to obtain uniform results.

Where the endpoint was taken to be the last gasp elicited by stimulation, there is no indication either of the exact nature of the stimulus or the means taken to obtain reproducible results. In addition, experience with use of electric

shock (39) would suggest that a stimulus of sufficient intensity and duration to provoke a gasp in a neonatal anoxic rat might also have other side effects.

Recently, Mayer (33) reported a number of newborns which had not been observed to breathe until after the fifth minute. Their condition was described as a "false state of anoxia" because radiography showed adequate pulmonary expansion and there was apparently adequate respiratory exchange, as a result of slight substernal undulations.

(From the biometric viewpoint, it was noted that with only two exceptions, the vast number of reports failed to provide any indication of the reproducibility of results, or of the extent of variation of the data from the published arithmetic means.)

(b) Blood Gas Studies

When newborn puppies were subjected to an atmosphere of pure nitrogen, Himwich et al. (25) observed that, after only five minutes, there was no measurable amount of oxygen in the arterial blood, although respiratory movements continued for another twenty or thirty minutes. Smith and Kaplan (46, 47) found no correlation between blood oxygen level and the period of delay before the first spontaneous breath, nor between the oxygen level at birth and its rate of subsequent change. Even with an adequate arterial oxygen saturation, (e.g., ninety-three per cent) prematures were observed by Graham et al. (19) to be breathing irregularly as though suffering from hypoxia.

Carbon dioxide studies have been equally equivocal. Eastman (13) found that, in the normal newborn, the range of carbon dioxide tensions (38-60 mm Hg P_{CO_2}) was extremely broad with no obvious relationship to respiratory activity.

(c) Neurological Signs

The system in which reversibility of the effects of neonatal anoxia appears to be critical is the central nervous system (6, 24).

Selle and Witten (44, 45) observed the pupillary responses in the isolated head and also the trunk reflexes in the spinal animal after decapitation. The former were stimulated by means of a bright light, the latter by the application of an allegedly painful electric shock, the stimulating electrodes being applied to one limb and to the cutaneous surface of the abdomen. Even if the tests had been performed on subjects in a more physiological state than that of decapitation, these techniques of stimulation would not be very reproducible when applied to small animals. Furthermore, there appears to be a basic fallacy with any interpretation of reflex activity under these circumstances, since an infant suffering from severe asphyxia neonatorum may have absent reflexes and still proceed to spontaneous or aided complete recovery (16, 39).

Electroencephalography would seem to have merit in this regard. However, there are two considerations that would preclude its application to this problem: (a) In depressed states, such as in heavy narcosis or after a convulsive episode, there may be a temporary but complete lapse of all electrical activity from the brain. This so-called silent period could result in a false interpretation. (b) In the failing central nervous system of a moribund subject, the rate of decrease in the amplitude of electrical activity is so gradual and the voltages may be so minute to begin with, that the electroencephalographic potentials would slowly become indistinguishable from electrical artefacts and there would be no distinct endpoint (15).

(d) Cardiovascular Signs

In the peripheral circulation, the determination of blood pressure in neonates, particularly of small species, is both time consuming and inaccurate, with available techniques. Swann (48) reported that he was unable to measure the pressures accurately in neonatal pups or to correlate the results with chances of surviving an anoxic episode.

Selle and Witten (40, 44, 45) and Himwich et al. (25) have observed persistence of cardiac activity in neonates after the onset of apnea. Indeed, in severely anoxic newborn human infants, the heart may continue to beat long after the baby is no longer capable of responding to resuscitatory efforts. However, when enzyme toxins were administered (26), there was abrupt termination of both gasping and cardiac activity. Selle (40) opened the thorax and observed the continued beating of the heart after gasping had ceased or after decapitation, and noted the time at which cardiac activity ceased. However, there are some serious objections to such drastic procedures. On the other hand, determination of electrical activity in the heart by electrocardiography can be carried out on the intact neonate with very little disturbance. Although electrocardiographs have been obtained by a number of investigators using foetuses, neonates and adult small animals, it does not appear to have been used as an index of cessation of cardiac activity in anoxic animals, prior to the present investigation.

C. PLAN OF PRESENT INVESTIGATION

The purpose of the present investigation was to study the effect of age on survival of anoxic neonatal rats, using as criterion the persistence of electrical activity in the heart. Cessation of cardiac activity is the common clinical criterion of death, and has been used by Selle (40) in assessing the survival of newborn rats in an atmosphere of nitrogen and following decapitation. He opened the chest and observed the beating of the heart directly. In the present investigations, it was felt desirable to avoid such drastic measures by recording electrical activity using standard electrocardiographic techniques in an intact animal. To accomplish this, apparatus was developed which permitted electrocardiographic recordings from up to eleven rats mounted in a closed chamber filled with nitrogen.

A. ANIMALS

The animals used were albino rats of the Wistar strain, obtained from the stock colony of the Department of Animal Husbandry of the University of British Columbia. A few rats of the Sprague-Dawley strain have also been included. The number of rats in each litter varied from six to fourteen, but only eleven could be accommodated in the apparatus at any one time. There was some difficulty in determining the exact time of birth when rats were born at night, and in some cases two hours were required for the delivery of the complete litter. However, in view of the wide scatter in "survival times", it was felt that estimation of the birth time within a few hours was sufficiently accurate for the purpose.

The rats in the age range from three to twelve hours have been grouped together, and the average value is plotted at the average time of seven hours. Those in the range from twelve to twenty-four hours have been plotted at the average time of twenty-one hours. Subsequent groups are arranged according to the closest day.

B. MEASUREMENT OF ELECTRICAL ACTIVITY OF THE HEART

Several types of electrodes have been used on small animals, each with certain limitations. Richards et al. (37) embedded needles in the skin. Agduhr and Stenström (1) used small zinc plates, amalgamated with mercury. They wrapped these around the limbs of adult mice, bandaging them with cotton wool dampened with saline. Under ether anaesthesia, the animal was transfixed to a cork platform by means of pins driven through the nose, tail and each of the limbs. Bauer (3) buried rectangular plates under the skin of young rabbits. However, to save time while working on foetuses, he fastened crocodile clips to their limbs.

Lombard (32) made recordings from adults of several small animals, by an entirely different system. She immobilized the anaesthetized subject in a

plastic sling, resting on its belly with the limbs hanging downward and dipping into small beakers containing 1 M. zinc chloride solution and zinc electrodes.

