A STUDY OF ANOXIA TOLERANCE IN NEWBORN PUPS USING DIRECT AND INDIRECT CALORIMETRY

by

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INTRODUCTION

The present thesis is an attempt to study the energy metabolism of newborn pups subjected to anoxia. More specifically, the aim is to evaluate the relative role of anaerobic and lowered metabolism in anoxic pups. This is because at present the many lines of evidence for both of these alternatives do not enable one to decide on the quantitative importance of either. Thus, the possibility that 80% of the pre-anoxic energy metabolism is maintained by anaerobic means seems equally plausible and likely as the possibility that there is an 80% decrease in energy metabolism during anoxia.

To study the energy metabolism of anoxic newborns, two principle techniques were employed. These were the methods of direct and indirect calorimetry. The details of the methodology and the results it yielded will be presented after a review of the pertinent literature.

HISTORICAL

Knowledge of the fact that infant mammals can survive longer periods of anoxia than adult members of the same species extends back several centuries. In 1651, William Harvey reported on the delivery of viable infants by caesarean section some time after the death of their mothers (44). The earliest experimental data on the neonate's anoxia tolerance appears in the writing of Robert Boyle (12). In 1670 Boyle wrote that:

"These tryals may deserve to be prosecuted with further ones, to be made not only with such kittens, but with other very young animals of different kinds; for by what has been related it appears, that those animals continued three times longer in the Exhausted Receiver, than other animals of that bigness would probably have done."

Robert Boyle also demonstrated that in a vacuum the flame of a candle or the life of an animal were extinguished (97). At this time the difference in anoxia resistance between adult and newborn mammals could not be fully appreciated because Boyle's observations preceded the concept of oxidative metabolism by more than a century.

By 1772 the respiratory gases had been isolated. Although Priestly contributed to their discovery, he interpreted respiration as "the phlogistication of dephlogisticated air" (44). In 1775 Antoine Lauret Lavoiser proposed that respiration is a combustion process, in which oxygen is combined in the body to form carbon dioxide and water, and

that this process is the source of animal heat (97). Although Lavoisier recognised the chemical identity of the oxidative process of carbon in a candle flame and of carbon in a guinea pig, he had erroneously assumed that oxidation occurs in the lungs.

The writing of Sir Ashley Cooper in 1790 are the first to call attention to the remarkable effect that varying temperature has on the effect of drowning newborn mammals (36). He submerged puppies and kittens for 10 minutes in water at four different temperatures. The animals submerged in the warmer baths were more active initially, but became inactive sooner than those placed in the cold bath. After the pups and kittens had been immersed for 10 minutes, he exposed their viscera. Animals that had been in baths of 100°F, or warmer, showed either no or only momentary myocardial and intestinal muscle activity while those submerged in 56°F, or colder water, showed myocardial activity for 19 to 30 minutes. This demonstration that cold enables the newborn and his individual organs to function for longer periods of anoxia has been confirmed repeatedly.

In 1812 Julien Legallois conducted a series of experiments on death by asphyxia (74). He concluded that "The existence of the individual is produced by a certain impression of the arterial blood" upon the tissues and that "Life can only be maintained by the continual renewal of this impression". Although he knew that the production and circulation of arterial blood depend on pulmonary and cardiac function respectively,

he could not define the nature of this "impression". He, like Lavoisier, was ignorant of the role the circulation plays in the exchange of respiratory gases.

Legallois demonstrated that the ability to survive a temporary deprivation of this "impression" depends on the particular species and diminishes rapidly during early infancy. He found that, "the asphyxia which rabbits can bear is about seven times longer at the moment of birth than at the adult age; it is nearly the same with dogs and cats; whereas the newborn guinea pig can only bear one which is scarcely double of that supported by the adult (74). He related this to the guinea pig having about twice as long a gestation period as the rabbit and being so mature that they can run and "chew grass on the day of their birth".

Taking cessation of gasping movements of the jaw as a criterion of death, Legallois found similar survival times when the production of "the impression" on the brain was interferred with either by submerging, removing the heart or opening the thorax.

This prolonged activity of the medullary respiratory centers[1] despite the interruption of the circulation points to a local tissue resistance to anoxia. Legallois also showed that decapitation produces asphyxia of head and trunk and that the two separated segments show

[1]He had discovered "that respiration depends upon a particular and small part of the medulla oblongata situated at a small distance from the occipital foramen and near the origin of the pneumogastric nerves" (74).

signs of life as long as they would if they were not disjointed but asphyxiated together. By maintaining the trunk on artificial respiration, he was able to elicit signs of life from it for several hours.

Legallois also recognized the importance of temperature in determining the anoxia resistance of mammals. He wrote "Cold modifies and protracts the phenomena of asphyxia, in a very remarkable manner, in very young animals. This is a curious fact, capable of important applications to the human foetus; and connected to the theory of hibernation in certain animals" (74). He intended to pursue this observation further, however, two years later he came to an untimely death at the age of 44.

In the first part of the nineteenth century, W. F. Edwards established a correlation between an animal's ability to resist anoxia and its response to a cold stress. He found that "If at the temperature between 10° and 20°C a new-born puppy be removed and kept an hour or two from its mother, its temperature falls considerably, and continues falling until, in the course of three or four hours, it stops a very few degrees above that of the surrounding air" (36). He determined that kittens, rabbits, and young sparrows show this same poikilothermic behavior until "the period of the opening of their eyes." Each of these poikilothermic animals was able to survive anoxic periods of about 30 minutes of anoxia. He strengthened his contention that poikilothermia and anoxia resistance are associated by showing that hibernating mammals, fishes, reptiles and amphibians, also have a relatively high

anoxia tolerance.

Edwards also confirmed the finding that cooling increases anoxia tolerance, however marked cooling below 10°C reduced survival in the newborn kittens.

The relation of anoxia resistance with poikilothermia plus the demonstration that shorter periods of anoxiaare lethal at warmer temperatures led him to the conclusion "that heat, whether internally or externally produced has the same influence on the duration of life in asphyxia" (36). He felt that "an individual in a state of asphyxia" should be cooled. In contrast to current teachings he wrote that, "If instead of cold, continued warmth were to be applied it would be one of the most effectual means of extinguishing life."

In 1832 Thomas Nunnelly described the process of recovery in kittens that had been submerged in 57°F and 100°F water for three and one half to ten minutes. Immediately after they were removed from the bath, the kittens were profoundly depressed, as evidenced by inactivity and slow gasping respirations. He pointed out that the longer the submersion the more pronounced the depression and the slower the recovery. Kittens submerged in the warm bath were more depressed and took longer to regain their ordinary temperatures than those kept in the colder water for an equal time (36).

The next investigator to call attention to the effect of temperature on anoxia resistance was Paul Bert who wrote in 1870, "that cat litter mates submerged in water at 20° survived 26 minutes, while at 36 degrees the time of survival was only 11½ minutes" (45).

Later Paul Bert (11) published data which show several distinguishing features between asphyxia in one month and three day old kittens. The asphyxia was produced by placing cats in closed vessels containing air at pressures from 750 to 134 mm Hg. He found that given the same amount of air per unit of body weight three day old kittens require about four times as long to asphyxiate as one month old cats. These experiments suggest two factors that may contribute to the increased ability of the young kitten to resist asphyxia. His data enables one to calculate the average rate of oxygen consumption during the period of asphyxia. The oxygen consumption per kilogram per unit time was four times greater in the one month old cats than in the three day old cats. The other finding was that the younger animals were able to extract relatively more oxygen from the environment. Thus, the lethal oxygen tension for the older group was 33 ± 3 mm Hg while for the three day old kittens it was 17 ± 1 mm Hg. The point of death for the cats in each group regularly came at these lethal oxygen tensions despite four fold or greater variations in chamber size, ambient pressure and carbon dioxide concentration.

Despite these early observations on the remarkable ability of newborn mammals to survive long periods of anoxia, compared to the adult a comprehensive explanation of the mechanisms involved has not been presented to date. However, twentieth century investigators have proposed a host of theories which will be reviewed in the subsequent section.

ANOXIA RESISTANCE AND IMMATURITY

During the first half of this century a number of studies appeared which confirmed the earlier observation that immature animals as judged by phylogeny or ontogeny have increased anoxia tolerance. With this finding there has also been a search for the particular distinguishing features which account for the difference in anoxia resistance.

The effect of age

Legallois found an 80% decline in the rabbit's anoxia resistance during the first ten days of life. Similar rapid decreases in anoxia tolerance have been demonstrated for other species by experiments conducted more than a century later.

The ability to survive anoxia at birth and the rate of decline of tolerance thereafter depends on the species. Most of the disparity in results reported by investigators working on the same species can be attributed to non-uniformity of experimental conditions. Thus, differences in ambient temperature, method of producing anoxia and criterion of death all will be reflected in the results.

Because the decline in anoxia resistance is so pronounced immediately after birth, some investigators have suggested that anoxia tolerance is an adaptive response to the hypoxic conditions of intra uterine life. Thus, with the escape from this hypoxic environment, at birth, the adaptive response is lost. A study by Glass et al (45) is of particular interest in this respect. They determined the time of the last gasp in newborn rabbits placed in nitrogen after gestation periods

ranging from 29 to 35 days. (In the rabbit, the gestation period can be known with accuracy since ovulation regularly occurs 10 hours after mating. The normal gestation period is 32 days.) It was found that premature rabbits had a greater anoxia tolerance than term rabbits. Rabbits that were one, two, and three days postmature had exactly the same decrease in survival time as term rabbits that had lived a corresponding time in the nest. Thus, the decline in anoxia tolerance is not a function of parturition age but is a function of conceptual age.

The same increased anoxia resistance of the premature has been found in monkeys (67, 122), lambs (30), and rats (14). Mabry (77) has reported on a 3 lb 4 oz infant who at the time of birth suffered 15 to 30 minutes of "total anoxia". Several psychologic and neurologic evaluations during the 20 months since then have failed to reveal a significant deviation from the norm. This baby demonstrated more than the usual anoxia tolerance, perhaps because he was an estimated five weeks premature. That postmature infants have diminished anoxia tolerance is suggested by the data of Walker (118) who found that, "Death from anoxia in association with difficult labour is extremely uncommon until after term, and 83% of all such deaths occur after the 41st week." This high incidence of anoxic deaths in postmature babies cannot be explained solely on the basis of more difficult labours with increasing fetal size, because the rise in difficult labours was much less than the increase in anoxic deaths with increasing maturity.

The effect of species

As Legallois stated the ability to withstand anoxia at birth depends on the maturity of the newborn. Thus, species such as the guinea pig which are relatively mature at birth survive only six minutes under anoxic conditions that allow the less mature rat to live 50 minutes. It is difficult to put a quantitative estimate on maturity, but qualitative criteria such as the amount of fur, eyes open and ability to maintain balance, place the several species tested in the same approximate sequence as they fall when listed according to decreasing anoxia resistance.

Estimates of the human infant's anoxia resistance range from 15 (75) to 30 minutes (118). The wide range probably reflects different degrees of anoxia and varying gestation periods. Since viable infants are not left unventilated willfully, the estimates of the human's anoxia resistance is based on unusual circumstances. An example of the sort of evidence upon which the estimate of human anoxia tolerance is based is an account of a viable infant being removed from the uterus 15 to 20 minutes after the mother suffered an accidental death (92).

In the rat and other species investigated, it has been shown that the time of death is very nearly the same regardless of whether anoxia is induced with nitrogen, helium, carbon monoxide, argon, hydrogen, nitrous oxide, cyanide, or submersion (140). In all these circumstances and as Fazekas writes, "in all species there is a loss of tolerance at a rate which seems to depend upon post natal development" (40). For example,

"the rapidly developing rat at 18 days of age exhibits the diminished adult tolerance while the more slowly maturing dog is more resistant than the adult at 30 days of age" (40).

That hypoxia tolerance is affected similarly by increasing age and more mature species was shown by Britton and Kline (14). Hypoxic conditions that enabled adult rats to survive 2.5 hours were tolerated by newborn rats for 16.5 hours. Young cats, dogs and rabbits also had a decline in hypoxia tolerance during the first few days of life toward the adult level. The most resistant were opossums of five days pouch age which lived for 50 hours in an atmosphere that kills the adult opossum in 40 minutes.

Resemblance of the newborn's nervous system to that of primitive animals

The nervous system of new-born animals appears to have a greater resistance to anoxia than that of adult mammals. This has been demonstrated by Kabat (69) who found that arrest of the cephalic circulation in adult dogs for more than six minutes is regularly associated with irreversible damage or death. Pups ten days of age on the other hand, had complete recovery after 20 minutes of cephalic ischemia. Not only could younger pups recover after longer periods of stasis, but the rate of recovery was much more rapid, within a day while the adult may show reversible residual effects for weeks. Another distinguishing feature was the persistence of nervous activity in the newborn throughout the period of ischemia while adult dogs go into a state of profound spinal shock after two minutes of arrested cephalic blood flow. Thus, Kabat

observed that "respirations continued during brain stasis for 20 to 30 seconds in the adult dog and five minutes in dogs eight to ten days of age" (69). The persistent activity of the newborn is not limited to respiration, but has been shown to apply to the corneal and flexion reflex. In ten day old pups "the flexion reflex could be elicited throughout the period of cephalic stasis, even though the stasis was continued for as long as 35 minutes" (69). Just as the head of newborn dogs, rats and mice (110) show persistent activity when circulation is interrupted; it is possible to elicit reflexes from the trunk of the mouse for extended periods. The newborn mouse has spinal reflexes in response to electrical stimulation for about 20 times as long as the 15 day old mouse (38).

The greater resistance of the newborn's brain has been viewed by Kabat as "another example of ontogeny recapitulating phylogeny", because lower forms of animals are known to be more resistant to anoxia and to spinal shock (69).

The role of perinatal anoxia in determining later mental function is currently receiving much attention. Some authors maintain that the anoxic newborn will either succumb or recover completely while others ascribe many of the neurologic deficits of older children to birth asphyxia. It seems reasonably certain that the statement by Schreiber from 1940 is no longer tenable. "Considerable clinical evidence supports the conclusion that the brain tissue of an infant can sustain much less oxygen deprivation than can the adult organ and, therefore, is more readily damaged from this particular cause than is adult cerebral tissue" (99).

Some aspects of immaturity which may be pertinent to anoxia resistance

Since immature animals differ from their mature counterparts in many respects, it is not difficult to find parameters which correlate with anoxia resistance. However, a correlation does not distinguish a causal from a coincidental association.

Newborn rats show an appreciable rise in the cholinesterase activity of the frontal cerebral cortex during postnatal development, which is inversely related to the decrease in anoxia tolerance (46). Other enzymes (such as succinic dehydrogenase, cytochrome oxidase and aldolase) as well as the cerebral oxygen consumption (40) do not increase markedly until the tenth day when anoxia resistance has already decreased from 50 minutes to ten minutes. Furthermore, thyroidectomy inhibits the cholinesterase rise and enhances the anoxia resistance. Also, in the guinea pig where anoxia sensitivity is high at birth, the rise in cerebral cholinesterase activity has already occurred in utero (46).

Britton and Kline (14) have suggested that "The relatively large size of the adrenal glands may be a factor in the superior resistance of young animals." They support this with the observation that six to seven day old rats have a 40 to 80% decrease in hypoxia tolerance after adrenalectomy when compared to sham operated controls. One-third grown rabbits had a slight increase in tolerance when treated with cortico-adrenal extract. These findings are consistent with the suggestion by Legallois, 150 years ago, that the newborn's asphyxia tolerance is "connected to the theory of hibernation in certain mammals" (74) for it has been found that "the adrenalectomized animal does not hibernate and dies

of cold as nonhibernants do" (72).

It has recently been suggested that the normally low prothrombin level of the newborn may help protect him from anoxia (33). This suggestion is based on the finding that during circulatory arrest "blood coagulates in the small vessels" and if adult dogs are given heparin or Varidase, a fibrinolytic activator, they can survive longer periods of stagnant anoxia and recover much more rapidly with fewer residual effects (27). Control dogs succumbed to ten minutes of circulatory arrest while some Varidase treated dogs had complete recovery after 15 minutes of the same insult. These authors propose that the mechanism of anoxic brain damage may involve the formation of multiple infarcts because of clot formation secondary to anoxic acidosis. That anoxia resistance is promoted by anticoagulants is of interest since the typical pathologic lesion of anoxia is perivascular hemorrhages.

Richter has promoted the non-functional state of the newborn's blood brain barrier as contributing to anoxia tolerance. "In the infant brain the lactic acid formed can quickly escape; but in the adult brain that may be considerably delayed by the blood-brain barrier, so that lactic acid accumulates; and values as high as 82 mg lactic acid/100 g brain have actually been found" (75). In the guinea pig the barrier to ferro cyanide is fully established at birth, whereas the permeability to this ion is high in the newborn mouse, rat, rabbit, cat, and dog, but decreases rapidly during the first days of life" (75). The newborn human and especially the premature has been shown to have a particularly

permeable blood-brain barrier which is also thought to render them susceptible to kernicterus.

There are a host of other unique attributes of the immature newborn which may play a role in his ability to withstand anaerobic conditions. An example is non-fusion of the cranial bones which may protect vital centers from the pressure of cerebral edema. Thus, anoxia produces an overall increase of 5 to 6% in the brain volume (75). Prevention of this cerebral edema by the administration of 50% intravenous sucrose has been shown to partially protect adult dogs and humans from the cerebral damage associated with hypoxia (23).

The theories presented up to this point have related the newborn's anoxia resistance to some aspect of immaturity. Although most of these studies have yielded interesting results, they also have shortcomings. There is a danger to mistake a correlation between anoxia resistance and some aspect of immaturity for a demonstration that the two are causally related. To establish a causal relation between a particular attribute and anoxia resistance, it is necessary to vary that one parameter while all others are held constant. Thus, the selection of a control animal becomes critical. Most of the studies on immaturity have compared newborns with more mature animals who differ from the newborn in many respects besides the one that is under question. It does not suffice to show that fetal hemoglobin, poikilothermic response, sparse fur, eyes still closed, and immature central nervous systems all correlate with anoxia resistance. Much more convincing is the comparison

of the anoxia resistance between groups of newborns who differ from each other only with respect to the one attribute that is being evaluated.

During the last two decades an increasing number of investigations have changed their emphasis from the general question of the relation between immaturity and anoxia resistance to the more specific question of what happens to the energy metabolism of the anoxic newborn.

Although this approach is just now being exploited, its genesis dates back to 1780 when Lavoisier demonstrated that respiration is the combining of oxygen with carbon and hydrogen and that this chemical combination is the source of energy. In general, the studies of the anoxic newborn's energy metabolism have proven to be a more direct approach to the problem and have been more amenable to the introduction of adequate controls.

Most of the proposed answers to the question of what happens to the anoxia newborn's energy metabolism can be placed into one of two schools of thought. The proposition championed by Himwich and others is that newborn mammals can survive several times longer without oxygen than adults because they obtain relatively more energy from anaerobic metabolism. The other thesis has emphasized not anaerobic metabolism, but a reduction in metabolism which can be thought of as a state resembling hibernation or suspended animation. In the next two sections the literature that is pertinent to each of these contentions will be reviewed.

ANOXIC MOBILIZATION OF ANAEROBIC ENERGY SOURCES

"Just a century ago Pasteur showed that the production of ethanol from glucose by yeast can occur under anaerobic conditions" (116). He emphasized the importance of the finding that living tissues can derive energy from glucose in the absence of oxygen. Meyerhof was one of the first to demonstrate that extracts of mammalian tissues can obtain useful energy in a similar manner by converting glycogen to lactic acid under anaerobic conditions.