Various types of amplifying and recording equipment were used by these investigators. In all cases, records were obtained from only one animal at a time.

In the following experiment, special apparatus was constructed which made it possible to place up to eleven neonates in a single chamber and record simultaneous electrocardiograph records from four at a time, without in any way disturbing the animals or the gas mixture. Each animal was held in place by a pair of electrodes which gripped the right foreleg and left hindleg, so that the potentials picked up would correspond to the Standard Limb Lead II of the Electrocardiograph. The apparatus is shown in Figure 4. Initially, brass electrode clips were used, but these were replaced later by limb clips, illustrated in Figure 5, which consisted of two arms of nickel-silver wire, and a coil spring which pressed the arms together. The latter electrodes did not corrode, and the tension could be adjusted so that it was just sufficient to hold the animal securely during the struggling which occurs in the early stages of anoxia.

The electrode connections were shielded, as was the bottom of the plastic box, to reduce the pickup of extraneous potentials. A special selector switch on the panel made possible connection of any desired electrode pair to the recording apparatus. Initially, electrocardiograph records were obtained from each animal in turn, using a standard clinical model of the Sanborn-Viso-Cardiette, with single channel amplifier and a direct writing thermal stylus. A full minute recording was made from each rat, and it was found that at least twenty minutes was required to obtain records from eleven animals. This gave an uncertainty in time of endpoint of at least twenty minutes. Fortunately, it was

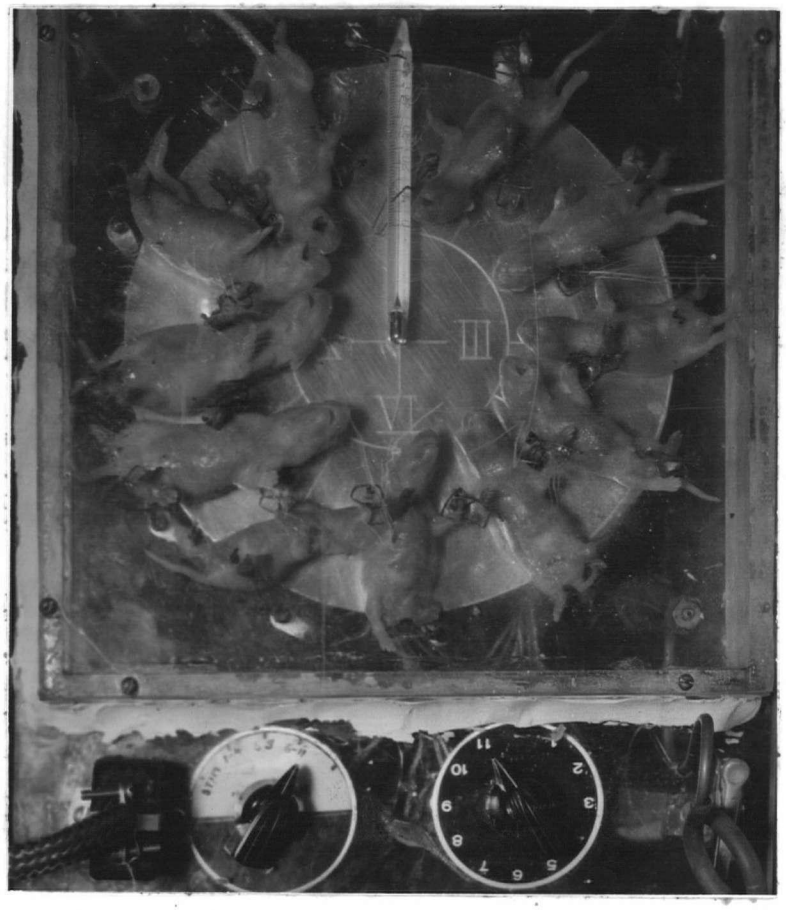

Fig. 4. Arrangement of Subjects in Nitrogen Chamber. The subjects are placed radially, immobilized by the electrodes. The thermometer is inside the chamber. The switch at lower left connects the electrodes to the amplifier-recorder, four pairs at a time. The switch at right connects an ohmmeter to the subjects, one at a time.

To follow page 16.

To follow page 16.

Fig. 5. Detail of Electrodes and Immobilization of Subjects, showing the design of the nickel-silver clips which, attached to the right foreleg and left hindleg of each subject, immobilized the animal and carried to the recorder-amplifier the Standard Limb Lead II of the Electrocardiogram.

possible to obtain the loan of an obsolete electroencephalograph machine* which provided a four channel amplifier and ink recorder, and made possible simultaneous observation of E.C.G.'s from four animals at a time. A full run took less than three minutes for a litter of eight rats, allowing a run of one minute duration on each of the two groups of four animals. For the full number of eleven subjects, a complete observation was possible every five minutes. Typical early and late recordings are shown in Figure 6.

Electrical resistance through each animal was measured after they were first attached to the electrodes and again at the end of the experiment. A volt-ohm-milliammeter, used for this purpose, showed that the resistance did not exceed more than 5,000 or 6,000 ohms in any case, although the amplifiers would still operate efficiently when the resistance was as great as 20,000 ohms. Resistance was reduced to a minimum with an electrode jelly.**

C. PRODUCTION OF ANOXIA

The newborn rats, held in place by the electrodes, were surrounded by an air-tight plastic box illustrated in Figure 7. The edges were sealed with plasticine. Tank nitrogen (testing 99.9% N_2) was humidified by passing through

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* Amplifier-recorder was manufactured by Electro-Physical Laboratories, Inc., 290 Dyckman Street, New York 34, New York, U.S.A. There was a separate five-stage push-pull amplifier on each of four channels. Each channel had a separate ink-writing galvanometer. Frequency response range was one quarter cycle to 4,000 cycles. The ink-writing galvanometers had a response that was uniform for 0 to 55 cycles. Paper speed originally was three cm. or six cm. per second but was modified for the present experiment to 1.5 cm. per second, allowing a full minute of recording to be made on a thirty-three inch length of paper. The apparatus was made available through the kindness of the E.E.G. Department, Vancouver General Hospital.

** Modified electrode jelly as formulated by the Dispensary of the Vancouver General Hospital. Composition:

Pulverized Tragacanth No. 1 A.A.	50 Gm.
Glycerin	400 Gm.
Sodium chloride	400 Gm.
Carbolic Acid	10 ml.
Pulverized Pumice	90 Gm.
Water	1600 ml.