The current concept of anaerobic metabolism is that most of the reactions of intermediary metabolism do not require molecular oxygen as a reactant and that they will continue in its absence (115). The energy yielding reactions require that hydrogen atoms and electrons be removed from substrate molecules and transferred to some acceptor. Under aerobic conditions the eventual acceptor is oxygen which combines with hydrogen to form water. In the absence of oxygen the oxidative enzymes become saturated with hydrogens and will remain in the reduced state unless there is another hydrogen acceptor.

The most generally agreed on anaerobic hydrogen acceptor in mammalian tissues is pyruvic acid, which can be converted to lactic acid by the addition of two hydrogen atoms. There are experimental results which would lead one to suspect the existance of other hydrogen acceptors which may be of greater quantitative significance (4). However, these acceptors have not been identified nor have they been demonstrated to play a role in the anoxic newborn.

As Villee has pointed out there is considerable evidence which suggests that "the fetus may owe its well-known ability to survive relatively long periods of anoxia during the birth process to energy derived from glycolysis" (114). The most direct evidence for the process of anaerobic glycolysis has come from the measurement of a decrease in the substrate, glucose, and an increase in the product, lactic acid, during anoxia.

Anoxic carbohydrate utilization

Villee has found that the fetal "brain has essentially no glycogen, but both liver and lung have rich stores of glycogen and enzymes to convert it to blood glucose and hence make it available to the brain" (114).

That these elevated levels of tissue glycogen actually correlate with anoxia resistance was shown by Dawes et al (30). They found a linear relationship between the mean survival time in nitrogen of guinea pigs, rabbits, and rats at varying ages and the total initial carbohydrate concentration in the cardiac ventricles. By sacrificing fetal lambs after varying periods of asphyxia, he demonstrated that there is a progressive decline in the cardiac and liver carbohydrate content and increase in the lactate concentration. Lambs of 121 to 146 days gestation age (term is about 147 days) showed biochemical plus histologic evidence of decreases in liver and cardiac carbohydrate content "to less than 50% of the control value within 15 to 25 minutes of tying the cord" (30). "Only in the liver and heart was there any suggestion that carbohydrate reserves had been exhausted in the terminal stages of anoxia" (30). The same preferential depletion of cardiac and hepatic glycogen during anoxia

has been shown to occur in newborn rats (71) and mice (49).

Lamb fetuses closer to term had a shorter anoxia resistance and lower initial cardiac and liver carbohydrate reserves which were depleted at a more rapid rate than those of younger fetuses (30). The results from other investigators also support the notion that the more immature mammals have relatively greater carbohydrate energy reserves which are consumed at a slower rate during anoxia" (106). Dawes "concluded that the maintenance of the circulation is of primary importance in anoxia", because "the decrease in cardiac carbohydrate with age is, to date, the only known change which exactly parallels the decrease in tolerance to anoxia" (30).

Subsequent work on newborn rats (106) and monkeys (29) lends support to the correlation between cardiac glycogen and anoxia resistance. The decrease in cardiac carbohydrate concentration had the same pattern as the decrease of survival time in nitrogen during the first 17 days of life. In contrast, liver carbohydrate fell rapidly to a minimum after the first day, at which age the survival time was still high, and later increased as the survival time fell (105). Rats exposed to the limit of their anoxia tolerance had almost depleted their heart carbohydrate. In the liver and blood the change in carbohydrate concentration was variable and in skeletal muscle, lung and skin it fell only slightly during anoxia (106). It was further found that fasting rats had a greater fall (about 40% with 12 hours fasting) in anoxia tolerance and heart carbohydrate than non-fasting litter-mates. After three, six, and twelve hour fasting, the percentage reduction in survival time and

in heart carbohydrate were closely similar; the percentage fall in liver carbohydrate was much greater (106).

Following a 25 minute anoxic episode at 36°C (where 28 min is fatal for the newborn rat) there is a gradual restoration of the cardiac carbohydrate to the control level during the first three hours of recovery. The resistance to a second anoxic episode during this recovery period increases in the same proportion as the increase in concentration of the heart carbohydrate (106). The concentration of carbohydrate in the liver remained low during the four hours after anoxia, although the blood lactic acid fell from a mean of 148 mg/100 ml to normal levels (10 mg/100 ml) within two hours (106). In summary, the work of Dawes and others show that anoxia resistance correlates with the heart carbohydrate concentration regardless of whether the latter is altered by age, species differences, starvation, glucose administration or previous anoxia.

However, this does not explain the observation that newborns can resist circulatory arrest to the head for longer periods than adults, which has been demonstrated in rats (55, 100), foetal monkeys (29), rabbits (74, 100), mice (96), and pups (69, 100).

The events leading to persistent respiration when the cephalic circulation is obstructed have been called the "tissue factor" in anoxia resistance. That there is also a "circulatory factor" is evidenced by the observation that the duration of gasping is doubled when the animal is asphyxiated with an intact circulation (28). The correlation between cardiac carbohydrate and anoxia resistance may rest in part on the "circulatory factor" bringing nutrient and removing metabolic products from the brain.

The importance of anaerobic glycolysis in promoting the newborn's anoxia resistance has been further established by determining the effect of altered blood glucose levels on anoxia tolerance. Thus, Himwich (58) working on rats found that "the survival period of infant animals eight to ten days of age is prolonged from 7 to 15 minutes, if they receive glucose before the inhalation of nitrogen." Since the adult rat does not show an increase anoxia tolerance with glucose injections (59), the factor limiting its survival is probably not limited glucose stores. The beneficial effect of glucose injection in young rats is most pronounced if the blood glucose is low initially or if the rat has had only one hour to recover from a previous anoxic episode (106). These experiments indicate that hyperglycemia facilitates the newborn's anoxia resistance; that hypoglycemia impairs it has also been shown.

In 1941 Himwich demonstrated that anoxia and hypoglycemia act synergistically in the newborn. "The survival time of newborn rats injected with insulin and subjected to the inhalation of nitrogen was reduced from 50 minutes to 25 minutes" (58). The 25 minutes of anoxia survival is still about 20 times the resistance of the adult rat. The effect of insulin to reduce, but not eliminate the newborn's anoxia resistance, was also shown in cats (59). This may be related to the observation that newborns develop less of a decrease in blood sugar with insulin injection and they have a greater ability to withstand hypoglycemia (58, 59). Thus, newborn rats given 600 to 2000 units per kg of insulin have a decrease in blood sugar from 91 mg% before injection to 36 mg% one hour later. They continue to live for periods of 10 to

16 hours in air while adult rats succumb within one and three-fourths to five hours after receiving 5 to 40 units per kg (59). By injecting insulin treated rats with glucose their anoxia resistance can be restored to normal (59).

It would appear that the effect of insulin is not so much on the myocardium, but more on the brain itself. Thus, although insulin in the dose of one unit per gram caused a marked decrease in blood glucose there was little effect on the heart carbohydrate concentration. Rats treated with this dose of insulin had a 50% decrease in the duration of gasping, but "the times for which the heart continued to beat were not significantly different" from saline injected controls (106). Asphyxiated mice also showed only scant or partial depletion of glycogen in the heart and liver if death was hastened by insulin injection (50).

That hyper and hypoglycemia have a direct action on the newborn brain was also suggested by W. A. Sallo (101) who rendered 12 to 15 day old rats hyper and hypoglycemic and then noted the duration of the gasps of the isolated head. The rats were divided into groups of 18 which were given different subcutaneous injections. He found that when young rats are decapitated there is an initial series of about eight rapid gasps followed by an apneic period of about 40 seconds. After this there is a second slower series of gasps which he calls the anaerobic series because its duration appears to depend more on age and carbohydrate stores available to the brain at the time of guillotining. By injecting insulin, he could reduce the duration of gasping by 92% whereas glucose injection

extending the gasping period by 30%. Rats receiving insulin plus glucose did not respond significantly different than saline injected controls.

Anoxic lactic acid production

In 1931 Eastman and McLane found that moderate birth asphyxia produced a two fold rise in umbilical vein and artery blood lactic acid levels (35). Since then the rise in serum lactate with anoxia has been demonstrated repeatedly in man, lamb (13, 15), mouse (95), rat (71, 95), cat, dog, hog, and rabbit.

That the lactic acid rise is not solely a shift from the extravascular fluids to the vascular compartment, but rather a production of new lactic acid was shown by Himwich in 1942. He reported that 40 minutes of anoxia raises the newborn rat's total lactic acid concentration from 40 mg% to 145 mg% (35).

Dawes (30) has observed three fold rises in the blood lactate of fetal lambs after interrupting gas exchange by tying the umbilical cord. It appears that the rate of lactate rise, just as the rate of carbohydrate depletion, becomes progressively less as anoxia is prolonged. Earlier work by Reiss (95) on rats and mice subjected to carbon monoxide poisoning also indicates that the rate of anaerobic glycolysis diminishes during the course of anoxia.

The finding that lactic acid concentrations in the anoxic newborn attains 500% increases while adult mice had only a 31% rise (95) has led some investigators to attribute the newborn's anoxia resistance to higher rates of anaerobic metabolism. However, the initial rate of

lactate accumulation and carbohydrate depletion are greater in older animals (30). This would suggest that although the anoxic newborn has relatively more total lactate accumulation this is attained by glycolysis at a slower rate and for a longer time than in the adult. In vitro studies confirm the finding that newborn tissues have a lower rate of glycolysis than adult tissues (22).

Because the rate of energy metabolism is less in newborn brain tissues on a wet weight basis, the lower rate of glycolysis can actually serve to meet a greater proportion of the total energy requirement. This has been suggested by Mandel et al (82) who did in vitro comparisons of the metabolism of adult and newborn brain. They found that while "the uptake of glucose in moles per unit of fresh weight increases from one for three days old, rats to two for adult animals, the uptake of oxygen increases from one to three and the lactic acid production only from 1 to 1.4. This allows an estimate of the aerobic and anaerobic degradation of glucose to be made. The data indicate that the proportion of glucose oxidized increases from 36% of the glucose consumed for three day old rats to 56% for adults, while the fraction degraded by glycolysis decreases from 45% to 33% respectively. It should be emphasized that a change in the energy metabolism similar to that in the brain has also been found in our laboratory to occur in the liver, kidney, and spleen". This shift from predominantly glycolytic to more aerobic metabolism with maturation has also been documented by other investigators (22, 111).

The demonstration that lactic acid concentration increases cannot be

taken as proof of the presence of anaerobic glycolysis in itself. This is because lactic acid levels can be elevated by other mechanisms such as respiratory alkalosis (93). What is needed to relate changes in lactic acid concentration to anaerobic metabolism is concomitant knowledge of the pyruvate concentration (62, 63, 64, 66). If there is a greater proportionate rise in lactate than in pyruvate then anaerobic glycolysis is presumably contributing to the animal's energy requirements. In one study (13) it was shown that the lactate to pyruvate ratio rises progressively during the course of asphyxia in lambs from an average control ration of 12 to 100 within 15 minutes of asphyxia. The failure of most investigators to carry out pyruvate determinations along with their lactate determinations makes it impossible to make quantitative estimates from their results of the amount of glycolysis. A prominent cause of changes in lactate or pyruvate which does not reflect an oxygen debt is changes in pH. Most estimates of anaerobic glycolysis in the newborn based solely on lactate accumulation are probably low. This is because the anoxic and asphyxic newborn regularly develops an acidosis which would tend to lower the concentration of plasma lactate and pyruvate and thus offset the effect of anaerobic glycolysis. Failure to appreciate the relation between pyruvate and lactate continues to lead some current investigators to the improbable conclusion that "anaerobiosis does not play any significant role in protection against anoxia" of premature infants (113).

In the work of Huckabee (63) a change in serum pH from 7.39 to 7.53

induced by sodium bicarbonate infusion was associated with a three fold rise in blood lactate and pyruvate levels in an adult dog. The anoxic changes in pH in the newborn are much greater. Probable factors contributing to this are an interference with the cell membrane function, increased acid production by anaerobic glycolysis and decreased CO_2 elimination from hypoventilation. Thus, it is not surprising that investigators have reported decreases in blood pH values of 0.40 units in asphyxiated term lamb fetuses after five minutes of anoxia (13, 30). Swann (108) showed that although the blood pH in pups declines from 7.34 to 6.62 within 17 minutes of anoxia the pup can recover from these hydrogen ion concentrations which are considered fatal for adult animals. Wilson et al have also reported on nine infants who recovered from an average blood pH of 7.1 in association with birth asphyxia (120).

Although this thesis does not explore the possibility, it may well be that the immediate cause of death in anoxic newborns is not depletion of energy reserves, but rather some other interference with homeostasis such as acidosis. Thus, making newborn rats and mice (96) anoxic by placing them in CO_2 reduces their survival time by 50% from what it is with carbon monoxide, nitrogen, helium, cyanide or nitrous oxide (40, 53, 95). This hypothesis gains support from the finding that blood glucose does not fall appreciably in the asphyxiated lamb (13) and rises during the course of anoxia in the pup (40) while the change is variable in the rat (106, 116). Furthermore, Dawes showed that in asphyxic fetal lambs "when glucose and sodium carbonate were administered together, the time taken for the blood pressure to fall from the initial level of

30 - 40 mm Hg down to 10 mm Hg was increased from a mean of 48 minutes to more than eighty minutes. This treatment was also effective in raising the blood pressure and heart-rate even when it was delayed for as long as 40 minutes after the cord had been tied" (22). Since glucose infusion alone did not prolong survival in these lambs, the process of anoxic death may not be simply depletion of energy reserves. As early as 1931 Reiss had shown that newborn rats and mice subjected to carbon monoxide poisoning reduce their carbohydrate content by less than 20% (95). He concluded that the point of death was characterized by a critical concentration of lactic acid which was the same whether the animal succumbed to anoxia after 15 to 25 minutes at 37° or after more than an hour at room temperature. However, if lactic acid accumulation is the lethal factor, then one would expect a diminution in anoxia tolerance if lactic acid were administered. Britton et al (15) found that the survival time of fetal lambs in hypoxia was approximately the same after blood lactate was elevated to 100 mg/100 ml by injection.

Toxic inhibition of anaerobic glycolysis

There are chemical agents which more or less specifically inhibit some of the enzymes of the glycolytic cycle. The magnitude of their inhibition as judged by interference with glucose utilization and lactate production is directly related to the decline in anoxia resistance which they produce.

Himwich (57) was the first to demonstrate this by injecting newborn rats with 1 mg of iodoacetic acid. This metabolic toxin blocks anaerobic glycolysis at the point where triosephosphate is converted to

phosphoglyceric acid. "After allowing 15 minutes for absorption, half of the total number of injected rats were placed in an atmosphere of nitrogen and the other half retained as controls respiring air." The infant rats placed in nitrogen had a reduction in anoxia resistance from the usual 50 minutes to near adult levels of three minutes. Injected rats breathing air were followed for 50 minutes during which time they continued to show signs of life.

This has been confirmed by Hicks (50), who was able to reduce the newborn's anoxia resistance to two minutes by allowing 30 minutes for the absorption of iodoacetate. His "control animals given the same doses of iodoacetate were unaffected in air and when killed 24 hours later showed no pathologic changes" on histologic examination. Subsequent studies by Fitzgerald show that the percent decrease in the newborn's anoxia tolerance is a direct function of the iodoacetate dose (43). That iodoacetate interrupts anaerobic glycolysis was further supported by the fact that while 50 minutes of anoxia usually brings the body lactic acid level to 145 mg%, an iodoacetate injected rat dying of anoxia maintains a lactic acid level of about 35 mg% (41).

Because iodoacetic acid inhibits other dehydrogenases besides 3-phosphoglyceraldehyde dehydrogenase (115), one cannot be certain that all of its effect is by virtue of blocking the glycolytic cycle. Thus, Fitzgerald found a decrease in oxygen consumption with iodoacetate injection which supports his statement that "in higher concentrations this reagent may inhibit a number of different enzymes of the oxidative portion of the cycle as well (e.g. succinic dehydrogenase)" (43).

By injecting ratlets with fluoride Himwich was further able to incriminate anaerobic glycolysis as the source of energy which permits prolonged survival in the young. Fluoride, by forming a complex with magnesium ions, will inhibit to some extent a number of enzymes in both the glycolytic and citric acid cycles which require magnesium as a cofactor (115). (For example enolase which catalyses the formation of phospho-enol-pyruvate from 2-phospho-D-glycerate (50).) Newborn rats injected subcutaneously with 0.5 mg of sodium fluoride per gram of body weight had a decrease in anoxia tolerance from 50 minutes to 16 minutes (55). Hicks was able to confirm this in mice and he showed that control animals could live in air for at least several hours with the same dose of fluoride (50).

Anaerobic energy production other than anaerobic glycolysis

To date all the biochemical evidence pointing to anaerobic metabolism in anoxic newborns has come from experiments dealing with anaerobic glycolysis. It is conceivable that other pathways of anaerobic energy production besides anaerobic glycolysis could play a role in the newborn's anoxia tolerance. This was suggested by Raiha who proposed "that in the human fetus the tissues have a less important carbohydrate oxidation than in post natal life, and that the anaerobic production of pyruvic acid from glucose furnishes a rich substrate for the synthesis of fat characterized by a low number of double bonds (94). Theoretically the synthesis of fat from carbohydrate could make oxygen available by the following reaction:

$$17\ C_6H_{12}O_6 \longrightarrow 2\ C_{51}H_{98}O_6 + 4\ H_2O + 43\ O_2$$

Raiha supported his proposal that the fetal synthesis of fat "has an oxygen sparing effect on the metabolism" by observations on fetal tissues. He cites the "rapid increase in fat which characterizes the human fetus during the three last fetal months". He assumes this fat was synthesized by the fetus since "the human placenta is absolutely impermeable to fat in the direction of mother to fetus". Consistent with the notion of a high rate of lipogenesis from pyruvic acid he has found "that the co-carboxylase content of the umbilical blood is about 50% higher than that of the maternal blood and that the co-carboxylase exhibits a downward trend after birth". On the basis of umbilical arteriovenous differences in O_2 and CO_2 content, he has calculated R.Q. values of "one or slightly higher, indicating a pure carbohydrate oxidation combined with fat production from carbohydrates.

Villee (94) attempted to quantitate "the maximum advantage the fetus could derive from fat synthesis", assuming "that no energy is required for fatty acid synthesis". The 360 grams of fat synthesized by the fetus in the last month could take up 18 moles of H_2 which would make "available 10.4 moles of energy rich phosphate. This represents about 80 calories of energy, the energy requirement of a 3 kg fetus for 16 hours" (117).

On the basis of *in vitro* studies with rat and human fetal tissues, Villee promoted the hypothesis "that the high rate of lipogenesis in the fetus is an adaptation to the low oxygen tension prevailing in fetal tissues during much of gestation" (117). Villee found that just before

birth the lipid content of rat livers was 1.58 ± 0.16 mg%, but by 18 hours after birth it increases to 3.72 ± 0.21 mg% (117). He further showed that fetal and newborn rat liver slices have a much greater capacity to incorporate labeled, citrate, pyruvate and acetate into fats. "Fetal liver synthesizes lipid from acetate twice as rapidly as adult liver under aerobic conditions and 20 times as rapidly under anaerobic conditions" (117). Although both adult and newborn liver showed a decrease in lipogenesis with anoxia, this was much more marked in the adult samples (117). The fetal liver samples had respiratory quotients of 1.39 which is higher than the adult rat's liver and is consistent with the higher rate of lipogenesis.

More recently Villee (116) has concluded "that lipogenesis can be of no significance in the ability of the fetus to withstand anoxia". "There is no reaction known in mammalian tissues in which molecular oxygen is evolved. The conversion of glucose to fat does not produce oxygen, but produces carbon dioxide and water as byproducts:

$$25\ C_6H_{12}O_6 + 5\ O_2 \longrightarrow 2\ C_{51}H_{98}O_6 + 48\ CO_2 + 52\ H_2O$$

Hydrogen atoms given off in the reactions of glycolysis or elsewhere might be utilized in the synthesis of fats, for hydrogen is required for the synthesis of fatty acids from the two-carbon unit acetyl co-enzyme A. In a sense, the synthesis of fat provides a hydrogen 'sink', a depot for hydrogen atoms, which is an alternative to their combination with oxygen to form water. Recent advances in our knowledge of the details of fatty acid synthesis have shown that the reactions require

energy in the form of ATP. Thus, although lipogenesis might utilize the hydrogens released in glycolysis and prevent the accumulation of toxic amounts of lactic acid, the process would not provide for a net release of energy under anaerobic conditions" (116).

The problem of non-lactate hydrogen acceptors warrants further investigation particularly in the light of the recent observation by Alpert et al (4) that the lactate which appears in anoxic adult rat liver slices accounts for only a small fraction of the total carbohydrate which disappears.

Aside from the present thesis there are no quantitative estimates of the anoxic newborn's energy metabolism. In the absence of such knowledge, it is difficult to say if the observed anaerobic glycolysis accounts for all or only part of the anaerobic energy production.

Oxygen stores

Some investigators have ascribed part of the anoxia resistance to the fact that the dissociation curve of fetal hemoglobin lies to the left of the adult hemoglobin dissociation curve. This is not likely to play a significant role in anoxia resistance in view of the fact that under conditions of anoxia the blood oxygen in pups is 0.3 volumes % (108) or less (40, 53) after six minutes of anoxia.

Even in asphyxia where the animal does not expire a portion of its oxygen stores with each respiratory effort, there is a rapid depletion of blood oxygen. In lambs near term, the blood oxygen saturation falls to 2% or less within five minutes of clamping the cord (13).

Fitzgerald (43) has attempted to quantitate the anoxic resistance which may be attained by virtue of oxygen stores. He writes, "Although the quantity of oxygen stored in the blood and tissues of an animal is usually considered too small to be of value in resisting anoxic conditions as indeed it is for an adult animal, this is not true for the newborn mouse". The actual time for which oxygen stores in the blood and other tissues of the newborn mouse may be sufficient would lie between the two extremes of 16 and 196 minutes." The first figure assumes that the animal exhales all of its oxygen stores until its blood oxygen content is 0.4 vol. % while with 196 minutes there would be no exhalation of O_2 stores. Both of these estimates are based on the premise that from the onset of anoxia the newborn mouse has a decrease in oxygen consumption to 0.5% (0.03 cal/g/hr) of the control rate at 35°C. That this is a rather untenable assumption was not evident in the absence of direct calorimetry to estimate the anoxic newborn's heat production. The other difficulty with these calculations is that they assume that the oxygen stores become completely depleted during the course of anoxia when in fact it is difficult to demonstrate further decreases in blood oxygen content after it reaches 0.1 vol. % during the first six minutes.

The inhibition of the cytochrome oxidase system has provided further evidence that oxygen stores doesnot play a significant role in the newborn's anoxia resistance. Cyanide is known to combine with the iron atom of the cytochrome enzymes and in this way it prevents the transfer of electrons from substrate molecules to oxygen (115). Thus, the survival timeof cyanide injected animals is independent of the oxygen supply. If

oxygen stores do not play a significant role in anoxia resistance, then death by nitrogen anoxia or cyanide poisoning should come at about the same time. Heiss (96) was the first to report that newborn mice lived for 32 minutes after hydrocyanic acid poisoning while adult rats died in less than a minute. Himwich repeated this by injecting 5 mg of sodium cyanide into newborn rats who continued to make respiratory efforts for 50 minutes in both air or nitrogen. The same amount of cyanide causes death in the adult rat in 10 minutes (57). This finding that the difference between the adult and the newborn remains nearly constant whether oxidative metabolism is interrupted by cyanide or pure nitrogen has been confirmed by Fitzgerald in mice (42). He further showed that the duration of gasping after cyanide injection is prolonged by keeping the newborn at lower temperatures. Although the newborn takes longer to die after an injection of cyanide the lethal dose on a weight basis is less than in the adult. That oxidative metabolism responds similarly in newborn and adult rats is further shown by experiments on excised cerebral tissues where "M/200 sodium cyanide inhibits 95% of the total oxygen consumption" in each (57). These experiments show that the newborn's anoxia resistance probably does not rest on oxygen stores or any other mechanism involving oxidative metabolism since some signs of life are maintained when the cytochrome oxidase system is inhibited.

ANOXIC SUPPRESSION OF ENERGY METABOLISM

To my knowledge there are no published reports that conclusively show a decrease in the rate of energy metabolism in the anoxic newborn. There is, however, considerable circumstantial evidence which indicates that the newborn responds to anoxia with a definite, but as yet, indeterminant decrease in energy metabolism.

Decreased activity

The most obvious sign of a decrease in the energy metabolism during anoxia is a marked diminution of all gross muscular activity. This has been documented for pups (109), kittens, rabbits, rats (50), lambs (30), mice (50), and humans (6). All will lay completely quiescent for one major part of the anoxic episode, except for a deep respiratory gasp between apneic intervals. Adult mammals in contrast will become restless, agitated, hyperpneic and begin to convulse shortly after losing consciousness, almost until the point of death. Swann and Brazier studied the process of anoxic death in 50 adult dogs (107). They found that an occasional dog could not be revived after two and one-half minutes of anoxia and all dogs were beyond resuscitation after four and one-half minutes of anoxia, despite oxygen insufflation. The duration of anoxia did not define the point of death as narrowly as did the systolic blood pressure. If the resuscitative procedure was instituted while systolic blood pressure was still above 100 mm Hg, then it was uniformly successful, but once systolic pressure was below 80 mm Hg, oxygen insufflation was of no avail. The decline from 100 mm Hg to 80 mm Hg blood pressure took

an average of 16 seconds. Thus, the adult dog maintains sufficient cardiac output and vascular tone to keep the systolic blood pressure above 100 mm Hg until 16 seconds before the point of death. The change from hyperpnea to apnea was more abrupt than circulatory failure and preceded it by 84 seconds on the average.

The same investigator (108) found a very different pattern in anoxic pups aged three to four days. Here the respiratory rate declines from a control of 30 to 5 per minute within the first minute of anoxia. By 18 minutes the pup is making one gasp every two minutes and, on occasion, can be successfully resuscitated after another eight minutes of anoxia. The heart rate and blood pressure also decline shortly after the onset of anoxia and continue to diminish gradually. All the pups with blood pressures above 10 mm Hg survived and some with half as high a systolic pressure were resuscitated.

Other newborn and fetal (30) mammals also distinguish themselves from the adult members of their species by showing a greater absolute and relative decline in cardiac, respiratory, and gross muscular activity shortly after the onset of anoxia. This has been shown in the case of rats (50, 106), mice (50), fetal lambs (47), dogs (40), monkeys (67). While the adult succumbs immediately after these activities become suppressed, the newborn will continue to show signs of life for considerable periods and on termination of the anoxia, it will return to its preanoxic state if it is still viable. In teleologic terms, it is as though the anoxic adult rapidly consumes all energy reserves in an effort to escape or overcome the oxygen deficiency while the newborn

conserves its energy in the "hope" that the anoxia will pass over.

Decreased rectal temperature

If the decrease in activity actually corresponds to a diminished metabolic rate, then one would predict a decline in the animal's heat production. Although this has not been measured in anoxic newborns, it has been implicated by the demonstration of a decrease in rectal temperature of human babies under conditions of oxygen deficiency. The data of McClure and Caton (79) indicates that three infants with apnea for the first three to five minutes after birth had decreases in rectal temperature from 3° to 5°F in the first 15 minutes of life. "Although the 'nonapneic' infant's temperature may also fall rapidly, the immediate fall is of less amplitude" (80).

This finding has been confirmed by Burnard and Cross (19) who followed the rectal temperature of two larger groups of newborns for a longer period and under more rigidly controlled conditions. The two groups differed only in that the 53 control babies breathed immediately while the 15 experimental babies failed to establish respiration within three minutes of birth. The only maternal anesthetic employed was chloral. There was a significant decline in the rectal temperature of both groups during the first six hours of life and a subsequent gradual return to the initial temperature in the next 14 hours. However, the mean decline in rectal temperature of the asphyxic group exceeded that of the control group by 2°F. There was no overlapping of the 95%

confidence limits of the two groups for the first 16 hours. They "conclude that a somewhat reduced rectal temperature is a natural phenomenon in babies suffering from asphyxia at birth. It seems probable that this lowered temperature is the result of a period of diminished metabolic rate, caused by the anoxia. If this is so, then the lowered temperature may have survival value".

Subsequent studies by Moore (87, 88) on three to 17 day old kittens show that they have a decline in rectal temperature on exposure to hypoxia at 30°C. In general, the longer and the more severe the hypoxia was the greater was the fall in rectal temperature. Within a few minutes after the cessation of hypoxia, the rectal temperature began to return to control levels.

Newborn premature humans (37, 103) have also been shown to have a decline in the rectal temperature to about 33°C under controlled hypoxia conditions of 15% oxygen for several days. Except for the previously cited studies on apneic newborns, there are no reports on the effect of anoxia on the newborn human or animal's rectal temperature. Presumably there is a decline which exceeds the hypoxic change.

Decreased heat loss

Since body temperature is a function of the rate of heat production minus the rate of heat loss we cannot equate a decline in body temperature with a decrease in the rate of heat production. It is conceivable that during anoxia the animal loses its peripheral vasomotor control to produce an increase in cutaneous blood flow. The resultant increased

rate of heat loss could account for the decline in body temperature. Thus, the only way to measure heat production is to monitor both body temperature and rate of heat loss. To my knowledge such an experiment has not been published for anoxic newborns. There is, however, one experiment on human infants that points toward a decrease in the rate of heat loss under hypoxic conditions. This is a study by Brodie et al (16, 17) which compared the heat loss of babies breathing air with that of babies breathing 15% oxygen for 50 minutes. Each group contained ten babies from three to seven days old. They found that the babies breathing air heated their plethysmograph an average of 5.65 ± 0.87°C above room temperature while those breathing 15% oxygen only elevated their plethysmograph temperature 3.59 ± 0.73°C above room temperature. Because the temperature gradient between plethysmograph and room was less in the hypoxic babies, they conclude that hypoxia decreases the heat production. The limitations of this study are that it offers only an index of heat loss rather than a quantitative estimate. This is because a given temperature gradient was not related to a corresponding rate of heat loss. Also while there was a 2°C difference in temperature gradient between the two groups, the mean room temperature was also 2°C colder in the air breathing babies which lost more heat. The fact that rectal temperatures were not measured in either group is compensated by the fact that they previously failed to get a significant rectal or skin temperature difference between infants subjected to identical conditions. Despite its limitations, this is the only published study of newborn humans or animals which makes an attempt to evaluate heat loss during diminished oxygen supply.

Decreased oxygen consumption of hypoxic newborn

Although attempts at evaluating heat production (of oxygen deficient newborns) by direct calorimetry have been limited to the measurement of rectal temperature and one qualitative study of heat loss, there are numerous experiments which have employed the principles of indirect calorimetry. The study of the newborn's energy metabolism under conditions of oxygen deficiency by indirect calorimetry is befraught with a number of limitations. The first is that the metabolic rate is not a simple function of oxygen consumption when anaerobic metabolism plays a significant role. If oxygen deficiency promotes anaerobic metabolism, then estimates of energy metabolism based on oxygen consumption become increasingly misleading the more pronounced the hypoxia. This consideration has restricted indirect calorimetry to hypoxic experiments since under anoxic condition there is no oxygen consumption to measure

This method of estimating metabolic rate makes the tenuous assumption that the caloric equivalent of oxygen remains constant. Theoretically this can vary between 4.5 and 5 cal/cc of oxygen, depending on the particular foodstuff being oxidised. Few investigators of hypoxia in the newborn have determined the respiratory quotient which would give an index of the appropriate caloric equivalent of oxygen and its variability. Studies (104) that have taken the respiratory quotient into consideration (25) obtained values below 0.70 in about 43% of the infants on the third day of life which has been considered to be "below levels compatible with normal metabolic processes". Since a number of careful investigators (9, 25, 90) have obtained the same result (in over 200 experiments

during the first week) with different methods of good reproducibility, the phenomenon does not appear to be experimental errors, but rather points to some as yet not understood aspect of newborn metabolism. Karlberg (70) has reviewed the subject and writes, "Immediately after delivery the respiratory quotient is almost one according to Hasselbalch's investigations in 1904. It then falls during the next few days to 0.70, but rises again, after a further lapse of a few days, up to about 0.86, i.e. the general mean value for infants. This implies, that in studies of the energy metabolism of newborn, both oxygen consumption and the production of carbon dioxide should be determined" (70). It would appear that attempts to attribute the low respiratory exchange ratio to experimental error or extrapulmonary gaseous exchange (25) should also explain why these factors would be more prominent at three days of age than before or after. Cross et al (25) has pointed out that explanations such as the excretion of carbon dioxide in the form of bicarbonate, conversion of fat to carbohydrate, and recovery from metabolic acidosis or respiratory alkalosis are not tenable on a quantitative basis. In 1931 Reiss (95) determined the respiratory quotient of mice and rats aged one to five days. The values for the entire animals as well as for liver slices fall below 0.7. His explanation that the low respiratory quotient is a reflection of a high rate of anabolism has not been confirmed nor rejected to date. That CO_2 fixation is not a process limited to the plant kingdom has been shown by Wendler and others (49).

In summary, there are two principal limitations in studying the newborn's energy metabolism under oxygen deficiency by indirect calorimetry. The first is that the more hypoxic the infant, the greater the potential for anaerobic metabolism and hence the greater the error in estimating total metabolism. The other objection is that the caloric equivalent of oxygen has been selected on the basis of theoretical values based on adult metabolism which may not apply to growing infants who have marked changes in the respiratory exchange ratio during the first week of life.

The first report that newborns breathing 15% oxygen decrease their oxygen consumption by 17% was published in 1955 (24, 26). Here as with the hypoxic decline in rectal temperature and heat loss, the initial observation was made on human newborns by Cross et al. Since then, other investigators have demonstrated a dependency of the oxygen consumption on ambient P_{O_2} in kittens (87, 88) and puppies (86). The magnitude of the decrease in oxygen consumption varied directly with the degree of hypoxia. Kittens exposed to 12% oxygen had a 20% decline in oxygen consumption while those exposed to 7% oxygen have about a 50% decrease (86). Since the decrease in oxygen consumption occurs within five minutes of the onset of hypoxia, it would appear that the subsequent gradual decline in deep body temperature is a consequence and not a cause, of the fall in metabolic rate (87). That changes in oxygen consumption precede changes in rectal temperature is also illustrated by the pattern of recovery from hypoxia. In general, the oxygen consumption rises dramatically, being back or beyond the preliminary value

within five minutes, while rectal temperature which may have fallen 3°C during hypoxia returns much more gradually to control values (68).

It thus appeared that the newborn respond differently from adults, since it was generally accepted that oxygen consumption of an adult mammal does not fall until death due to hypoxia is imminent (in the region of 6% oxygen) (52).

More recent work by Hill (51, 52) has shown that what was thought to be a distinguishing feature between adult and newborn can in part be attributed to differences in size rather than differences in maturity or age. Her work indicates that under hypoxic conditions the thermo regulatory response of both kittens (aged 1 - 40 days) and adult guinea pigs is inhibited. "For instance, in an environment at 26°C a newborn kitten consumes about twice as much oxygen as at its neutral temperature which is around 34°C. When the oxygen content of the atmosphere breathed by a newborn kitten in an environment at 26°C is reduced from 21 to 10% the animal's oxygen consumption falls sharply to nearly half its former value, and then remains steadily at that new level for as long as the animal is hypoxic. Meanwhile, the animal's rectal temperature falls slowly and approximately exponentially. On returning the oxygen level to 21%, the kitten's oxygen consumption promptly doubles, and its rectal temperature starts to rise toward the control level. If this experiment is repeated at the neutral temperature, the finding is entirely different. Reduction of the oxygen content of the inspired air to 10% now produces no reduction in oxygen consumption, and the animal's rectal temperature

does not fall. Adult guinea pigs show a similar phenomenon" (51).

There is one distinguishing feature between kitten and guinea pig. This is that a mild decrease in the ambient temperature below the neutral temperature will evoke a greater rise in metabolic rate in the kitten than in the guinea pig. This is because the kitten's homeothermic mechanism rely more on regulation of heat production than on control of heat loss (51). The ability to control heat production appears to develop before the ability to regulate heat loss in both mammalian phylogeny (85) and in the ontogeny of humans (7, 18, 32), pups (68, 81), pigs (61, 89), rats (109), and sparrows (31).

If an animal's homeothermic state is dependent on control of heat loss whether because of increasing maturity, increasing body size, or small thermal stress, it will not show an appreciable decline in metabolic rate with hypoxia of more than 6% oxygen. If the role of increased heat production is prominent however, as it is in small creatures, immature animals or in the face of an appreciable cold stress, then the effect of hypoxia on metabolic rate becomes more pronounced. This concept rationalises several apparent inconsistencies. Thus, the lamb was found to have a constant oxygen consumption in a warm environment until the arterial oxygen saturation fell below 35% (1, 2) while the relatively less mature human infant has a decrease in oxygen consumption with much milder degrees of hypoxia at room temperature. Because the lamb was not required to maintain a high level of heat production, it maintained normal levels of metabolic activity until relatively severe state of hypoxia was

induced. The metabolic machinery of the infant in the cooler environment apparently begins to fail at relatively higher levels of ambient oxygen concentration as a consequence of the greater demand for heat production. It is also consistent with the finding that under cold conditions small adult mammals are more apt to have a decrease in oxygen consumption with hypoxia than larger animals. To summarize, "in all cases the type of response obtained is determined largely by the level of metabolism immediately before the induction of hypoxia; and the level of metabolism at rest is in turn determined by the environmental temperature (52), plus the maturity of the animal's homeothermic mechanisms, and its size. These findings do not justify the prepetuation of the concept that gaseous metabolism is independent of variations in barometric pressures (112)".

Further investigation is required to answer the question of whether the hypoxic decline in oxygen consumption represents interference with other aspects of energy metabolism besides thermal regulation. An *in vitro* study on kitten liver slices by Longmuir suggests that during the first two weeks there is a "two-or three-fold reduction in the oxygen concentration below which tissue respiration is limited" (76).

Since newborn and small adult mammals respond similarly to hypoxia, it seems questionable that the difference in anoxia resistance can be related to the hypoxic decline in oxygen consumption as has been suggested. However, it may be that at room temperature the newborn will become more hypothermic since his temperature regulation relies primarily on heat production.

The effect of temperature on anoxia resistance

The increased anoxia resistance of newborn animals at cold temperatures has been observed by almost every investigator of the problem since 1790 when Sir Astley Cooper (36) called attention to it. At first this may seem hard to reconcile with the finding that moderate cooling generally increases the oxygen consumption of neonates more than that of adults. However, since anoxia renders the animal poikilothermic, we would not expect an increase in metabolism on cooling, but rather a decrease by virtue of the Q_{10} effect. Thus, the metabolism an anoxic animal has to support is not that of thermogenesis, but that of maintaining its vital functions and structural integrity. One would anticipate an increase in the anoxia resistance at lower temperatures if the energy required to maintain biochemical integrity is diminished more than is the supply of energy. This would appear to be the case in newborn and adult (65) mammals plus poikilotherms all of whom show a greater anoxia resistance at lower temperatures. Schneider (98) has studied the effect of cooling the adult rabbit brain on its metabolism. At 37°C the brain could recover from ten minutes of anoxia while at "26°C the revival time after ischemia of the brain is between 30 and 40 minutes! At the colder temperature the rate of glycolysis was much less and yet the depletion of phosphocreatine and ATP proceeds more slowly.