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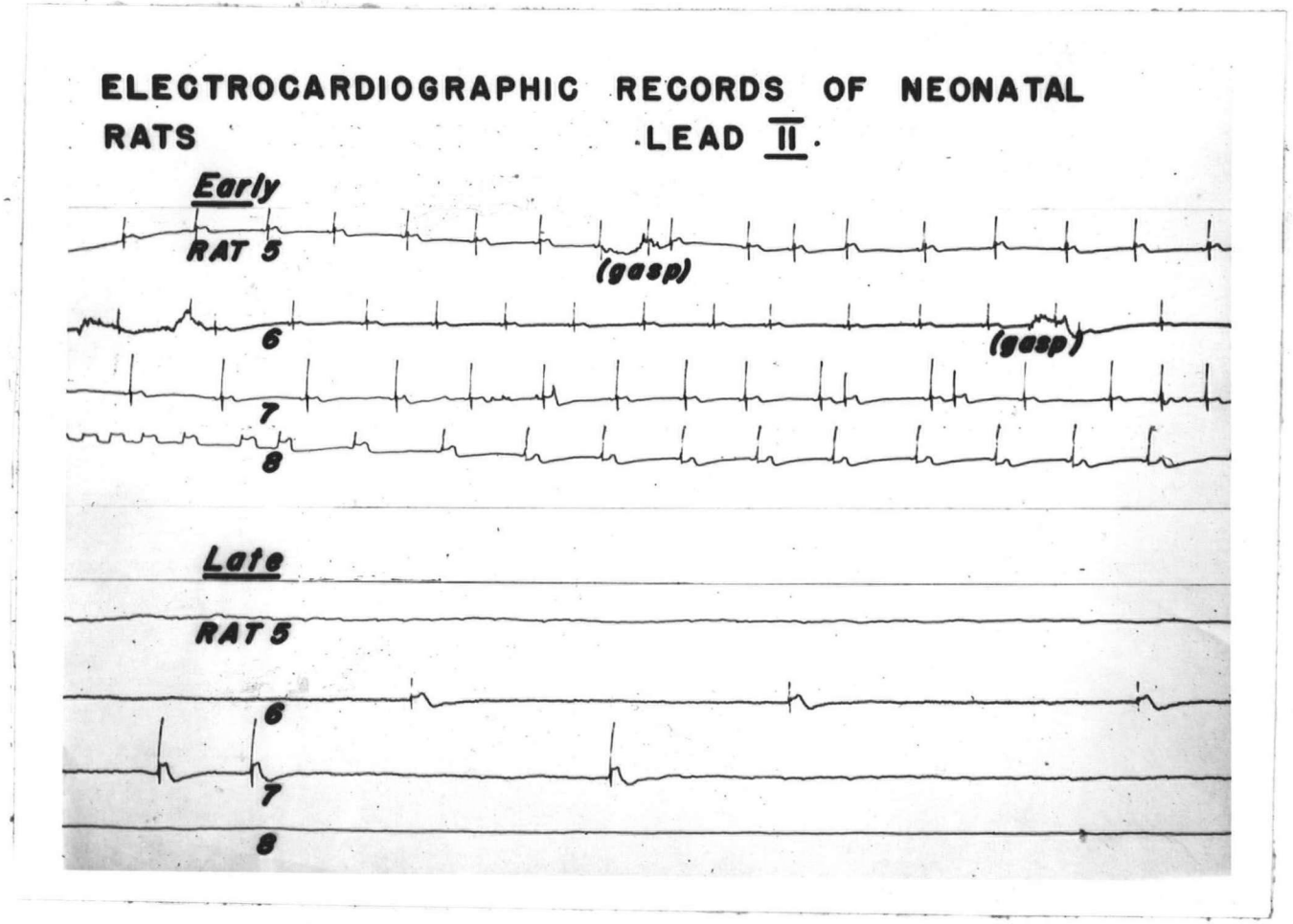

Fig. 6. Electrocardiographic Records of Neonatal Rats, showing tracings from four-day-old rats. In the earlier set, the subjects had been anoxic for several minutes. Their heart rate was approximately 40 per minute. In the later set, amplitude was set to a greater degree than in the earlier group. Activity persisted in No.6 and No.7 but the endpoint had been reached in No.5 and No.8.

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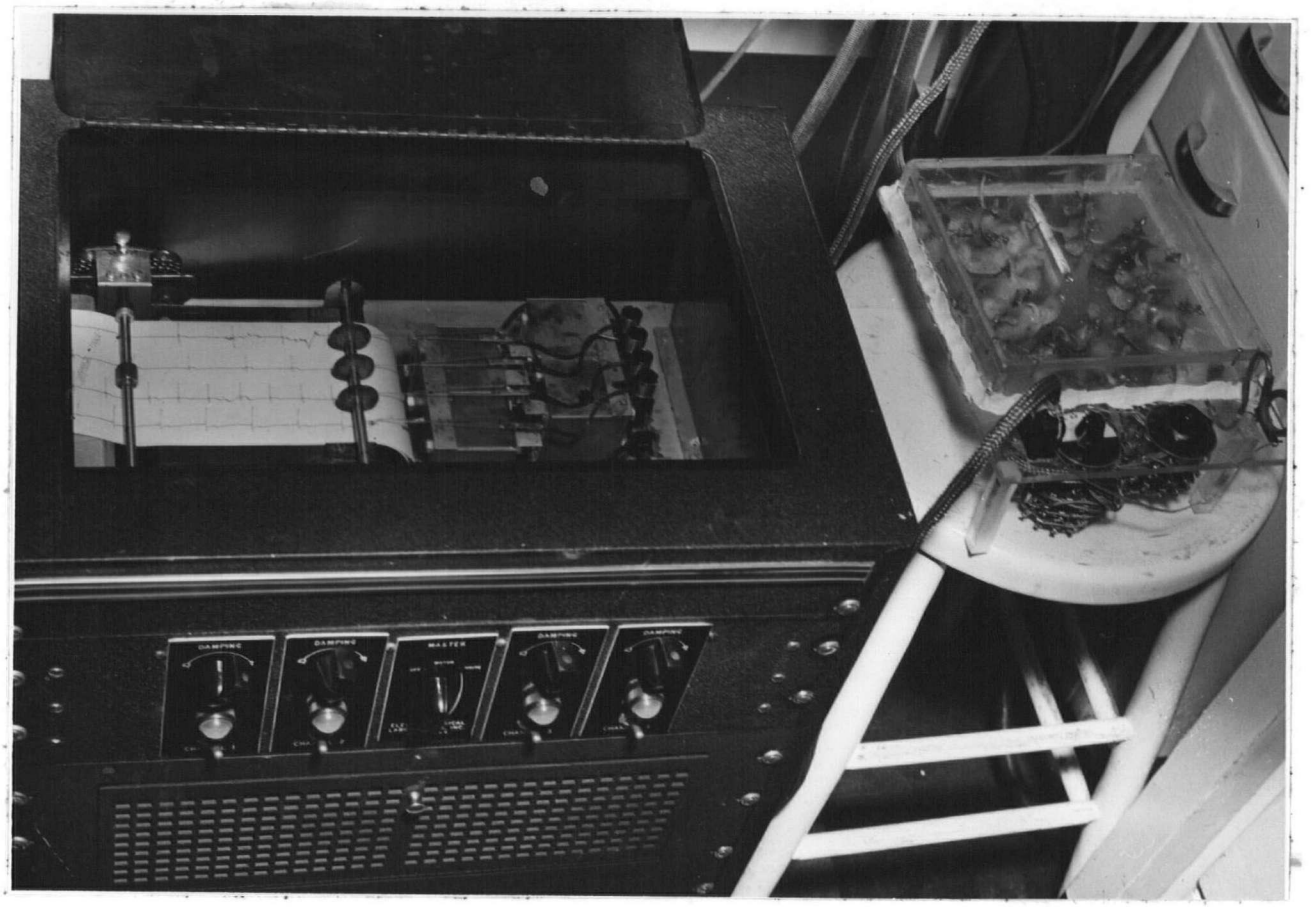