Several investigators (53,95) have shown that raising ambient temperature 10°C above room temperature reduces the newborn rat's anoxia tolerance from 50 minutes to 20 minutes. Decreasing the rat's colonic temper-

ature to 9°C on the other hand extends the anoxia survival to two hours (3). The resistance to cellular anoxia after cyanide poisioning is also extended by cooling (42). The inverse relation between ambient temperature and anoxia resistance has also been shown to apply to kittens (36), pups (36), guinea pigs (83), and humans (119). Reducing the caloric temperature of pups from 37°C to 15°C extended the time for lethal anoxia exposure four to seven fold (84).

It is difficult to say if the marked effect of temperature on anoxia resistance is primarily by regulating the rate at which anaerobic sources are depleted or the level at which energy must be supplied to sustain life. Measurements of the total anoxic heat production at different temperatures might throw some light on this question. Whether either or both of these alternatives are correct is reasonably certain that at lower temperatures the anoxic metabolic rate is diminished. Thus, respiratory efforts occur less frequently at lower temperature and with sufficient cooling may be arrested for 30 minutes only to resume on rewarming (102). While the frequency and duration of gasping of isolated rat heads were influenced by warming or cooling the total number of gasps was little altered (102). This suggests that temperature does not influence the amount of anoxic metabolism, but rather its rate. This is supported by the finding that at lower environmental temperatures the rate at which cardiac carbohydrate is depleted is slower (106). Reiss (95) found that lactate accumulation in newborn rats was more rapid at higher temperatures than at room temperature, but at the time of death it was the same.

Because the metabolic rate appears to diminish when anoxic animals are cooled, it has been suggested that the poor thermal insulation and small size of newborns facilitates cooling during anoxia and so promotes anoxia resistance (78). Besides the reports on human infants (19) where the rectal temperature fall followed the asphyxic episode by some time, there are no published studies on the magnitude of temperature drop with anoxia. An answer to this question may document one of the factors that probably plays an increasing role the longer the anoxic episode. Although the adult fails to show the decline in body temperature, his limited anoxia resistance is probably not related to this. Generally the adult succumbs to acute anoxia at a time when even the small newborn has a negligible decrease in body temperature. Also, even if the newborn's temperature is maintained at the adult level, he can survive for considerable periods. Thus, five minutes after the anoxic death of the mother, viable rats have been delivered (40). Similar observations on removing viable infants from pregnant, but dead mothers were reported for man and rabbits as early as 1651 by William Harvey (48). This has also been confirmed in cats (40).

Quantitation of anaerobic glycolysis

If glycolysis is the only anaerobic source of energy, then biochemical estimates of the amount of anaerobic glycolysis should give an index of the anoxic energy production. As pointed out previously, marked rises in lactic acid may result from minor pH changes in the absence of oxygen deficiency (62). Since anoxic and asphyxic newborns

generally develop an appreciable acidosis serum lactate may reflect anaerobic glycolysis only poorly. Also, Villee has pointed out that lactic acid "may be produced from pyruvic acid derived from alanine or from oxaloacetic acid as well as from that produced from hexoses, so that we cannot accept the rate of accumulation of lactic acid as an exact measure of the rate at which hexoses have been metabolized in the glycolytic cycle" (116). Despite these limitations, attempts have been made to relate the anoxic and hypoxic lactate accumulation to a rate of energy production. Cross has "noted that anaerobic glycolysis will give only about 7% of the energy obtained by the oxidative breakdown of glucose, and it is thus a most uneconomical method in an infant, whose supplies of glucose at birth are necessarily limited" (26). This is because while the complete oxidation of glucose yields 38 energy rich phosphate bonds, degradation to pyruvic acid gives a net gain of only two energy rich phosphates per glucose molecule (116).

Villee has calculated that the lactate accumulation during 30 minutes of anoxia in rats could "represent ten percent of the basal energy requirement" (114). He feels that "it is unlikely that this amount of energy would suffice for the observed survival of the ratlet" (116). His estimates of anaerobic glycolysis based on glycogen disappearance are a little higher. One day old rats had a "fivefold increase in the net utilization of liver glycogen under anaerobic as compared to aerobic conditions". This is "less than half the amount necessary to equal aerobic energy production" (71). The disparity in the two estimates

cannot be attributed to urinary loss of lactate since with the anoxic decrease in blood pressure there is a decline in urine production. As Dawes demonstrated in fetal lambs 15 minutes of anoxia decreased the urine output from 0.2 - 0.3 ml/min to less than 0.01 ml/min (30). All the estimates of anaerobic glycolysis to date leave one with the conclusion that the anoxic newborn has a marked decrease in energy production unless other anaerobic energy sources play a significant role. Further evidence that the decrease in oxygen consumption of hypoxic kittens is not compensated by a corresponding increase in anaerobic glycolysis is afforded by the failure to demonstrate an oxygen debt (52, 88). Although there is generally a rise in oxygen consumption in the immediate post hypoxic period, this occurs only while the temperature is returning to control levels. Also this small elevation in oxygen consumption is just enough to account for the extra heat required to elevate the body temperature and its much less than the decrease in oxygen consumption during hypoxia. Thus, it cannot be maintained that an oxygen debt has been paid back, only that a heat debt has been paid back (52). The absence of significant elevations in oxygen consumption in the immediate post hypoxic period does not exclude the possibility of significant amounts of anaerobic glycolysis, particularly since the notion that an oxygen debt is payed back immediately has had experimental doubt cast on it (5). In this regard it is of interest that in seals emerging from 15 minutes dive "only one-half of the debt is paid off in a comparatively short time, and it would appear that the seal is able

to extend the payment over a prolonged period during which the metabolism is only slightly above normal (73).

Fitzgerald (43) has shown that the newborn can live for some time on limited oxidative metabolism despite the inhibition of anaerobic glycolysis. He injected newborn mice with doses of 60 mg/kg of iodoacetate which completely inhibits glycolysis and causes a marked decrease in oxygen consumption to less than 5% of the control rate. The mice "survived for about an hour in air by tolerating an extremely low oxygen utilization in the absence of glycolysis".

Because the brain is the most sensitive organ to anoxia in adult animals, a number of investigators have focused on the metabolic characteristics of newborn brain. These studies show an increase in both the rate of glycolysis and oxygen consumption with growth. Newborn rat brain has an oxygen consumption which is one-third to two-thirds of the adult rat brain oxygen consumption on a wet weight basis (60). Pups show similar changes in brain oxygen consumption with growth (56). Most of this difference can be explained on the basis of decreasing water content of cerebral tissues with increasing age. The average percent dry weight in a one week old pup's (56) and rat's (34) brain is about 12% while in the adult animal it is 22%. Calculated on a dry weight basis, the increase in metabolic rate with maturation is not evident in rats (54).

Although the overall changes in oxidative metabolism of newborn and adult brain can be interpreted in the light of decreased water

content with age there are some other differences. In the pup the cortex has the lowest oxygen consumption and the medulla the highest, this is reversed in the adult (56). It appears that the portions of the neuraxis which develop later in phylogeny and ontogeny have a relatively greater oxygen requirement (60), and immature animals which are not dependent on these structures are more resistant to anoxia.

In vitro studies support the notion that the anoxic newborn has a decrease in metabolism. Chesler and Himwich (22) found that in brain slices from five day old rats anaerobic glycolysis (as estimated from anoxic lactic acid formation) provided only about one-fourth of the energy which could be obtained from oxidative metabolism (as calculated from oxygen consumption). The disparity between oxidative and anaerobic glycolysis is two to three fold greater in older rats brain tissue. The more marked Crabtree effect (stimulation of anaerobic glycolysis when oxidative phosphorylation is interferred with) of newborn brain may contribute to their anoxia resistance.

THEORETICAL CONSIDERATIONS OF METHODS

THEORETICAL CONSIDERATIONS OF METHODS

To study the energy metabolism of anoxic newborns, two principle techniques were employed. These were the methods of direct and indirect calorimetry.

Direct calorimetry

By the technique of direct calorimetry one attempts to follow energy metabolism by monitoring the rate of heat production. Thus, energy which is not dissipated as heat, but is stored in the form of increased structural complexity (ex. ionic gradients and chemical bonds) will not be measured by this method. In short, direct calorimetry reflects the rate of catabolism, but not the rate of anabolism.

Heat production can be calculated from heat loss and body temperature according to the following equation:

$$\text{rate of heat production} = \text{rate of heat loss} + \text{rate of change in body heat}$$

If the body temperature remains constant, then heat production and heat loss to the environment are equal. Because the anoxic newborn has appreciable decreases in body temperature, it was important to estimate changes in body heat content. This was done by obtaining continuous records of rectal temperature. Since the body has a thermal gradient from core to surface, there is apt to be an error in estimates of body heat content based on temperature measurements from one point. In adult rats, (which are about the same size as pups) this error has been found to be about 7.5% (20). Since the present studies were

not concerned with the total body heat, but rather with the rate of change in body heat per minute, the 75% error may not be applicable. During anoxic episodes where there is a rapid fall in body temperature and a marked decrease in cutaneous perfusion the rectal temperature is apt to lag behind the mean body temperature.

If in a given minute the change in rectal temperature differs from the change in mean body temperature by just 0.10°C, then there will be an 83 cal/min/kg error in the value calculated for heat production. Compared to the usual rates of heat production this amounts to a 100% error. In the present calculations the changes in rectal temperature per minute were estimated to the nearest 0.005°C from a line which was visually fitted to the plot of rectal temperature against time. This eliminated some of the random error, but the systematic error due to a possible difference in phase between rectal temperature and mean body temperature was still present. Generally the greatest decrease in rectal temperature occurred in the colder bath and was associated with lower values for heat production.

The value of 830 cal/kg was selected as the specific heat for the pup's tissues. There are no figures in the literature on the specific heat of newborn animals. The figure of 830 cal/kg best defines the specific heat of adult rats (20). This was determined by placing dead members of that species in a bomb calorimeter and measuring the heat loss associated with a given change in body temperature. Since newborn tissues are normally more hydrated than those of adults, their specific heat may be higher. However, 830 cal/kg is the value that was

used because it gave good agreement between the expected and measured heat loss of our calorimeter when it contained cooling dead pups.

As long as the changes in body heat content are small, relative to the rate of heat loss, a given error in either the specific heat or the estimate of mean body temperature will reflect itself as a small error in the calculation for heat production. With these limitations in estimating body heat, in mind, the equation for the rate of heat production can be rewritten as it was used in this thesis:

$$\text{rate of heat production} = \text{rate of heat loss} + (830 \text{ cal/kg}) \left(\text{change in rectal temperature/min}\right)$$

By obtaining continuous records of heat loss and rectal temperature before, during, and after anoxic episodes, it was possible to estimate the rate of heat production for the same periods.

Indirect calorimetry

Although the earliest work on indirect calorimetry measured carbon dioxide production, most of the recent experiments have determined oxygen consumption. This is because techniques of measuring oxygen have improved and it has become increasingly appreciated that the body stores of carbon dioxide can undergo appreciable fluctuations thereby complicating the situation.

The following equation defines the relation between oxygen consumption and aerobic energy metabolism:

$$O_2 \text{ consumption} \times \text{caloric equivalent of } O_2 = \text{caloric value of } O_2$$

Most investigators employ a factor of 4.8 cal/cc of oxygen as the

caloric equivalent of oxygen. In this thesis the value of 4.8 cal/cc of oxygen was also used.

By combining the information from direct and indirect calorimetry, it was hoped that increases and decreases in the animal's energy reserves could be estimated. Thus, during period of anoxia where oxygen consumption is zero, the rate of heat production would be a measure of the rate of anaerobic metabolism.

METHODS AND MATERIALS

METHODS AND MATERIALS

The animals used in the experiments were pups ranging in age from ten hours to three days. Older pups generally did not fit into the animal chamber. The weight range of the pups was 330 to 567 g. The three bitches who gave birth to the mongrel pups were obtained at a local dog pound and then kept in individual metal cages before, during, and after the birth of the litter.

The recording of rectal temperature was carried out with a rectal thermistor. This temperature sensitive resistance had a response time of two seconds. It was potted in the tip of a 1 mm O.D. vinyl tube with a 48 cm long lead. The thermistor tip was dipped in K-Y lubricating jelly and then introduced a minimum of 2 cm past the anal sphincter. The rectal thermistor was held secure by taping the lead to the length of the tail. After the experiment, the position of the thermistor was always checked and when it had dislodged, the experiment was excluded. Figure 1 and table 1 depict the non- linear relation between ambient temperature and thermistor signal. This was obtained by taping the thermistor tip to the mercury bulb of a standardized thermometer and submerging both in water of varying temperature. The thermistor thermometer is potentially more sensitive than 0.01°C which was the limit of resolution of our standard thermometer.

In order to measure the rate of heat loss, a gradient layer calorimeter was constructed after the principles described by Benzinger et al (10). The unit is simply two copper shells with a spiral of 270

Figure 1. Thermistor thermometer calibration

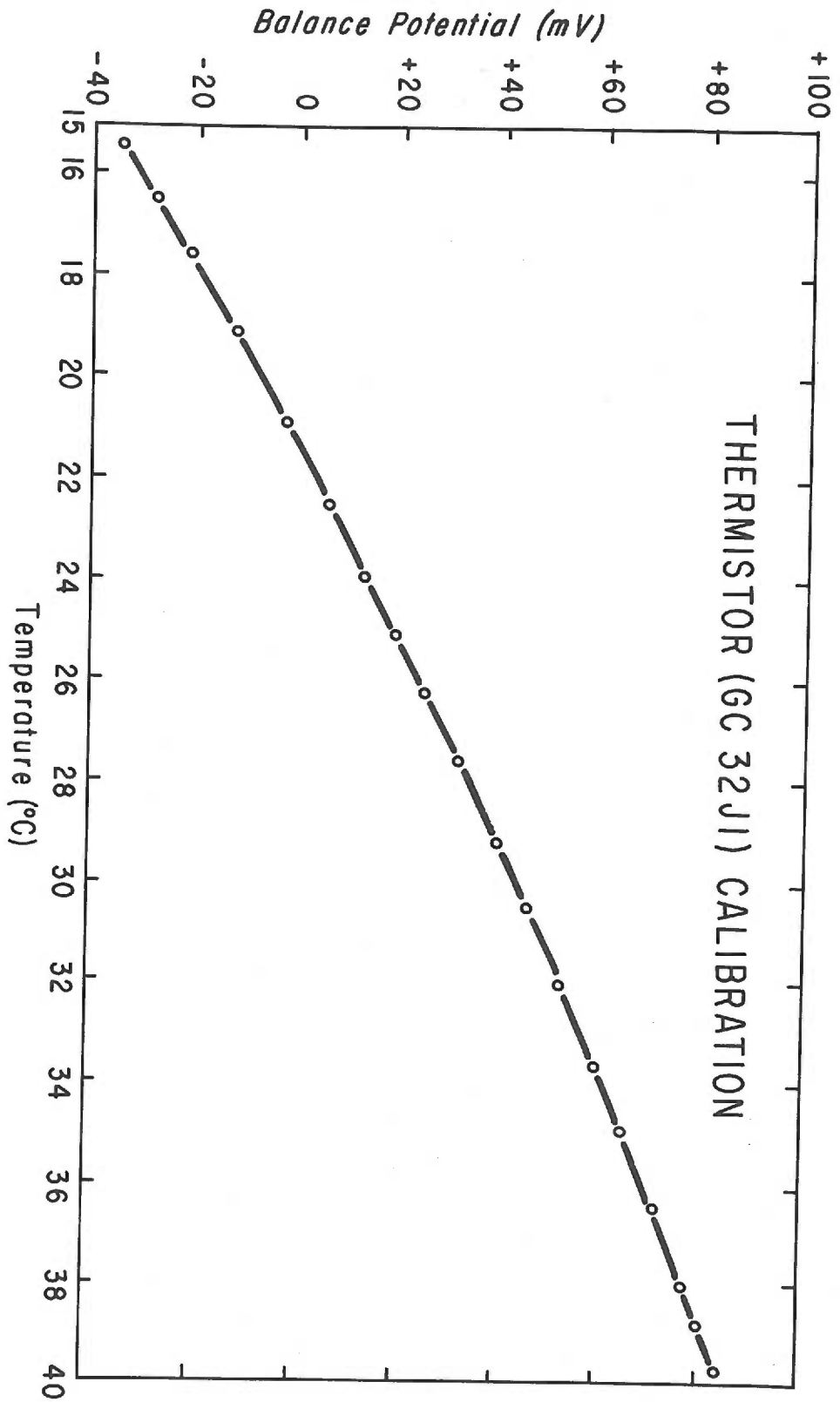

THERMISTOR (GC 32J1) CALIBRATION
Balance Potential (mV)
+100
+80
+60
+40
+20
0
-20
-40
Temperature (°C)
15
16
18
20
22
24
26
28
30
32
34
36
38
40

TABLE 1

RECTAL THERMOMETER CALIBRATION
(Thermistor GC 32J1)

Temperature °C	Balance Potential (mV)
15.47	-34.45
16.53	-28.05
17.60	-21.53
19.15	-12.52
21.01	- 2.03
22.55	+ 6.26
24.00	+13.68
25.15	+19.66
26.30	+25.59
27.63	+32.30
29.23	+40.00
30.50	+46.00
32.03	+52.80
33.70	+60.20
35.00	+65.80
36.50	+72.00
38.00	+78.10
38.80	+81.30
39.77	+85.16

TABLE 2

THERMISTOR FLOW METER CALIBRATION

Flow cc/min S T P D	Balance Potential (mV)
32.5	-90.00
51.9	-80.00
69.0	-70.00
86.1	-60.00
104.5	-50.00
122.8	-40.00
142.9	-30.00
165.7	-20.00
191.8	-10.00
220.5	00.00
253.2	+10.00
295.7	+20.30
347.8	+30.00
414.2	+40.00
490.0	+50.00

thermocouples between them. The layer of thermocouples was constructed by weaving a copper-constantan ribbon over and under an odd number of 1/64" thick plastic strips which were laid length-wise on the insulated exterior of the inner copper shell. The thermocouple ribbon was 1 cm wide with half of the width being copper and the remainder constantan. As this ribbon was interlaced through the plastic strips notches were placed along its length across its midline from alternating edges. In the end there were 270 thermocouple junctions in series with all the copper to constantan junctions on one side of the strips and all constantan to copper junctions on the other surface. Over this pattern of thermocouples the outer copper sleeve was slipped. Both copper shells were electrically insulated from the thermocouples by covering their adjacent surface with Clearseal. To keep the two tubes concentric an "O" ring was placed between them an inch from each end. The cavity between the ring and the end of the cylinders was sealed with a plastic. The chamber's internal diameter is 8.9 cm and the distance between the plastic ends is 21.3 cm.

One end of the unit was set in a permanent plastic seal. In this seal were imbedded the gas inlet and outlet tubes plus the leads to the thermopile. The other end of the chamber was fitted with a plastic lid of the same dimensions as the plastic seal. Each plastic end was 3.0 cm thick and reflected light on its inner surface so as to minimize radiant heat loss. The lid had a small hole for the rectal thermistor lead.

The animal was supported on a grated metal sleeve to minimize direct contact with the cooler chamber wall. The animal tray in turn was separated from the chamber wall by two "O" rings. Calibration showed that changing the heating element or its position in the chamber did not influence the calorimeter response. The calorimeter was calibrated by placing a nichrome wire inside the chamber. By varying the amperage going through this resistance and measuring the potential drop across the wire it was possible to measure the electrical energy dissipated in the chamber. This was converted to a rate of heat flow by the following equation:

$$\text{cal/min} = \text{volts} \times \text{amps} \times 60 \times .238$$

The estimated standard deviation of b, the slope is 0.05. The estimated standard deviation of a, the intercept is 0.005. As figure 2 depicts, the response was linear over a range of energy inputs which exceeds that encountered in the subsequent experiments. By the method of least squares a regression line of the calorimeter signal (x) on the caloric output or rate of heat loss (y) was obtained:

$$y = 38.56x - 1.23$$

Table 3 shows that varying the ambient temperature from 20.3°C to 29.7°C and the gas flow from 150 to 220 cc/min did not produce significant deviations from the linear relationship between heat input and calorimeter signal. This is to be expected since 200 cc of saturated air will remove only about 0.003 small calories on being warmed from 20.5°C to 22°C (provided it is not further saturated). To minimize heat loss via vaporization the gas was humidified at the bath temperature

Figure 2. Calorimeter calibration

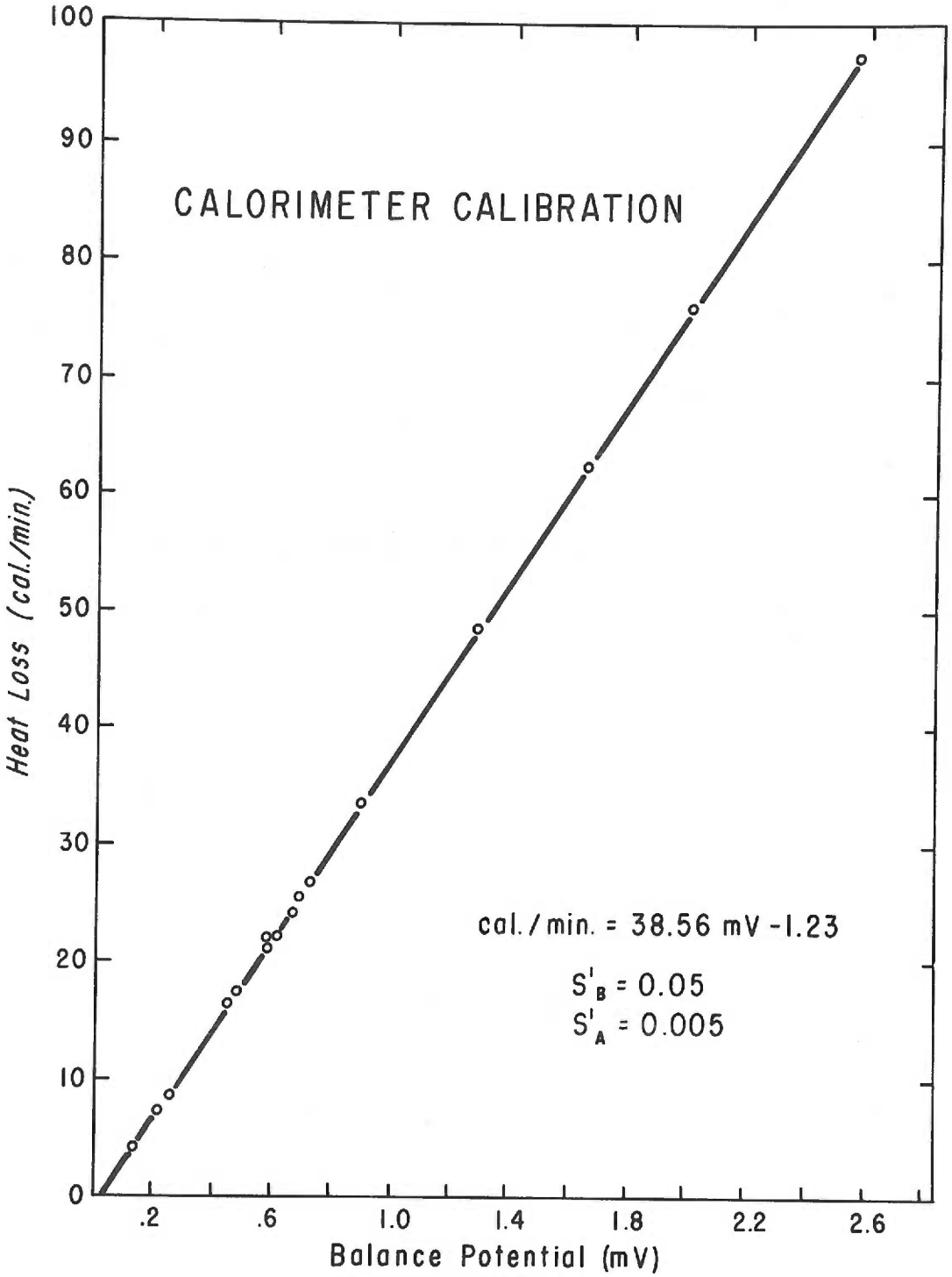

CALORIMETER CALIBRATION
Heat Loss (cal./min.)
Balance Potential (mV)
cal./min. = 38.56 mV -1.23
S'B = 0.05
S'A = 0.005
0
10
20
30
40
50
60
70
80
90
100
.2
.6
1.0
1.4
1.8
2.2
2.6

TABLE 3

CALORIMETER CALIBRATION

Bath Temp. °C	Gas Flow cc/min	Current amps	Potential volts	Heat Loss cal/min	Balance Potential mV
29.7	165	———	———	———	.035
20.3	158	———	———	———	.039
29.7	186	———	———	———	.031
29.7	No Flow	———	———	———	.032
29.7	No Flow	———	———	———	.031
29.7	150	.059	5.15	4.34	.140
29.7	150	.078	7.00	7.80	.228
29.7	186	.080	7.50	8.57	.260
29.7	220	.113	10.40	16.78	.463
29.7	158	.113	10.54	16.86	.473
29.7	220	.114	10.50	17.09	.473
20.3	166	.128	11.75	21.48	.588
20.3	155	.130	11.97	22.22	.599
29.7	154	.130	12.00	22.28	.607
20.3	159	.136	12.60	24.47	.676
20.3	159	.101	17.70	25.53	.689
29.7	220	.143	13.05	26.65	.723
29.7	154	.163	14.50	33.75	.901
20.3	186	.199	17.00	48.31	1.290
29.7	220	.220	19.90	62.52	1.657
20.3	158	.240	22.20	76.08	2.000
20.3	157	.283	24.10	97.39	2.562

just before it entered the chamber. This was accomplished by passing the gas into the base of a water filled jar through a fritted glass tube. By placing a thermistor near the point where the gas leaves the chamber it was found that generally the effluent gas is aroung 1.5°C warmer than the temperature of the entering gas. At the flow rates employed, the heat dissipated by warming and further saturating the gas is about 0.25 cal/200 cc which is less than 1% of the observed heat loss.

The 50% response time of the calorimeter is about four minutes. Despite the slow response time, the calorimeter can follow the changes in the animal's heat loss reasonably faithfully. This is because changes in the rate of heat loss are much slower even in the dead pup where heat production has stopped.

Combining the regression equation for calorimeter output with the factor for body heat, the equation for heat production takes the following form:

$$\frac{\text{Heat production}}{\text{cal/min/kg}} = \frac{38.56x - 1.23}{\text{body wt kg}} + \left(\begin{matrix}\text{°C change in rectal}\\ \text{temperature/min}\end{matrix}\right)\left(\begin{matrix}830\\ \text{cal/kg}\end{matrix}\right)$$

The oxygen consumption was obtained by the equation:

$$\frac{O_2 \text{ consumption}}{\text{cc/min/kg}} = \frac{\left(\begin{matrix}\%\ O_2\\ \text{entering}\end{matrix}\right)\left(\begin{matrix}\text{inflow}\\ \text{cc/min}\end{matrix}\right) - \left(\begin{matrix}\%\ O_2\\ \text{leaving}\end{matrix}\right)\left(\begin{matrix}\text{effluent}\\ \text{cc/min}\end{matrix}\right)}{\text{body weight kg}}$$

The equation was simplified according to the following considerations:

1) The oxygen concentration (at STPD) of the gas used in each experiment was constant at 23.69% O_2 as determined by Scholander analysis.

2) The volume leaving the system will differ from inflow volume by only 0.5% per ± 0.1 change in R.Q. at the flow rates employed here. This is within the limits of error of the present instrumentation.

3) Changes in gas volume from changes in water content or temperature do not enter into the calculations because bath flow and P_{O_2} were measured in terms of dry gas at STP.

4) The oxygen analyzer responded linearly to P_{O_2} which was then converted to percent oxygen by the equation:

$$\% O_2 \text{ of dry gas} = \frac{k \times mV \times 100}{B - P_{H_2O}}$$

Where k was the calibration constant in mm Hg per mV analyzer output.

The final equation for oxygen consumption used was:

$$O_2 \text{ cons/min/kg} = \text{flow/min/kg} \left[23.69 - \frac{2765(mV)}{B_{Press} - P_{H_2O}} \right]$$

Gas flow was measured by a self heating thermistor, which was placed in the path of the inflowing gas. The thermistor's resistance changed in proportion to the cooling that varying rates of gas flow produce. Figure 3 depicts the electrical circuit of the thermistor flow meter. A reproduceable, non linear calibration curve was obtained once the unit was submerged in ice water. Table and figure 4 show the calibration which was obtained using a Collins spirometer as a volumetric standard. The advantages of the thermistor flow meter are that it covers a wide range of gas flows with considerable sensitivity

Figure 3. Circuit for thermistor flow meter

CIRCUIT FOR THERMISTOR FLOW METER

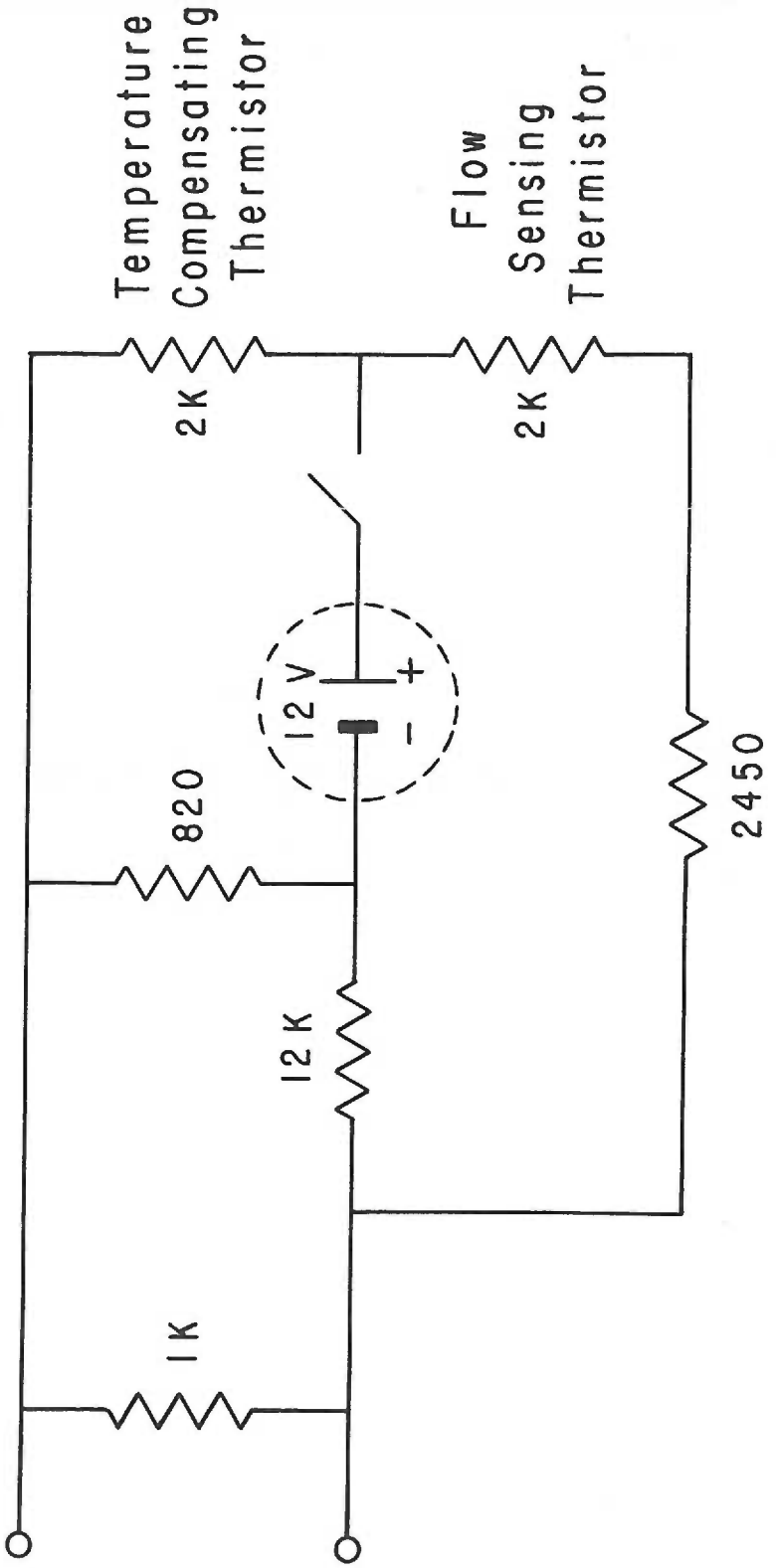
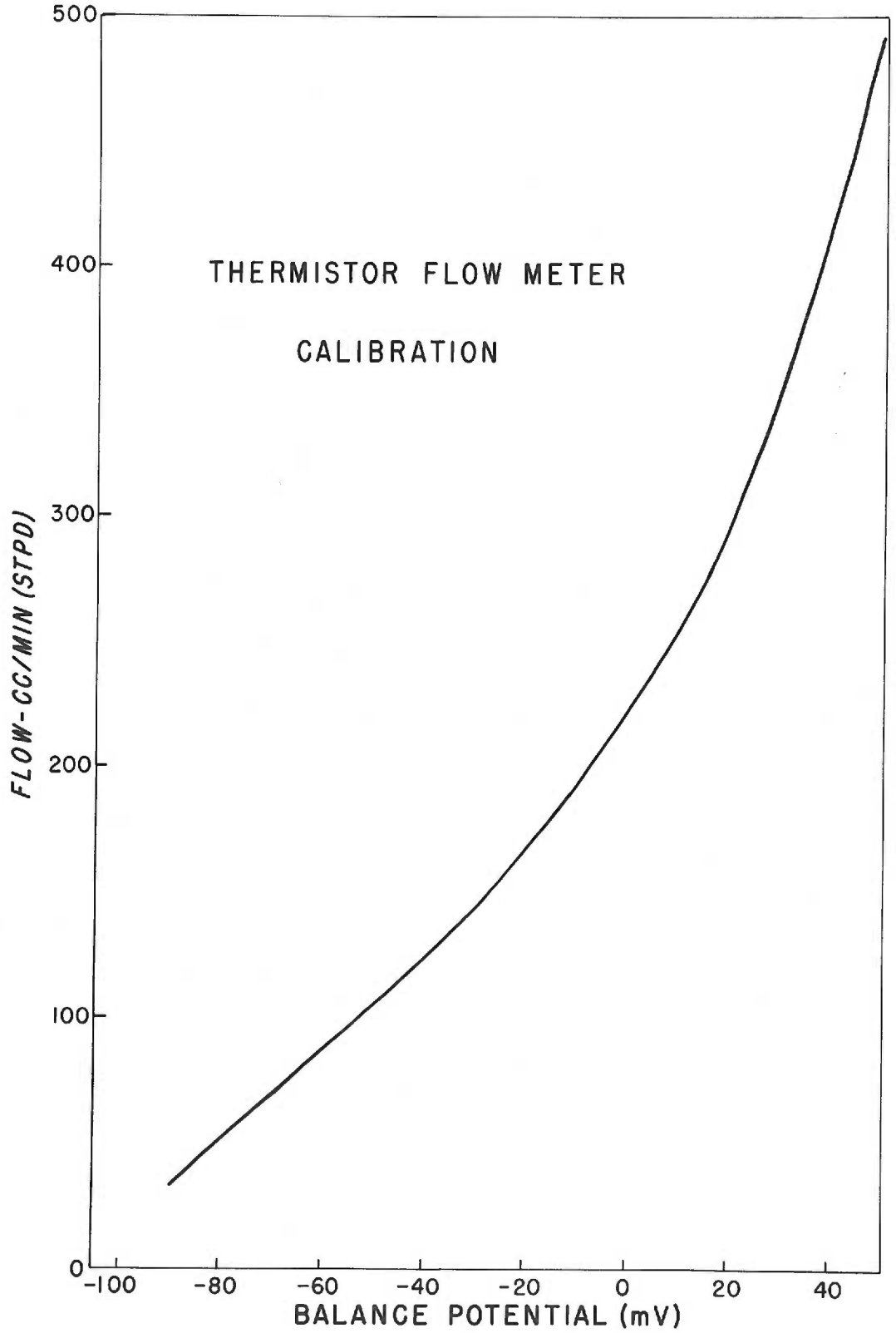


Figure 4. Thermistor flow meter calibration


THERMISTOR FLOW METER
CALIBRATION
FLOW-CC/MIN (STPD)
500
400
300
200
100
0
-100
-80
-60
-40
-20
0
20
40
BALANCE POTENTIAL (mV)

and gives a steady signal despite pressure changes from the animal's respiratory efforts.

For the anoxic episodes 99.7% N_2 was used. The shift from compressed air to nitrogen was generally brought about in 2 to 3 minutes. It entailed switching the reduction valve to the nitrogen tank and increasing the flow rate to 2 l/min. The flow rate was returned to the control level once the oxygen analyzer indicated that all of the oxygen had been flushed out of the chamber. To terminate the anoxic episode the reverse procedure was followed. The duration of an anoxic episode was defined as the interval from when the analyzer indicated zero oxygen until the time when the chamber was first perfused with air again.

The oxygen concentration of the out-flowing gas was obtained by passing 60 cc/min of the effluent through a Beckman model F-3 oxygen analyzer which had been calibrated with gas mixtures of known oxygen content. The sample gas was pumped to the analyzer with a low capacity suction pressure pump. The flow was regulated to 60 cc/min with a Nupro valve between the pump and the analyzer. The gas flow rate was indicated by a flow-meter distal to the analyzer. By injecting a sample of gas into the chamber it was found that the maximum response appeared on the analyzer in about 50 seconds. To compensate for this delay the oxygen analyzer signal was related to the rectal temperature, heat loss, and gas flow rate of the previous minute.

Because the newborn's anoxia tolerance is influenced so markedly by ambient temperature, attempts were made to evaluate the effect of this variable on the parameters determined in these experiments. To do this, half of the experiments were conducted with the chamber in a 20.2 °C water bath and for the remainder the temperature was 29.45°C. The bath temperature was maintained within ± 0.1°C by an infra red light and a stirrer. To provide the cooler bath the heating of the lamp was balanced by running cold tap water through a heat exchange coil in the bath.

Barometric pressure was determined during the course of each experiment. Gas flow, effluent P_{O_2}, rectal temperature, and heat loss were recorded with a four channel Grass Polygraph. In each case a low level D-C preamplifier was used.

RESULTS

RESULTS

The experiments yielded qualitatively uniform results. A significant quantitative compilation of the data from the individual animals was precluded by virtue of the sample size, and variability in ambient temperature, duration of anoxia as well as animal age and weight. Because each animal served as its own control before the anoxic episode, meaningful conclusions can be drawn from individual animals even though between runs there are almost as many variables as experiments.