Fig. 7. Recorder Connected to Electrodes Housed in Airtight Plastic Box, showing 11 subjects in the nitrogen chamber. The circuits lead through the respective amplifiers and terminate in the four direct-writing galvanometers, photographed in the process of tracing electrocardiographic patterns.

a water bottle and then flooded through the chamber escaping at the opposite corner through a tube leading to a water bubbler escape (Figure 8). This provided a water seal, and the slow bubbling indicated the rate of flow of nitrogen and maintained the pressure just slightly above atmospheric. The escaping gas was tested from time to time by a Beckman Model D oxygen analyzer and at no time was the oxygen concentration found to exceed 0.5%.

D. TEMPERATURE CONTROL

The plastic nitrogen chamber was enclosed in a wooden box 2' x 1½' by 2', with the top composed of a ¼" transparent plate which could be removed. The heat was applied by a sixty watt electric light bulb situated beneath a wooden baffle and was regulated by a Fenwall thermostat. Temperatures inside the nitrogen chamber were measured with a thermometer and remained within the range 25.0 ± 1.0° C.

E. PROCEDURE

While the temperature in the chamber was stabilizing and the amplifier was warming up, the baby rats were separated from their mother, weighed, and an identifying number was written on each head. Electrode jelly was placed on the legs which were connected to the electrodes so that the animals were held on their backs with the heads directed towards the center of the panel. The electrical resistance through each animal was then measured and in no case exceeded 6,000 ohms. The four channels of the amplifier were standardized and calibrated to give a deflection of one centimeter per millivolt. A short trial run was made before the chamber was sealed with plasticine. The nitrogen valve was opened and the time recorded as the starting point of the anoxic period. The apparatus was then placed in the constant temperature box.

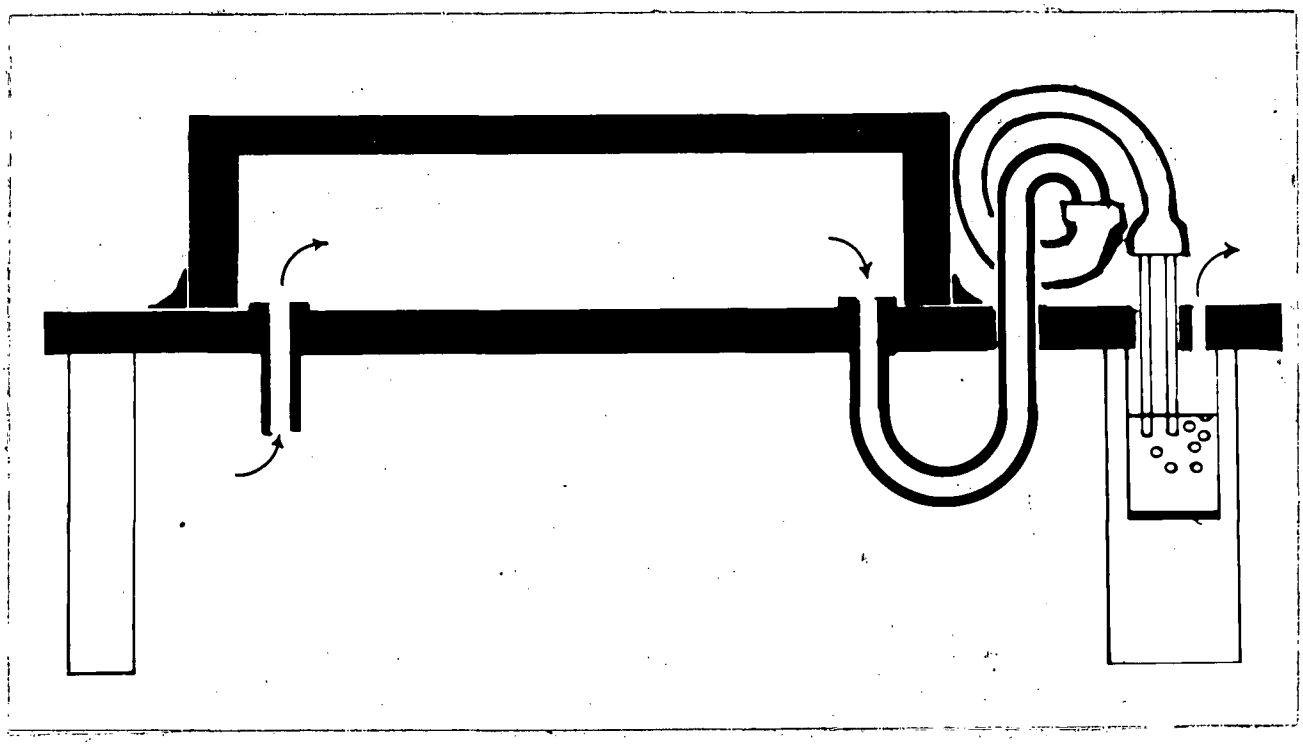

Fig. 8. Airtight Chamber Showing Path of Nitrogen Flow. The gas follows the circuit indicated by arrows. The under-water escape arrangement is shown at right in a well that was bore out of one of the supporting legs of the apparatus.