Rectal temperature:

In every experiment there was a definite fall in rectal temperature associated with the anoxic episode. The fall in temperature was about 1°C in the warm water bath (29.45°C), while in the cold water bath (20.2°C) the decline in rectal temperature was as much as 3°C. Characteristically, the fall in rectal temperature became increasingly pronounced during the course of the anoxic period and extended into the recovery period. In 9 out of 12 of the experiments the maximum rate of rectal temperature fall occurred in the early part of the recovery period. This is most likely a reflection of improving cardiac function bringing relatively stagnant cool blood from the periphery to the core. The depression of circulation with anoxia has been well documented (108). The change in the relation between rectal temperature and mean body temperature associated with changes in distribution of blood flow severely limited the quantitative interpretation of the data

on heat production.

In all six experiments in a warm environment, the minimal rectal temperature was reached by the 12th minute of the recovery period and generally before then. The six cold experiments had a falling temperature curve extending a minimum of 18 minutes into the recovery phase and usually much longer.

In 10 of the 12 runs there was a subsequent rise in rectal temperature towards the control level. The two other experiments continued to show slight decreases in rectal temperature during recovery at a reduced rate.

Heat loss

The rate of heat loss during the control period was about 75 cal/min/kg in four of the six animals in the warm water bath. Puppies in the cold bath had significantly higher rates of heat loss, greater than 100 cal/min/kg. The two youngest pups in the cold bath had continuously falling rectal temperatures during the control period and showed much lower rates of heat loss.

During the anoxic period there was typically a slight decline in the rate of heat loss. In five of the experiments there was a sudden burst of measured heat loss toward the end of the anoxic episode. The rapid appearance and subsequent disappearance of this signal was apparently the characteristic calorimeter response to the passing of stool, meconium, or urine. Fecal material or urine was always found in the chamber after an experiment was completed during which this

sudden rise in heat loss was observed.

Generally the rate of heat loss started to increase immediately after the anoxia at a time when rectal temperature was often still falling. This apparent discrepancy may be due to the dissociation between rectal and cutaneous temperature plus the increasing insensible heat loss with improved ventilation.

Heat production

Relatively small changes in rectal temperature led to marked variations in calculated heat production since 0.1°C change in temperature corresponds to 83 calories of heat per kilogram. Thus, the derived curve of heat production shows spurious fluctuations to the extent that the rectal temperature changes were out of phase with the mean body temperature. This was particularly pronounced during periods of presumed decreasing and recovery circulatory function during and after the anoxia. For example, if mean body temperature declined gradually by 0.15°C over a period of minutes, but the corresponding change in rectal temperature occurred later during a one minute interval, then 125 calories would be subtracted from the heat loss for that interval and a negative quantity for heat production would result.

Despite this limitation in the measurement of heat production, the simple calorimeter gave surprisingly good correlation between direct and indirect calorimetry during periods of steady state. During the control period the six pups in the warm bath maintained levels of heat production below 100 cal/min/kg, generally about 75 cal/min/kg. The

four older pups in the cold bath had a control level of heat production of about 1.25 cal/min/kg. The two pups who entered the cold bath on the first day of life were unable to maintain thermal homeostasis and showed a progressive decline in heat production as rectal temperature decreased.

Within the first two minutes of anoxia the rate of heat production regularly began to decrease toward levels that were always below 50% of the control period. With the drop in rectal temperature during the early recovery period, there was often a corresponding dip in the heat production curve to below zero. During the actual anoxic period, heat production was always a positive value. After less than 10 minutes of recovery all of the pups showed a rise in the rate of heat production and a return toward control levels. The subsequent course correlated well with the temperature curve. Animals which returned rapidly to control temperature also exhibited rates of heat production above the control level. Other pups, who made a sluggish or incomplete thermal recovery, had heat production rates at or below the control level. Two pups, who succumbed to the anoxia, had a rapid drop in heat production to near zero. The finding that heat production continued to hover about zero for up to four hours of cooling served as a check on the calorimeter, and the value for the animal's specific heat.

Oxygen consumption

During the control period the caloric value of the oxygen consumed correlated closely with the heat production but was almost always a few calories above it. This cannot be explained by the error in estimating

mean body temperature, since the excess observed by indirect calorimetry was present over several hours regardless of whether rectal temperature was stable, rising or falling.

Immediately preceding and following the period of absolute anoxia is a three minute span of hypoxia during which time it was not possible to carry out indirect calorimetry because of the changing gas composition and flow associated with the flushing. During the flush periods there was regularly a slight dip in the heat loss curves for which no correction was made since its magnitude and duration were small.

In all experiments the initial post anoxic measurements for oxygen consumption were markedly depressed in comparison to the control values. In 11 of the 12 experiments the initial caloric value of the oxygen consumed fell in the range of 25 $\pm$ 6 cal/min/kg which was generally less than half of the control value. The rise in post anoxic oxygen consumption always exceeded the rise in heat production. The disparity between the two was least in the pup with the shortest anoxic period. The area between the heat production curve and the oxygen consumption curve was always greater than 200 calories. (Henceforth, whenever comparison is made between oxygen consumption and heat production, oxygen consumption will be described in terms of its caloric value.)

In several of the experiments there was a temporal association between a rising rectal temperature and a meeting of the lines describing heat production and oxygen consumption. This can be interpreted as showing that at least part of an oxygen debt is paid back before the pup pays back a heat debt. Alternatively, it may mean that the oxygen debt is an

artifact from the distortion in the heat production curve during the period of falling rectal temperature. In five of the experiments spontaneous rises and falls in rectal temperature occurred later in the recovery period. On each occasion the calculated change in heat production was associated with a corresponding change in the measured rate of oxygen consumption. This close correlation between direct and indirect calorimetry was also present for gradual changes in body temperature.

Oxygen debt

An oxygen debt can be defined as the calories of heat produced by the animal during the period of anoxia. As a corrollary, repayment of the oxygen debt is defined to be the excess of the caloric value of the oxygen consumed over the heat produced during the recovery period. In every experiment the heat production during the anoxic interval was less than the heat production for a corresponding control period (table4). The percent decrease in heat production varied from 23% for the shortest anoxic period of 5.5 minutes (Exp 27-B) to 65% for the longest anoxic episode of 16.7 min (exp 21). The estimates for anoxic heat production ranged from 250 cal/kg to 770 cal/kg which is always more than the 65 cal/kg that could be obtained from a generous estimate of the oxygen stores (39).

The heat produced during the anoxic interval has a rough quantitative correlation to the excess of oxygen consumed over heat production in the early recovery period. Although there is considerable individual

TABLE 4

Heat production during anoxia and recovery

Exp. #	Ambient temp. °C	Duration of anoxia min	Expected heat production cal/kg	Observed heat production cal/kg	Decrease in heat production* %	Caloric value of excess O_2 consumed cal/kg	O_2 debt paid off** %
21	20.2	16.7	710	250	65	265	106
25	20.2	12.0	985	475	52	615	130
28-A	20.2	10.0	1250	710	43	650	92
28-B	20.2	10.0	1250	720	43	620	86
29	20.2	10.0	1150	700	39	700	100
13-A	20.3	10.0	1210	770	36	430	56
13-B	29.6	9.3	720	470	35	290	62
22	29.4	11.5	834	370	56	240	65
23	29.4	9.0	765	510	33	320	63
24	29.4	9.0	835	490	41	560	114
27-A	29.4	7.0	710	520	27	650	125
27-B	29.4	5.5	410	315	23	210	67

*Percentage decrease in heat production = $100 \left(\frac{\text{expected heat production} - \text{observed heat production}}{\text{expected heat production}}\right)$

**Percentage oxygen debt paid off = $100 \left(\frac{\text{caloric value of excess oxygen consumed}}{\text{observed heat production}}\right)$

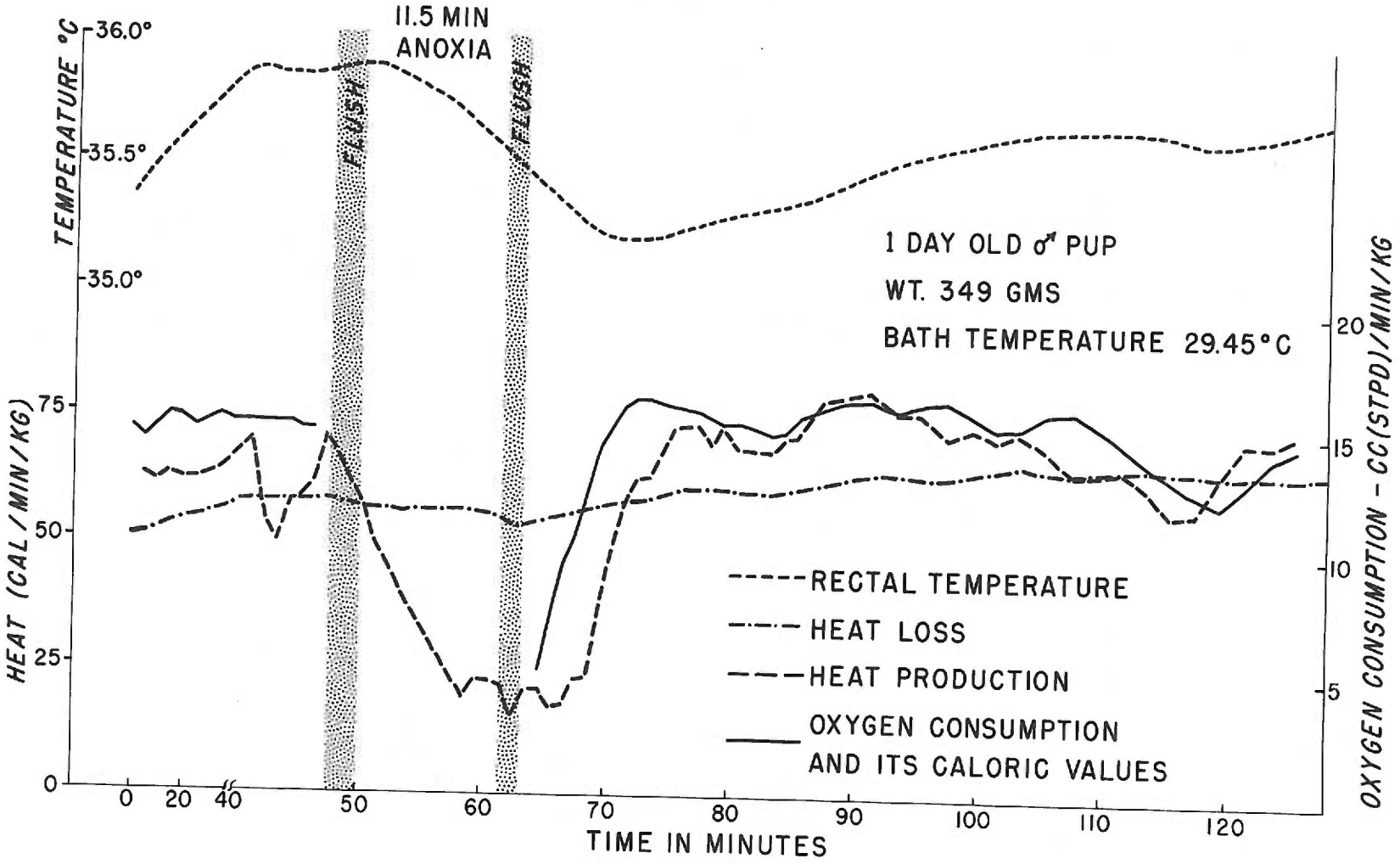
variation, the overall agreement between the two estimates of an oxygen debt is within 12% when all the experiments are taken together. The individual experiments show a correlation coefficient of $r = 0.69$. Considering the sample size, there is less than a 0.006 probability of getting such a correlation by chance alone.

The subsequent 12 figures illustrate the foregoing generalities and point up some of the individual variations.

In experiment 22 (fig 5), the one day old pup showed an initial rise in rectal temperature and heat loss during the control period. The low temperature observed shortly after removal from the animal quarters, was not uncommon in the first 24 hours. The slight dip in the rectal temperature later in the control period was probably spurious since the calculated fall in heat production was not accompanied by a change in oxygen consumption as occurred at 120 minutes. With the onset of anoxia, both rectal temperature and heat production began to decline. Within the first ten minutes of recovery, both parameters started to return toward control levels. The rate of heat loss decreased only by about 3 cal/min/kg during the 11.5 minute anoxic episode.

The conventional estimate of an oxygen debt as a rise in oxygen consumption during the recovery period above control levels reveals only a negligible oxygen debt in this puppy. However, the pup's metabolic response to anoxia appears quite different when an oxygen debt is redefined as a disparity between oxygen consumption and heat production. During the anoxic period the heat production curve outlines an area

Figure 5. Experiment 22. 11.5 minute anoxic episode with more than a 50% decrease in metabolism. There was a concurrent dip in oxygen consumption and heat production with the fall in rectal temperature at 118 minutes.


11.5 MIN
ANOXIA
FLUSH
FLUSH
TEMPERATURE °C
36.0°
35.5°
35.0°
HEAT (CAL/MIN/KG)
75
50
25
0
1 DAY OLD ♂ PUP
WT. 349 GMS
BATH TEMPERATURE 29.45°C
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION
AND ITS CALORIC VALUES
OXYGEN CONSUMPTION - CC(STPD)/MIN/KG
20
15
10
5
0
20
40
50
60
70
80
90
100
110
120
TIME IN MINUTES

corresponding to 370 calories which is more than a 50% decrease in heat production relative to control level. If this is an incurred oxygen debt, it corresponds roughly with the 240 calories by which oxygen consumption exceeds heat production during the immediate recovery period.

Experiment 23 (fig 6, table 6) was very similar in general pattern except that this litter mate was one day older and maintained a higher body temperature and rate of heat loss. Again there was unequivocal evidence for an anoxic suppression of energy metabolism as judged by the fall in rectal temperature, rate of heat loss, calculated heat production, and the initial post anoxic suppression of oxygen consumption. In this case the return to control body temperature came somewhat later and was associated with a peak in the oxygen consumption curve at that time.

Experiment 13-A (fig 7, table 7) was conducted in the cold bath with a three day old pup. While the previous two experiments in warm baths showed less than a 1°C fall in rectal temperature, this pup with a comparable anoxic period had a 3°C fall in rectal temperature. During the early recovery period rectal temperature takes a particularly steep dip and the corresponding negative values for heat production probably reflect a drop in mean body temperature, which actually occurred during the anoxic period. This shift in the depression of the heat production curve contributes to the uncertainty of estimating the magnitude of pay off of the oxygen debt. There was, however, a marked rise

Figure 6. Experiment 23. Nine minute anoxic period. During recovery, oxygen consumption exceeds heat production in a fashion suggesting the pay off of an oxygen debt.

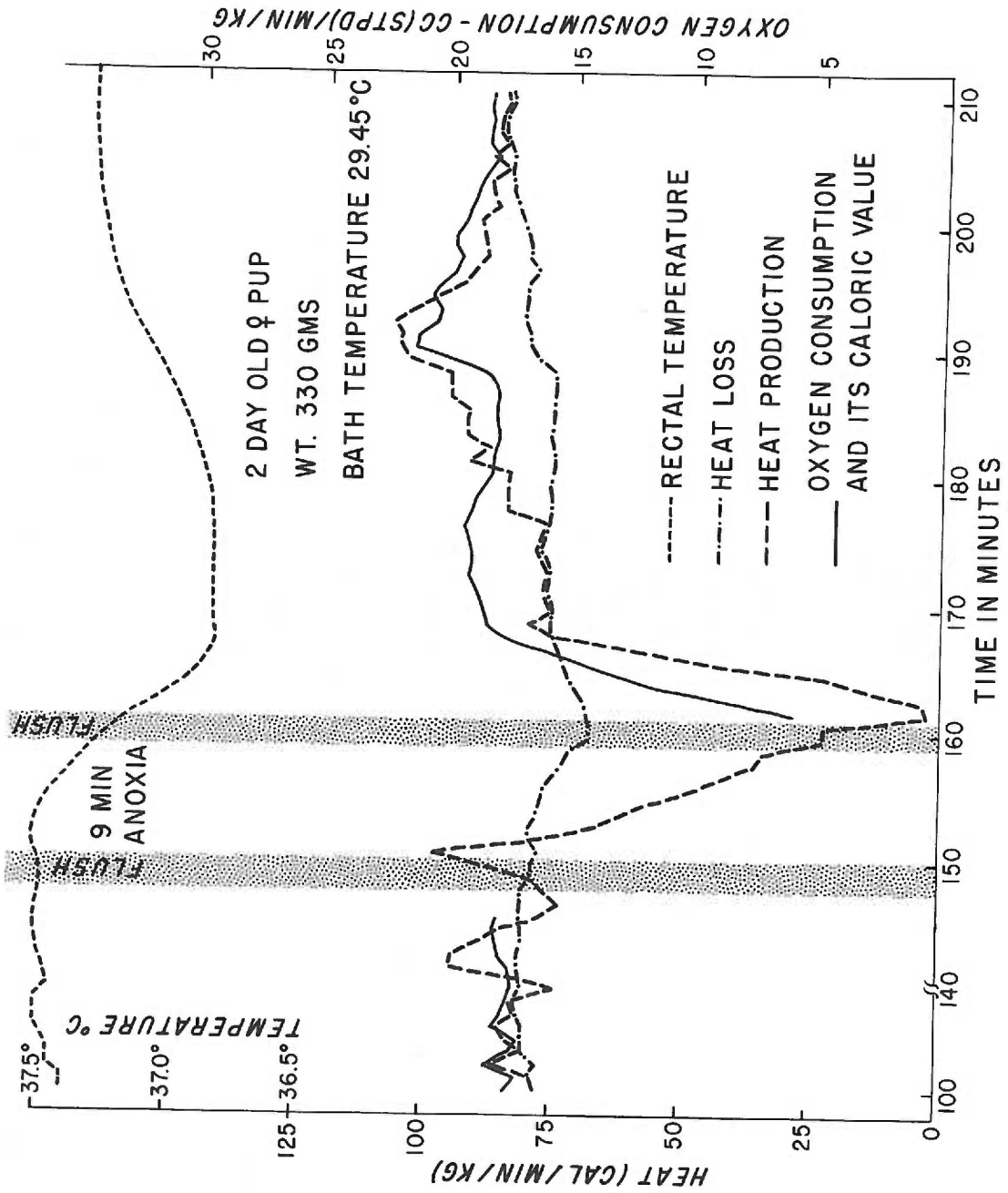
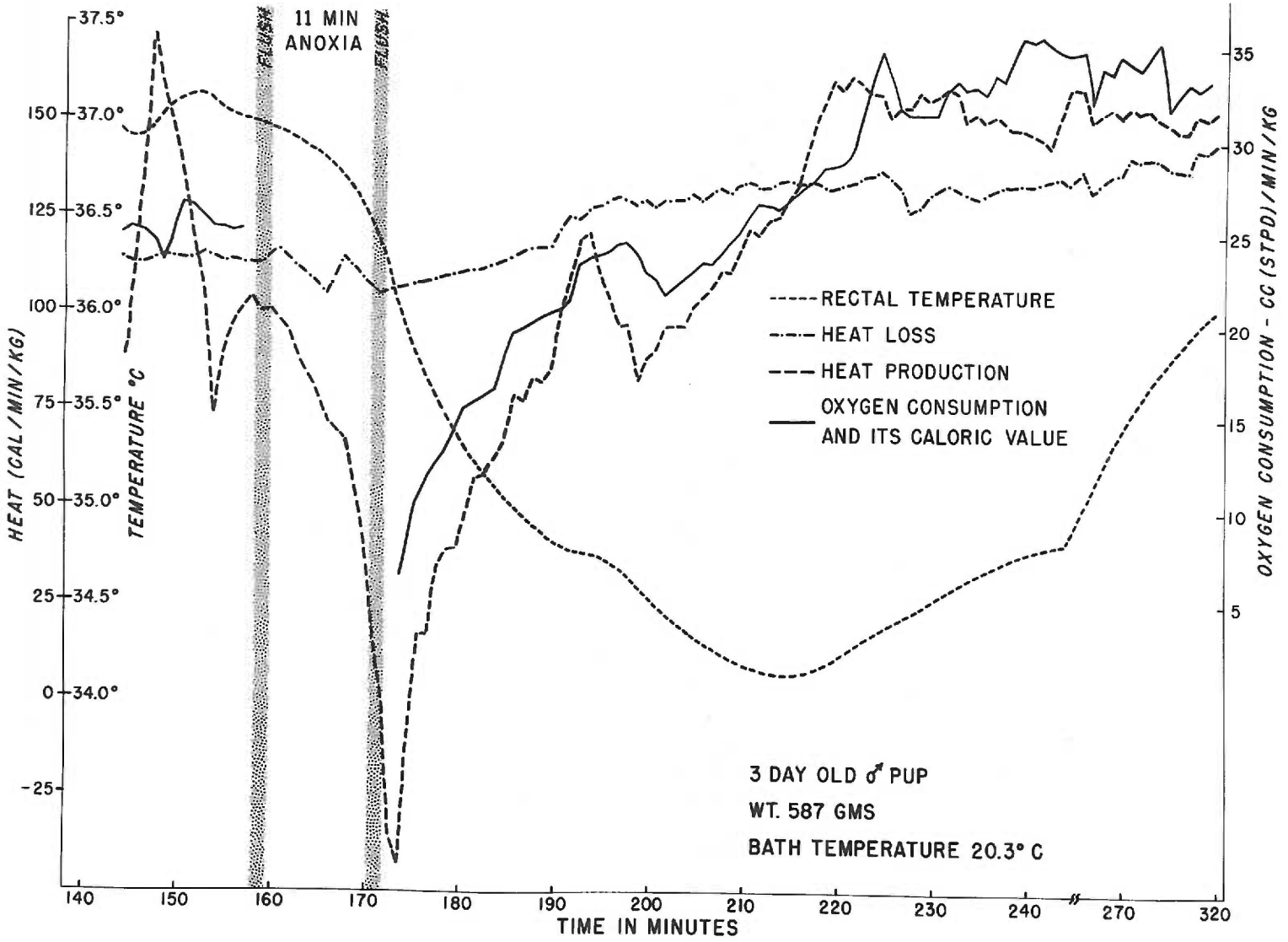

OXYGEN CONSUMPTION - CC(STPD)/MIN/KG
30
25
20
15
10
5
2 DAY OLD ♀ PUP
WT. 330 GMS
BATH TEMPERATURE 29.45°C
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION
AND ITS CALORIC VALUE
FLUSH
9 MIN
ANOXIA
FLUSH
TEMPERATURE °C
37.5°
37.0°
36.5°
125
100
75
50
25
0
HEAT (CAL/MIN/KG)
100
140
150
160
170
180
190
200
210
TIME IN MINUTES

Figure 7. Experiment 13-A. 11 minutes of anoxia in the colder bath. The animal showed a marked fall in rectal temperature. Note the high rate of oxygen consumption and heat production during the period of thermal recovery.


11 MIN
ANOXIA
FLUSH
FLUSH
37.5°
37.0°
36.5°
36.0°
35.5°
35.0°
34.5°
34.0°
150
125
100
75
50
25
0
-25
TEMPERATURE °C
HEAT (CAL/MIN/KG)
35
30
25
20
15
10
5
OXYGEN CONSUMPTION - CC (STPD)/MIN/KG
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION
AND ITS CALORIC VALUE
3 DAY OLD ♂ PUP
WT. 587 GMS
BATH TEMPERATURE 20.3° C
140
150
160
170
180
190
200
210
220
230
240
270
320
TIME IN MINUTES

in oxygen consumption above control levels, which correlated well with the corresponding rise in heat production.

Experiment 13-B (fig 8, table 8) is a second anoxic episode in the same pup over six hours after the first anoxia and in the warm environment. The difference in the control periods plus the drop in oxygen consumption, heat production, and heat loss from about 150 cal/min/kg at the end of the previous graph to about 75 cal/min/kg in the second control period, reflects this pup's homeothermic response. As in the other experiments, this pup showed the typical fall in heat production during the anoxic episode with a suppression of oxygen consumption immediately afterwards. Here again the major portion of the post anoxic rise in oxygen consumption is not an oxygen debt, but rather the energy expenditure of generating a rise in temperature.

A third anoxic episode for nine minutes in the warm bath at 626 minutes proved fatal, indicating decreasing anoxia tolerance on successive exposures.

Experiment 21 (fig 9, table 9) demonstrates the poikilothermic behavior of one day old pups in the cold bath. During the course of 8.7 hour control period, rectal temperature declined from 32.55°C to 25.56°C. The 7°C fall in rectal temperature was associated with a 40% to 50% decrease in oxygen consumption, heat loss and heat production. This pup's hypothermia enabled him to tolerate the longest period of anoxia and hence, the results are not as subject to the errors introduced by rapid changes in state. The anoxic heat production of 250

Figure 8. Experiment 13-B. Same pup as in figure 4. Experiment repeated in the warmer bath. At the higher ambient temperature the animal showed a marked decrease in metabolic rate. Note that much of the post anoxic rise in oxygen consumption is associated with the restoration of body temperature rather than the pay off of an oxygen debt. (See text)

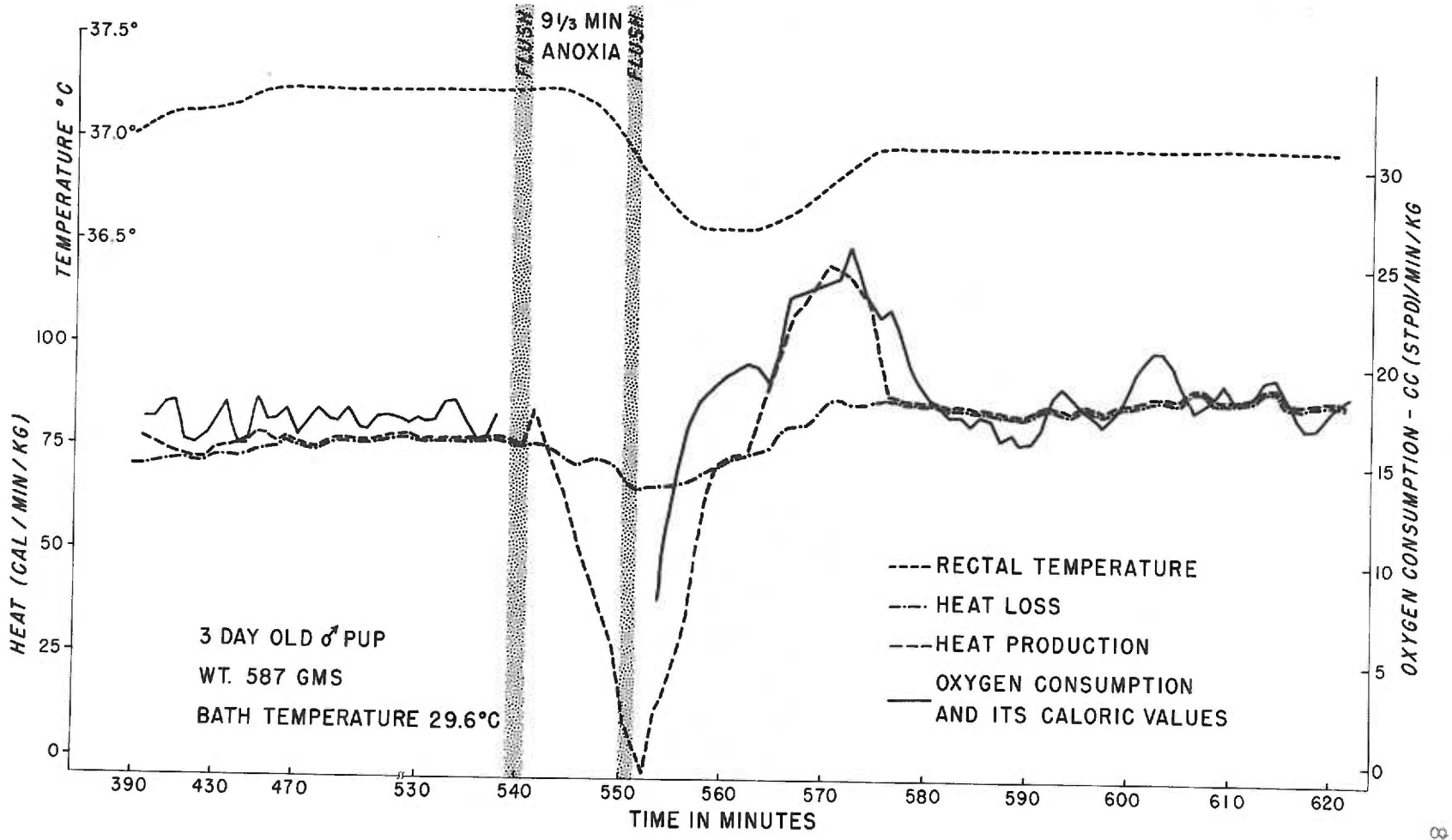
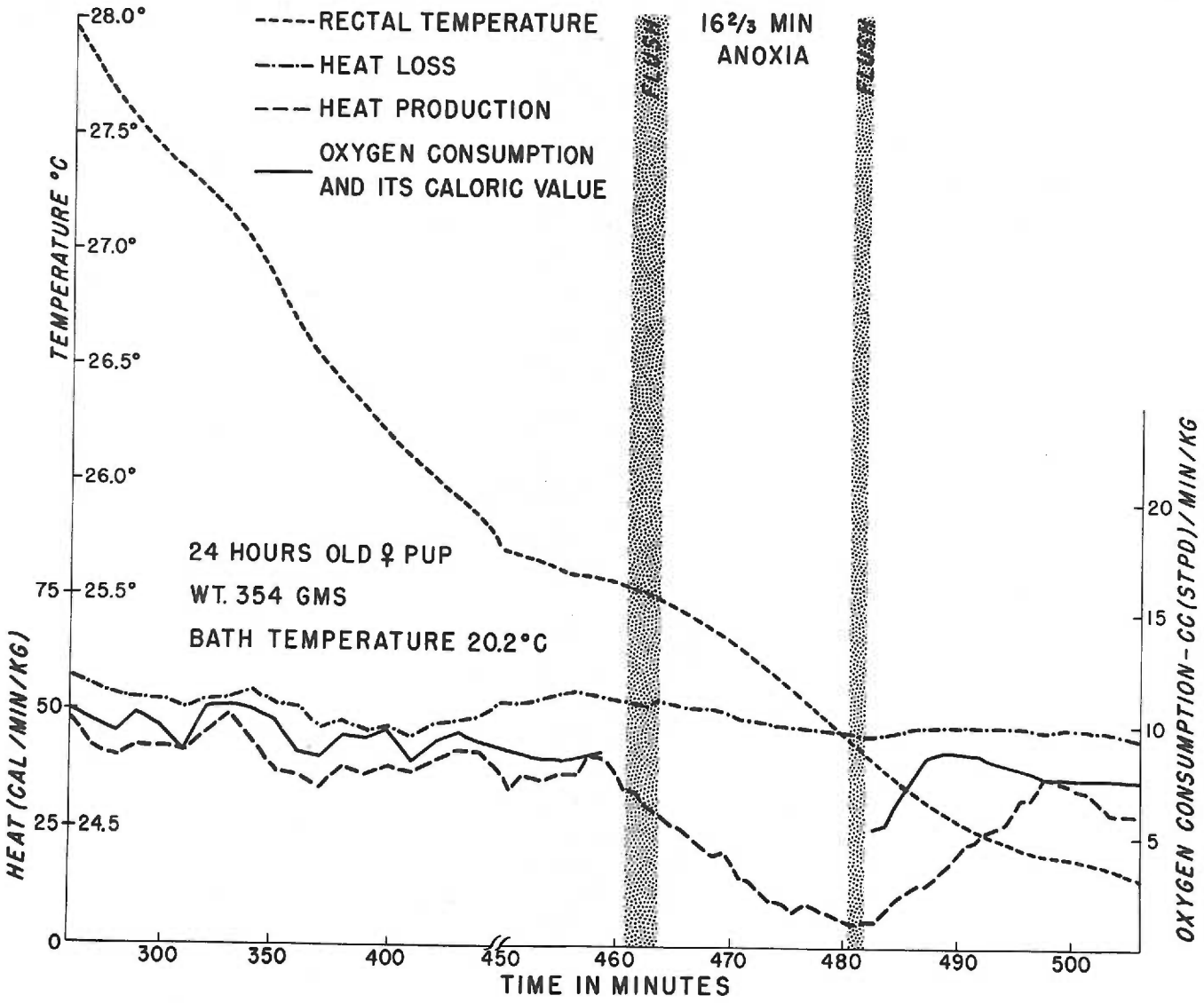

9⅓ MIN
ANOXIA
FLUSH
FLUSH
37.5°
37.0°
36.5°
TEMPERATURE °C
HEAT (CAL / MIN / KG)
OXYGEN CONSUMPTION - CC (STPD)/MIN/KG
100
75
50
25
0
30
25
20
15
10
5
0
390
430
470
530
540
550
560
570
580
590
600
610
620
TIME IN MINUTES
3 DAY OLD ♂ PUP
WT. 587 GMS
BATH TEMPERATURE 29.6°C
----RECTAL TEMPERATURE
-·-·- HEAT LOSS
---HEAT PRODUCTION
OXYGEN CONSUMPTION
AND ITS CALORIC VALUES

Figure 9. Experiment 21. Prolonged anoxia during hypothermia in a one day old pup who was unable to maintain thermal homeostasis. The anaerobic heat production was less than 50% of control levels and was quantitatively equal to the caloric value of the excess oxygen consumed during the early recovery.


-----RECTAL TEMPERATURE
-·-·-HEAT LOSS
- - - HEAT PRODUCTION
OXYGEN CONSUMPTION AND ITS CALORIC VALUE
16⅔ MIN ANOXIA
FLUSH
FLUSH
TEMPERATURE °C
28.0°
27.5°
27.0°
26.5°
26.0°
25.5°
24.5
24 HOURS OLD ♀ PUP
WT. 354 GMS
BATH TEMPERATURE 20.2°C
HEAT (CAL/MIN/KG)
75
50
25
0
OXYGEN CONSUMPTION - CC (STPD)/MIN/KG
20
15
10
5
300
350
400
450
460
470
480
490
500
TIME IN MINUTES

cal/kg was 35% of control levels and agreed within 6% with the excess oxygen consumption of the recovery period. This same puppy was the subject for experiment 24 two days later by which time he had gained 33 g. and overtly recovered from the hypothermia and 16.7 minutes of anoxia.

In experiment 24 (fig 10, table 10) the puppy had a 41% decrease in heat production during the first anoxic episode. In this case the excess oxygen consumption was 14% greater than the 490 cal/kg heat produced during anoxia. The caloric value of the oxygen consumed was also calculated for the lowest caloric equivalent of 4.5 cal/cc of oxygen. It can be seen that the excess oxygen consumption is 16% less than the anoxic heat production under these circumstances. The caloric equivalent of oxygen varies with the substrate, from a maximum of 5.0 cal/cc for carbohydrates to a minimum of 6.5 cal/cc for proteins. Thus, the overall conclusions are valid regardless of a possible change in substrate since the oxygen debt pay off is present even when calculated in terms of the minimum caloric equivalent. By the time of the second shorter anoxic episode, the pup had not restored his normal tolerance. The measured heat loss during the four hours of cooling accounted for most of the observed decrease in temperature and hence, the calculated heat production remained close to zero.

In experiment 25 (fig 11, table 11) the young puppy had a falling rectal temperature in the cold bath. The excess oxygen consumption covers the incurred oxygen debt whether 4.5 or 4.8 is taken as the

Figure 10. Experiment 24. Two consecutive exposures to anoxia in a three day old pup. There was decreased tolerance to a second shorter anoxic episode. The level of heat production remained close to zero throughout the period of cooling of the dead pup. The oxygen debt pay off correlates with the amount of anaerobic heat production regardless of whether 4.8 or 4.5 cal/cc is taken as the caloric equivalent of oxygen.

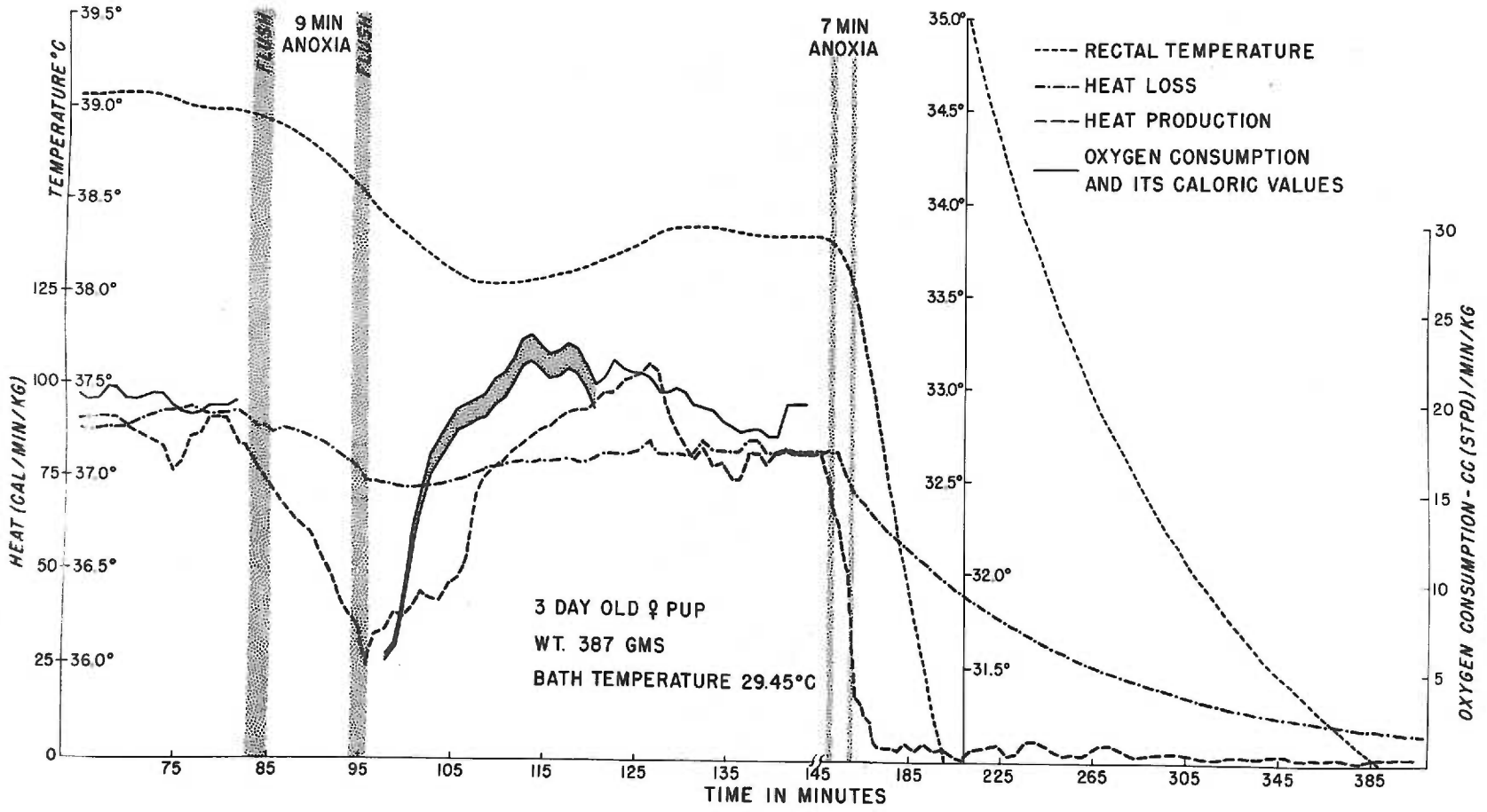

9 MIN ANOXIA
7 MIN ANOXIA
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION AND ITS CALORIC VALUES
TEMPERATURE °C
HEAT (CAL/MIN/KG)
OXYGEN CONSUMPTION - CC (STPD)/MIN/KG
TIME IN MINUTES
3 DAY OLD ♀ PUP
WT. 387 GMS
BATH TEMPERATURE 29.45°C

Figure 11. Experiment 25. 12 minutes of anoxia in a hypothermic pup. Although indirect calorimetry alone would not provide evidence of an oxygen debt, the combination of direct and indirect calorimetry showed close agreement between the oxygen debt incurred during anoxia and the excess oxygen consumption of recovery. Note the coincident decrease in oxygen consumption and heat production with the fall in temperature at 300 minutes.