To follow page 18.

One minute records were run on each rat at intervals of approximately ten minutes. When the amplitude of the deflection corresponding to the QRS complex had diminished to 0.2 centimeters, the amplifier gain on the appropriate channel was increased so that ultimately a point was reached at which the one centimeter deflection corresponded to 0.1 millivolts. The endpoint for any animal was taken as the time of the last recorded electrical activity from the heart. As the endpoint was neared, the frequency of observations was increased to one every five minutes.

A small potential error was introduced by adopting this endpoint, for there may have been a final E.C.G. spike during the five minute interval prior to the next recorded and "silent" run, in which no electrical activity was observed.

However, since the error was uniform in all groups, and was small in comparison with the individual variation in the data, it was felt that it should not seriously affect the validity of the results. Its elimination would have required continuous and simultaneous recordings from all animals during the entire anoxic period.

During the early part of the run, only two or three ECG complexes were recorded on paper during each observation. However, as the endpoint approached, records were made for a full minute. When, finally, two consecutive one minute runs failed to show any recognizable electrocardiographic activity from any member of the litter, the experiment was discontinued. The calibration of the recorder was rechecked, and the apparatus was then removed from the constant temperature box and a final gas sample taken and analyzed to confirm the absence of oxygen in the chamber. The resistance through each animal was also measured to insure that good electrical contact had been maintained throughout the experiment.

Despite careful shielding and grounding of connections the broad band corresponding to a 60 cycle induced current occasionally appeared on the record. In this case the interferance was screened out by turning on the filter circuit contained in the amplifier. Careful attention was also given to any deflections on the record that might have been due to extraneous potentials from neighbouring equipment. These were identified and carefully distinguished from the electrical potentials arising in the heart.

III. OBSERVATIONS AND RESULTS

Following the onset of anoxia, there was a brief period of hyperactivity lasting three to five minutes, during which there were wide fluctuations in the electrical base line with clearly recognizable ECG complexes superimposed. This was followed by a period during which there was no perceptible movement of the animal with the exception of occasional gasps, which occurred irregularly and at progressively longer intervals until, finally, no further activity could be observed. After ten minutes, there was no body activity to disturb the electrical base line, with the exception of occasional gasps, and even these disappeared long before the electrocardiographic endpoint was reached.

The duration of ECG activity was determined as described above, and the individual data have been plotted as a scatter diagram in Figure 9, in which each point represents the results from a single animal. The mean values for each age group and the standard errors of the means have been calculated according to the procedure described by Snedecor* and are represented in Table IV and plotted in Figure 10.

The "t" test of significance was applied to comparison of the observed differences. It was observed that the time of persistent electrocardiographic activity in the first group (up to 12 hours postnatal) was significantly greater than in any of the other groups (probability "p" of the difference occurring by chance was less than 1 in 100 in all cases). The time was double that observed in the four day group. On the other hand, no significant difference ("p" $>$ 0.05) could be demonstrated between the four day group, and the succeeding age groups.

*Snedecor, G.W.: Statistical Methods Applied to Experiments in Agriculture and Biology, Ames, Ia., Collegiate Press, Inc., 1937, pp. 50-55.

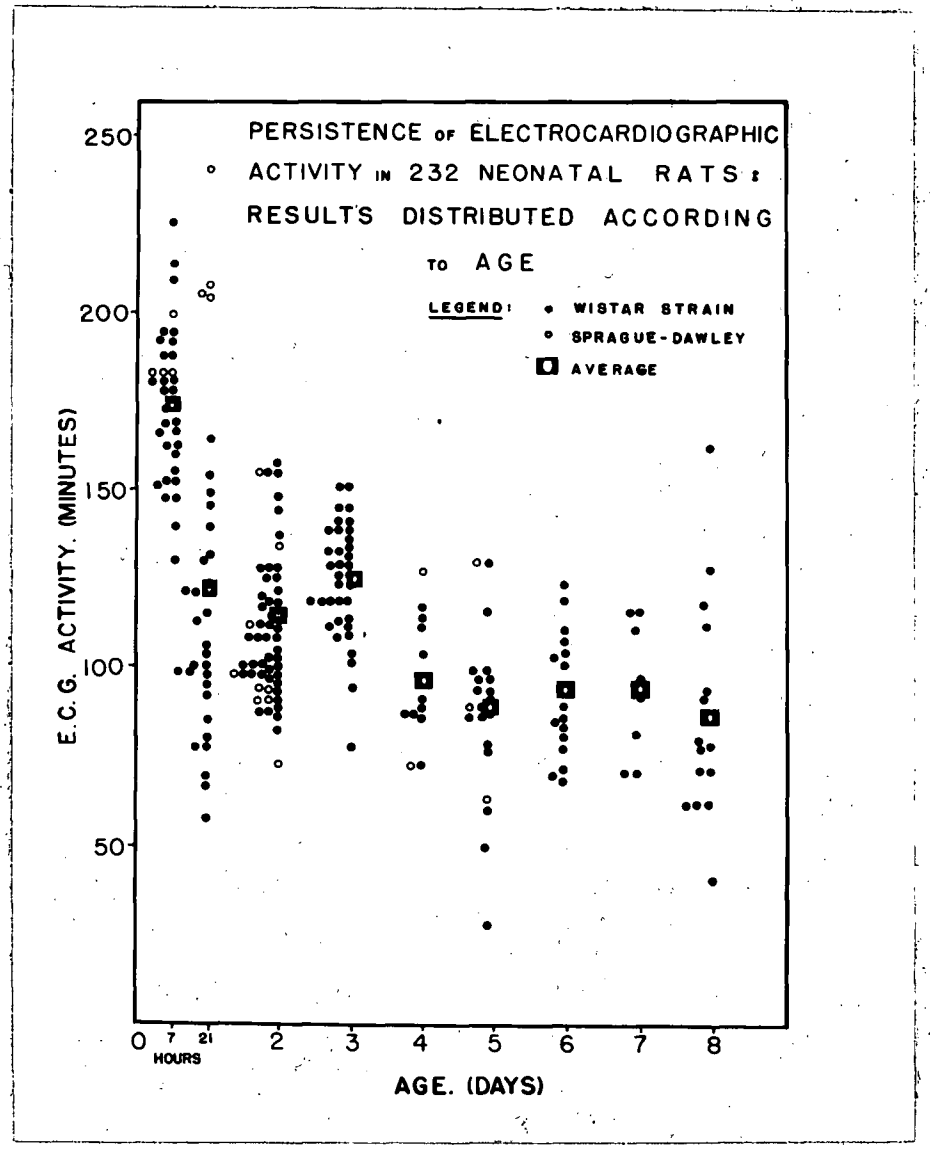

Fig. 9. Scatter Diagram, Showing Individual Data Relative to Persistence of Electrocardiographic Activity.

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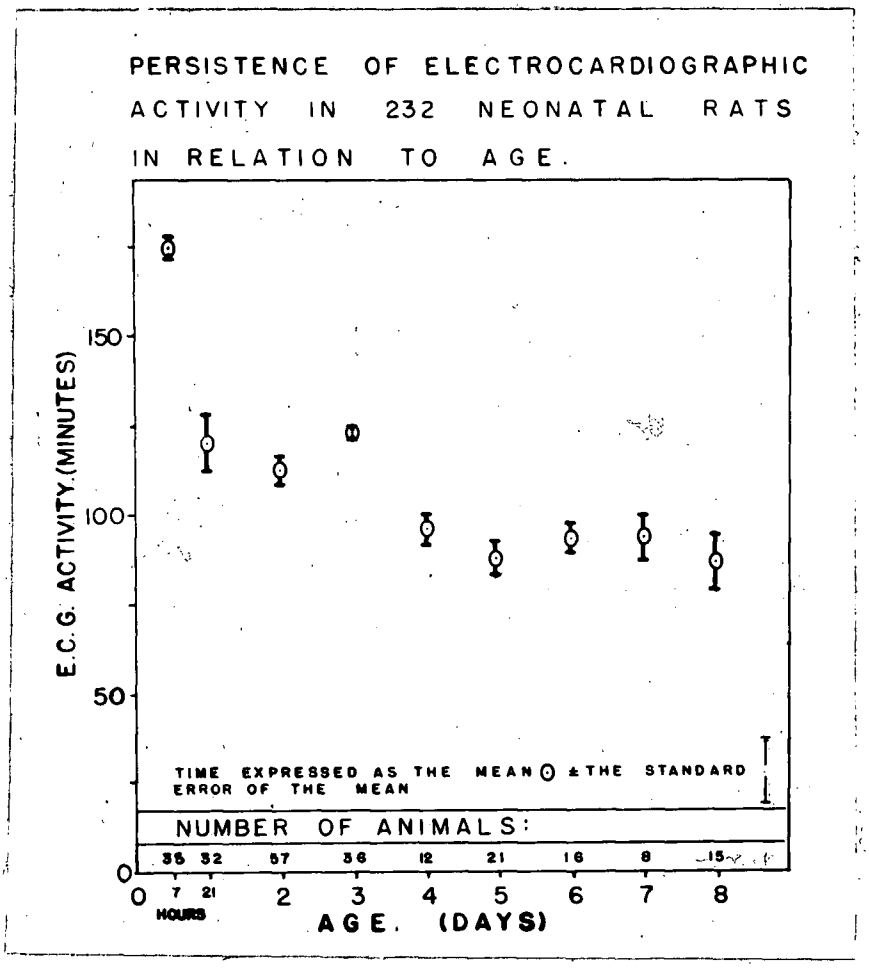

Fig. 10. Chart, Showing Persistence of Electrocardiographic Activity, According to Age Group.

Following page 21.

TABLE IV

PERSISTENCE OF ELECTROCARDIOGRAPHIC ACTIVITY IN 232 ANOXIC NEONATAL RATS.

AGE GROUP	NUMBER IN GROUP	MEAN TIME (Minutes)	± STANDARD ERROR OF THE MEAN
7 Hours	35	174.7	± 3.6
21 Hours	32	121.1	± 8.1
2 Days	57	113.6	± 4.1
3 Days	36	124.9	± 2.4
4 Days	12	95.5	± 4.9
5 Days	21	87.5	± 5.2
6 Days	16	92.9	± 4.6
7 Days	8	94.2	± 6.8
8 Days	15	87.2	± 8.1

IV. DISCUSSION

In this study of 232 neonatal rats rendered anoxic by exposure to an atmosphere of tank nitrogen in a closed chamber, the persistence of electrical activity in the heart appeared to decrease with post-natal age, the sharpest decline occurring in the first day of life. In Table V. the results obtained in this series have been compared with data reported by other investigators on the "survival time" in a number of anoxic newborn animals. There is good agreement between the values obtained in this series, using persistence of ECG activity as the criterion of "survival", and those reported by Selle (40) in which persistence of heart beat was observed in the open chest of a spinal animal. Cardiac activity persists longer than gasping activity in the whole animal or in the isolated head. Under the same conditions, "survival of activity" in rat, mouse and rabbit is similar, while that in the dog and guinea pig is considerably shorter. This may be related to the more mature condition at birth in the latter animals, and particularly in the case of the guinea pig, as has been suggested by Fazekas (14). However, in all species there is a decrease in "survival time" following the first day of life. It may be that this is the result of a shift in metabolic pathways occurring during this period of rapid adjustment to extrauterine life.

TABLE V

PERSISTENCE OF CERTAIN ACTIVITIES IN ANOXIC NEONATAL ANIMALS

Method and Species	Post-Natal Age (Days):								
	0.5 ±	1	2	3	4	5	6	7	8
Gasping activity of the whole animal (18):									
Guinea Pig	4.5	4.5	4	4	3.5	3.5	3.5	3	3
Dog	31				17			14	
Rabbit		27	20	17	13	11	13	9	7
Mandibular movements of the isolated head:									
Rat (43)		27	24					12	
Mouse (49) ± S.E.		27.5 ± 2.2		24.3 ± 1.7				12	9.6 ± 2.1
Cardiac activity (exposed heart) (40):									
Dog		58		42				31	
Rabbit		113		84				58	
Rat		103		96	102			99	
(electrocardiographic):									
Rat (present invest.) ± S.E.	174.7 ± 3.6	121.1 ± 8.1	113.6 ± 4.1	124.9 ± 2.4	95.5 ± 4.9	87.5 ± 5.2	92.9 ± 4.6	94.2 ± 6.8	87.2 ± 8.1

V. SUMMARY

A technique was developed for studying the effect of anoxia in the newborn rat with particular reference to persistence of electrical activity in the heart. In contrast to previous investigations in this field, no drastic surgical procedures were used, and the animals were held in a relatively undisturbed state in a closed temperature regulated chamber which could be filled with a gas mixture of any desired composition. Eleven newborn rats could be placed in the chamber at the same time under the same conditions, and electrocardiographic recordings could be obtained simultaneously from four animals at a time.

Anoxia was produced by flushing and filling the chamber with tank nitrogen (99.9% N_2), and the period of persistence of electrocardiographic activity was determined taking as endpoint the last recorded electrical potential from the heart. Two hundred and thirty-two rats were used, ranging in age from three hours to eight days post-natal. The results obtained agree substantially with those reported by other workers using cruder methods. The "survival time" of electrocardiographic activity in the four day old group was only half that observed in newborn rats less than twelve hours postnatal, the difference being highly significant. However, there was no further significant change in "survival time" during the period from four to eight days.

The technique developed should prove useful in studying many problems of neonatal physiology.

VI. ACKNOWLEDGEMENTS

The author is indebted to Professors D. H. Copp, E. C. Black and J. F. McCreary for their guidance and advice during the period of post-graduate training and in the present project, to Mr. A. J. Honour and Mr. H. Hallam for invaluable technical advice and assistance, and to Miss Katherine Hoskin for all of the photography and the reproductions.

The electroencephalograph was loaned from the EEG Department of the Vancouver General Hospital through the kindness of Dr. N. Auckland. Mr. R. St. L. Ferguson was constantly available to correct maintenance problems and to advise on the detection of artefactual wave forms.

The author is indebted to the National Research Council of Canada for financial assistance provided through a Graduate Medical Research Fellowship.

VII. BIBLIOGRAPHY

1. AGDUHR, E. and N. Stenström: The Appearance of the Electrocardiogram in Heart Lesions Produced by Cod Liver Oil. Acta Pediatrica 8: 493-610, 1928/29.

2. AVERY, R.C. and J. M. Johlin: Relative Susceptibility of Adult and Young Mice to Asphyxiation. Proc. Soc. Exper. Biol. & Med. 29: 1184-1186, 1931-32.

3. BAUER, D.J.: The Effect of Asphyxia upon the Heart Rate of Rabbits at Different Ages. J. Physiol. 93: 90-103, 1938.

4. BELL, W.B., L. Cunningham, M. Jowett, H. Millet and J. Brooks: The Metabolism and Acidity of the Foetal Tissues and Fluids. Brit. M. J. 1: 126-131, 1928.

5. BERT, P.: Leçons sur la physiologie de la respiration, profesées au Museum d'histoire naturelle, Paris, J. B. Ballière et fils, 1870, quoted by HIMWICH, H.E.: Reference (22) of this bibliography.

6. BRINKMAN, R.: Factors Relevant to Foetal and Neonatal Anoxic Tolerance, Anoxia of the Newborn Infant, A Symposium Organized by the Council for International Organizations of Medical Sciences, Established under the Joint Auspices of U.N.E.S.C.O. and W.H.O., 123-126, Oxford, Blackwell Scientific Publications, 1953.

7. BRITTON, S.W. and R. F. Kline: Age, Sex, Carbohydrate, Adrenal Cortex and Other Factors in Anoxia, Am. J. Physiol. 145: 190-202, 1945.

8. COURVILLE, C.B.: Asphyxia as a Consequence of Nitrous Oxide Anesthesia. Medicine 15: 129-245, 1936.

9. CREERY, R.D.G. and T. J. Parkinson: Blood Glucose Changes in the Newborn. Arch. Dis. Child. 28: 134-139, 1953.

10. DIXON, K.C.: The Effect of Rise in Temperature on the Carbohydrate Catabolism of Cerebral Cortex, Biochem. J. 30: 1483-1488, 1936.

11. EASTMAN, N.J.: Foetal Blood Studies. III The Chemical Nature of Asphyxia Neonatorum and its Bearing on Certain Practical Problems, Bull. Johns Hopkins Hosp. 50: 39-50, 1932.

12. EASTMAN, N.J. And C. M. McIane: Foetal Blood Studies. II The Lactic Acid Content of Umbilical Cord Blood under Various Conditions, Bull. Johns Hopkins Hosp. 48: 261-268, 1931.

13. EASTMAN, N.J., E. M. K. Geiling and A. M. DeLawder: Foetal Blood Studies. IV The Oxygen and Carbon Dioxide Dissociation Curves of Foetal Blood, Bull. Johns. Hoplins Hosp. 53: 246-254, 1933.

14. FAZEKAS, J.F., F. A. D. Alexander and H. E. Himwich: Tolerance of the Newborn to Anoxia, Am. J. Physiol. 134: 281-287, 1941.

15. FERGUSON, R.St.L.: Personal communication to the author.

16. FIAGG, P.J.: The Treatment of Postnatal Asphyxia, Am. J. Obst. & Gynec. 21: 537-541, 1931.

17. GIBBERD, G.F.: Mechanism, Prevention and Treatment of Asphyxia in the Newborn Infant, Anoxia of the Newborn Infant, A Symposium Organized by the Council for International Organizations of Medical Sciences, Established under the Joint Auspices of U.N.E.S.C.O. and W.H.O., 26-41, Oxford, Blackwell Scientific Publications, 1953.

18. GIASS, H.G., F. F. Snyder and E. Webster: Rate of Decline in Resistance to Anoxia, Am. J. Physiol. 140: 609-615, 1944.

19. GRAHAM, B.D., Helen S. Reardon, J. L. Wilson, M. U. Tsao and Mary L. Baumann: Physiologic and Chemical Response of Premature Infants to Oxygen-Enriched Atmosphere, Am. J. Dis. Child. 79: 371-372, 1950; Pediatrics 6: 55-71, 1950.

20. HALDANE, J.S.: Quoted by J. Barcroft: Anoxaemia, Lancet 2: 485-489 (Sep.4) 1920.

21. HENDERSON, Y: Quoted by E. S. Taylor, D. Govan and W. C. Scott: Oxygen Saturation of the Blood of the Newborn as Affected by Maternal Anesthetic Agents, Am. J. Obst. & Gynec. 61: 840-854, 1951.

22. HIESTAND, W.A. and Helen R. Miller: Further Observations on Factors Influencing Hypoxic Resistance of Mice, Am. J. Physiol. 142: 310-314, 1944.

23. HIESTAND, W.A., R. D. Tschirgi and Helen R. Miller: The Influence of Glycotropic Substances on Survival of the Primitive Respiratory Center in the Ischaemic Rat Head, Am. J. Physiol. 142: 153-157, 1944.

24. HIMWICH, H.E.: Brain Metabolism and Cerebral Disorders, Baltimore, Williams & Wilkins, Inc., 1951.

25. HIMWICH, H.E., F. A. D. Alexander and J. F. Fazekas: Tolerance of the Newborn to Hypoxia and Anoxia, Am. J. Physiol. 133: 327P, 1941.

26. HIMWICH, H.E., A. O. Bernstein, H. Herrlich, A. Chester and J. F. Fazekas: Mechanism for the Maintenance of Life in the Newborn during Anoxia, Am. J. Physiol. 35: 387-391, 1942.

27. HIMWICH, H.E., J. F. Fazekas and F. A. D. Alexander: Hypoglycemia in the Intact Rat, Am. J. Physiol. 133: 328P, 1941.

28. HIMWICH, H.E., J. F. Fazekas and F. A. D. Alexander: Effects of Cyanide and Iodoacetate on Survival Period of Infant Rats, Proc. Soc. Exper. Biol. & Med. 46: 553-554, 1941.

29. LeGALLOIS, J.J.C.: Experiments on the Principle of Life, and Particularly on the Principle of Motions of the Heart, and on the Seat of this Principle, Translated by N. C. and J. G. Nancrede, Philadelphia, M. Thomas, 1813, Quoted in Reference No. (18) of this bibliography.

30. LeLONG, M.: Foreward, Anoxia of the Newborn Infant, A Symposium Organized by the Council for International Organizations of Medical Sciences, Established under the Joint Auspices of U.N.E.S.C.O. and W.H.O., xi-xv, Oxford, Blackwell Scientific Publications, 1953.

31. LeLONG, M. and R. Laumonier: Histological and Histochemical Evolution of the Foetal Lung: Its Relation to Anoxia in Premature Infants, Anoxia of the Newborn Infant, A Symposium Organized by the Council for International Organizations of Medical Sciences, Established under the Joint Auspices of U.N.E.S.C.O. and W.H.O., 61-68, Oxford, Blackwell Scientific Publications, 1953.

32. LOMBARD, Elna A.: Electrocardiograms of Small Mammals, Am. J. Physiol. 171: 189-193, 1952.

33. MAYER, M.: Clinical Correlations in Prenatal and Postnatal Anoxia, Anoxia of the Newborn Infant, A Symposium Organized by the Council for International Organizations of Medical Sciences, Established under the Joint Auspices of U.N.E.S.C.O. and W.H.O., 1-25, Oxford, Blackwell Scientific Publications, 1953.

34. McGINTY, D.A.: The Regulation of Respiration, XXV Variance in Lactic Acid Metabolism in the Intact Brain, Am. J. Physiol. 88: 312-325, 1929.

35. MILLER, J.A.: Factors in Neonatal Resistance to Anoxia, I Temperature and Survival of Newborn Guinea Pigs under Anoxia, Science 110: 113-114, 1949.

36. MORISON, J.E.: Foetal and Neonatal Pathology, London, Butterworth, 1952.

37. RICHARDS, A.G., E. Simonson and M. B. Visscher: Electrocardiogram and Phonocardiogram of Adult and Newborn Mice in Normal Conditions and under the Effect of Cooling, Hypoxia and Potassium, Am. J. Physiol. 174: 293-298, 1953.

38. SCHREIBER, F.: Apnea of the Newborn and Associated Cerebral Injury, J.A.M.A. 111: 1263-1269, 1938.

39. SEGAL, S.: Unpublished data.

40. SELLE, W.A.: Influence of Age on Survival of Respiration, Spinal Reflexes, Pupillary Responses and Heart Action, Proc. Soc. Exper. Biol. & Med. 48: 417-419, 1941.

41. SELLE, W.A.: Effects of Various Chemical Agents on Survival of Primitive Respiratory Mechanism, Proc. Soc. Exper. Biol. & Med. 51: 50-52, 1942.

42. SELLE, W.A.: A Simple Technique for Studying the Periodicity and Survival of the Respiratory Center, Proc. Soc. Exper. Biol. & Med. 54: 291-292, 1943.

43. SELLE, W.A.: Influence of Glucose on the Gasping Pattern of Young Animals Subjected to Acute Anoxia, Am. J. Physiol. 141: 297-300, 1944.

44. SELLE, W.A. and T. A. Witten: Survival of the Respiratory (Gasping) Mechanism in Young Animals, Am. J. Physiol. 133: P441, 1941.

45. SELLE, W.A. and T. A. Witten: Survival of Respiratory (Gasping) Mechanism Subjected to Anoxia, Proc. Soc. Exper. Biol. & Med. 47: 495-497, 1941.

46. SMITH, C.A.: The Effect of Obstetrical Anesthesia upon Oxygenation of Maternal and Foetal Blood, with Particular Reference to Cyclopropane, Surg. Gynec. & Obst. 69: 584-593, 1939.

47. SMITH, C.A. and E. Kaplan: Adjustment of Blood Oxygen Levels in Neonatal Life, Am. J. Dis. Child. 64: 843-859, 1942.

48. SWANN, H.G.: Studies in Resuscitation, Section III, Asphyxia Neonatorum, AF Technical Report No. 5972, U.S. Air Force Air Materiel Command, Wright-Patterson Air Force Base, Dayton Ohio, Aug. 1949.

49. THOMS, R.H. and W. A. Hiestand: Relation of Survival Time of the Respiratory Gasping Mechanism of the Isolated Mouse Head to Age, Proc. Soc. Exper. Biol. & Med. 64: 1-3, 1947.

50. TYLER, D.B. and A. VanHarreveld: The Respiration of the Developing Brain, Am. J. Physiol. 136: 600-603, 1942.

51. WARBURG, O., K. Posener and E. Negelein: Über den Stoffwechsel der Carcinomzelle, Biochem. Ztschr. 152: 309-344, 1924, Quoted in Reference No. (24) of this bibliography.

52. WEGMAN, M.E.: Trend in Infant Mortality Rates, Pediatrics 6: 673-675, 1950.

53. WILSON, J., Helen Reardon and M. Murayama: Anaerobic Metabolism in the Newborn Infant, Pediatrics 1: 581-592, 1948.