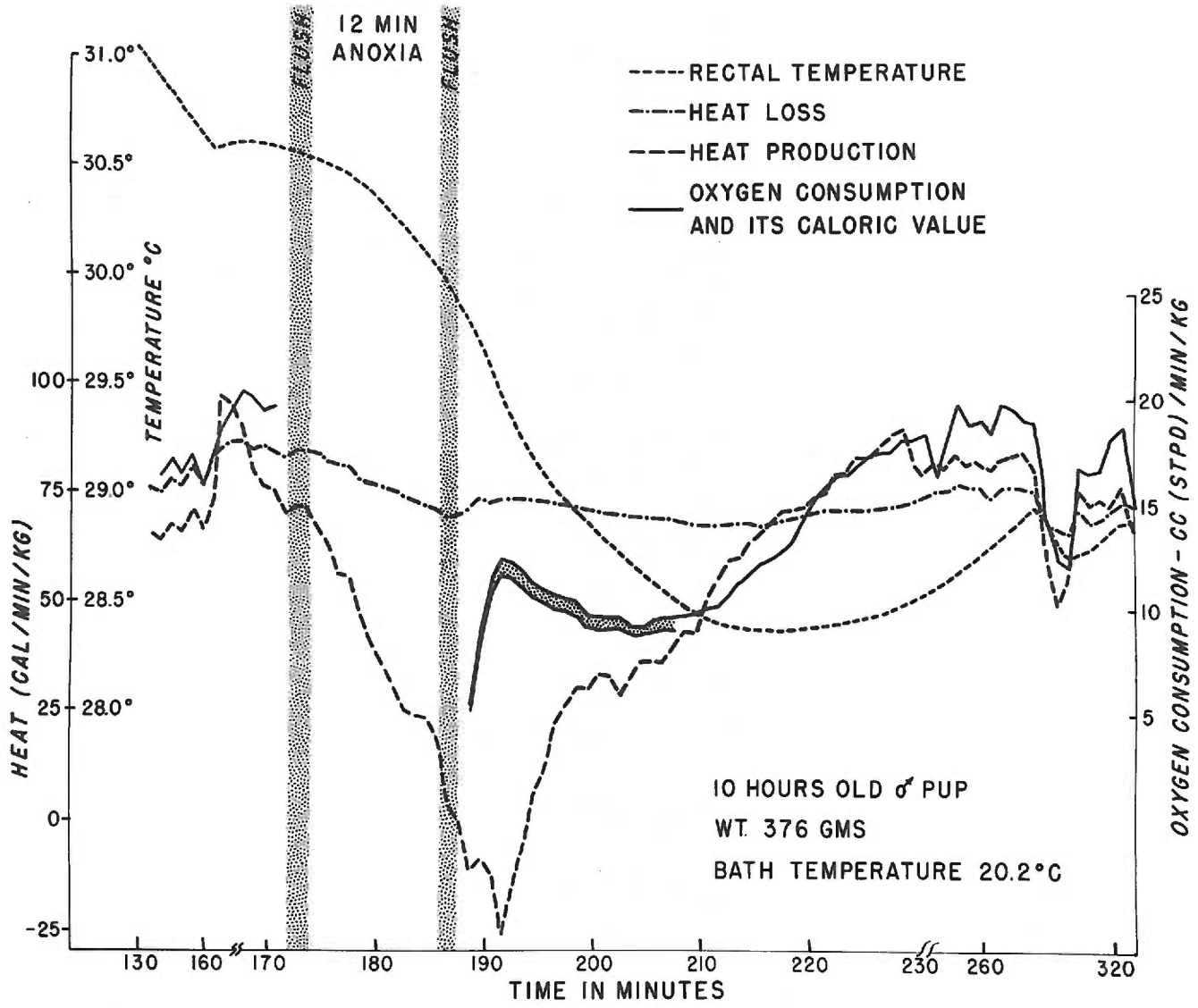
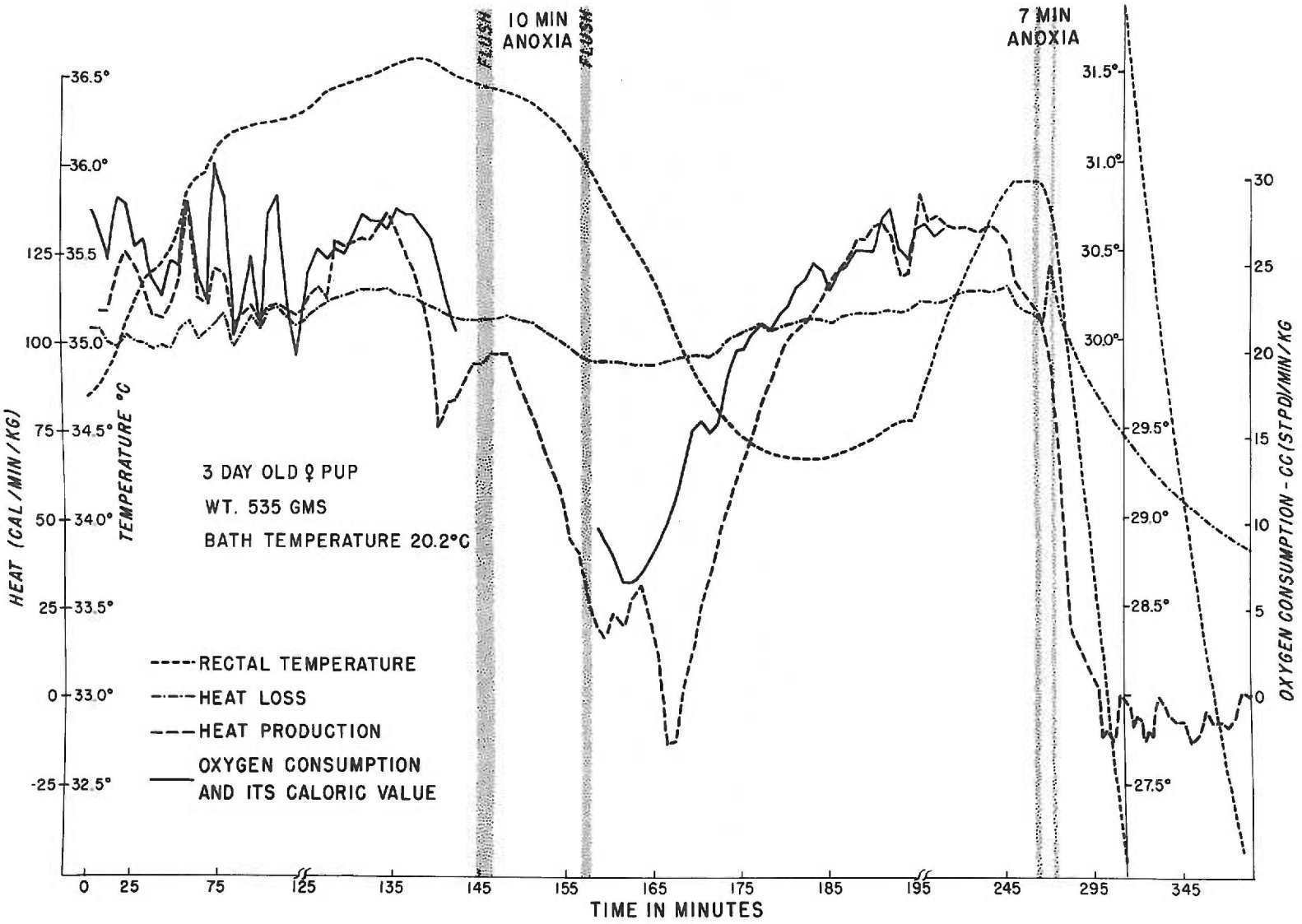

12 MIN
ANOXIA
FLUSH
FLUSH
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION
AND ITS CALORIC VALUE
TEMPERATURE °C
HEAT (CAL/MIN/KG)
OXYGEN CONSUMPTION - CC (STPD) /MIN/KG
31.0°
30.5°
30.0°
29.5°
29.0°
28.5°
28.0°
100
75
50
25
0
-25
25
20
15
10
5
10 HOURS OLD ♂ PUP
WT. 376 GMS
BATH TEMPERATURE 20.2°C
130
160
170
180
190
200
210
220
230
260
320
TIME IN MINUTES

Figure 12. Experiment 29. Two consecutive anoxic exposures in a three day old pup. The estimate of an oxygen debt during anoxia and the pay off following anoxia were 700 calories as measured by both methods of calorimetry. This was significantly less than the 1450 calories of a corresponding control period. The heat production remained close to zero while the pup was cooling after anoxic death from the second, shorter exposure.


10 MIN ANOXIA
FLUSH
FLUSH
7 MIN ANOXIA
HEAT (CAL/MIN/KG)
TEMPERATURE °C
OXYGEN CONSUMPTION - CC (STPD)/MIN/KG
3 DAY OLD ♀ PUP
WT. 535 GMS
BATH TEMPERATURE 20.2°C
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION AND ITS CALORIC VALUE
TIME IN MINUTES

caloric equivalent of oxygen. The level of oxygen consumption remained below control levels throughout the immediate recovery period when the oxygen debt pay off occurred. The pup in experiments 28-A and 28-B (figs 13 & 14, tables 13 & 14) survived a second longer episode than was tolerated by the older litter mate in experiment 29 (fig 12, table 12). This is consistent with previous reports of decreasing anoxia tolerance with increasing age. In each of the anoxic episodes in experiment 28, there was more than a 40% decrease in heat production and greater than 85% pay off of the oxygen debt incurred.

Experiments 27-A and 27-B (figs 15 & 16, tables 15 & 16) is another series of two consecutive anoxic episodes of shorter duration and in the warm bath. These two experiments illustrate the added information that can be obtained about an oxygen debt when the conventional approach of indirect calorimetry is supplemented with direct calorimetry. If the rise in post anoxic oxygen consumption alone is taken as a measure of the magnitude of an oxygen debt, then the second shorter anoxic episode had the greater oxygen debt. By direct calorimetry it becomes evident that after the first anoxic episode the pup's temperature continued to fall while in experiment 27-B there was a rapid rise in rectal temperature with a corresponding increase in the rate of heat production. By focusing on the difference between direct and indirect calorimetry, one finds that the shortest anoxic exposure had the smallest oxygen debt paid back and the quickest thermal recovery.

Figure 13. Experiment 28-A. The steepest fall in rectal temperature occurred after the anoxia and negative values for heat production were derived. The most likely explanation for these spurious values of heat production is that heat was redistributed within the body in such a fashion as to permit a fall in rectal temperature without a corresponding transfer of heat to the calorimeter.

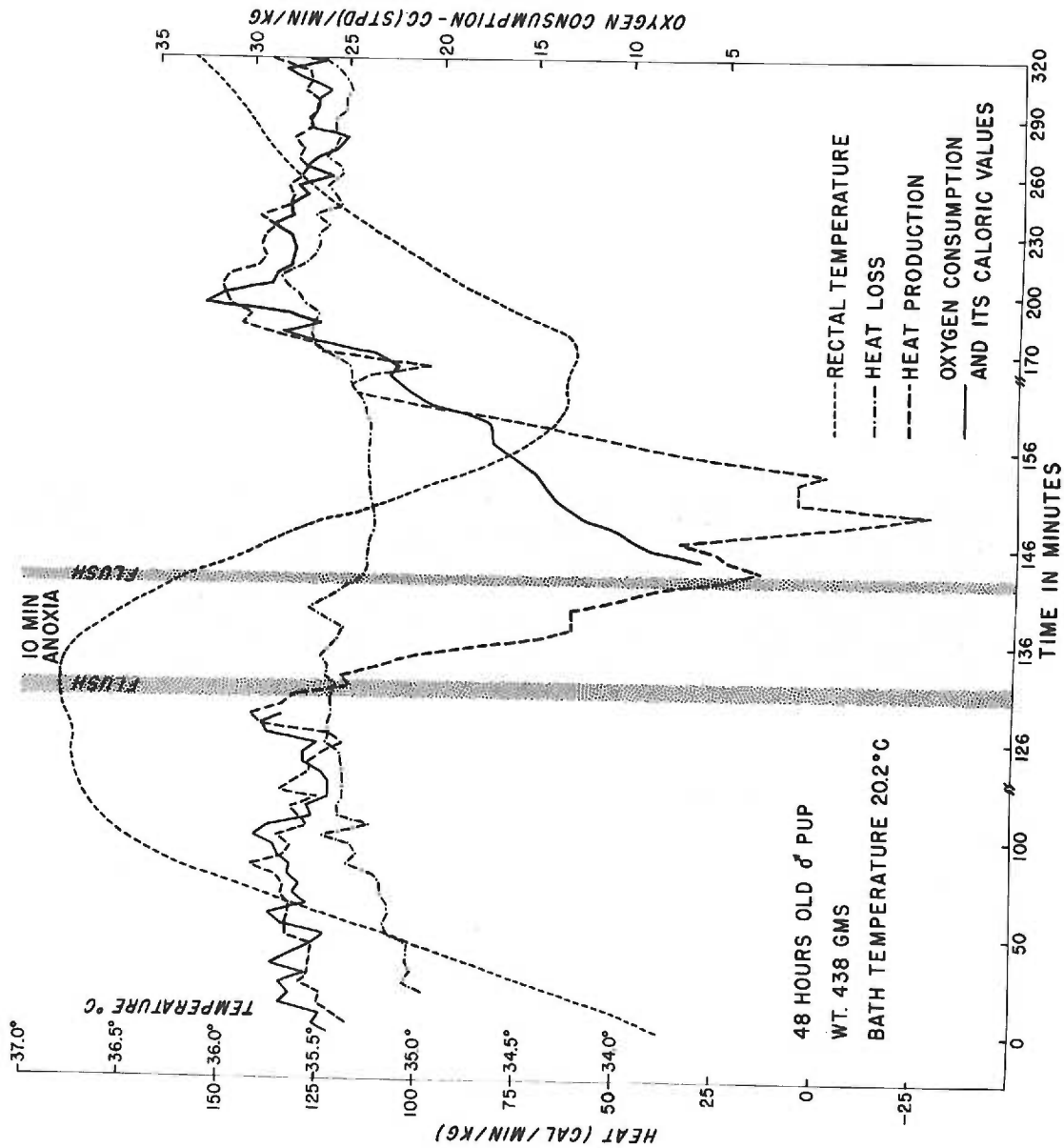

OXYGEN CONSUMPTION - CC(STPD)/MIN/KG
35
30
25
20
15
10
5
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION
AND ITS CALORIC VALUES
FLUSH
FLUSH
10 MIN
ANOXIA
TEMPERATURE °C
37.0°
36.5°
36.0°
35.5°
35.0°
34.5°
34.0°
HEAT (CAL/MIN/KG)
150
125
100
75
50
25
0
-25
48 HOURS OLD ♂ PUP
WT. 438 GMS
BATH TEMPERATURE 20.2°C
0
50
100
126
136
146
156
170
200
230
260
290
320
TIME IN MINUTES

Figure 14. Experiment 28-B. Second consecutive anoxic episode in the cold bath. This two day old pup from experiment 28-A showed better anoxia tolerance than the three day old pups of Figures 7 and 9. There was an abrupt increase in heat loss at the time (340 minutes) of reflex defecation.

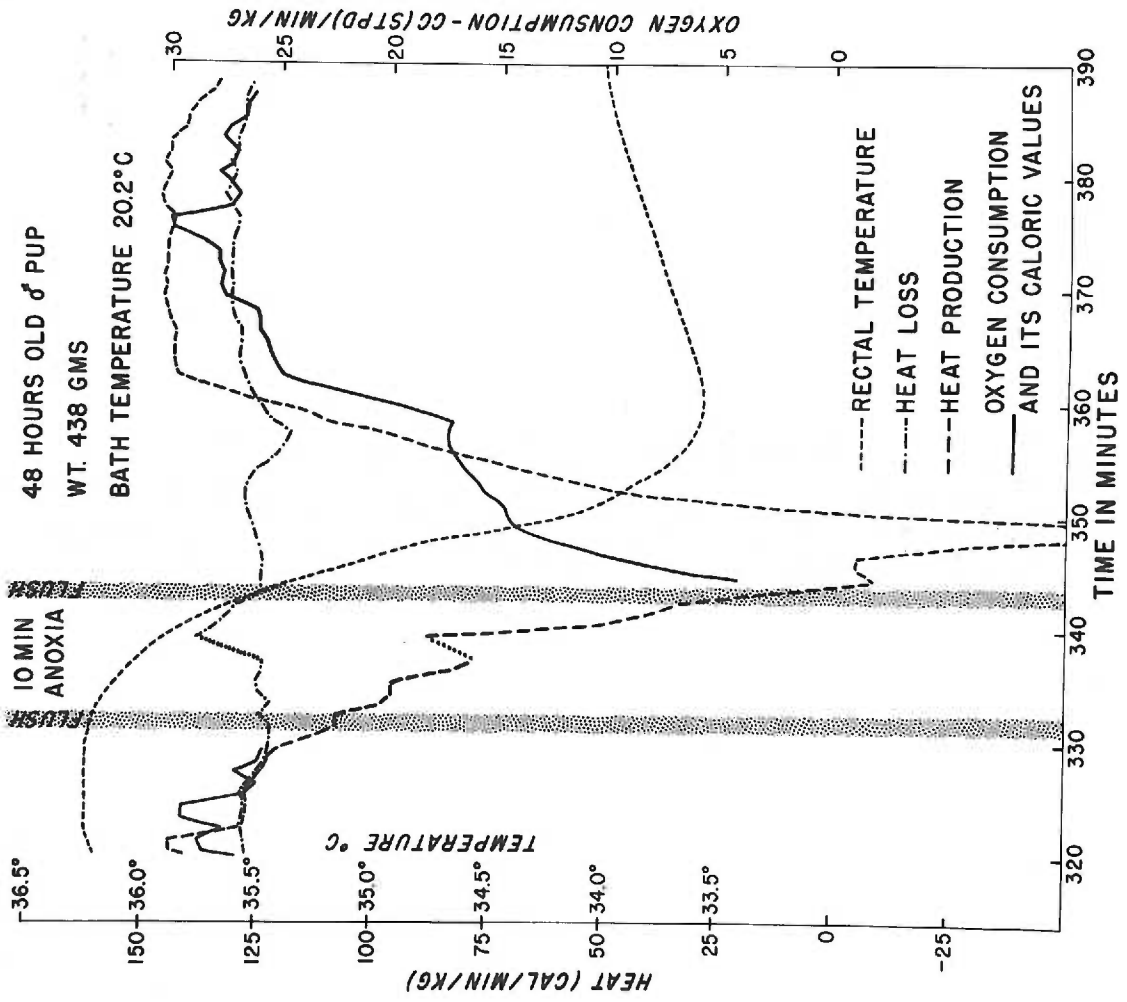

48 HOURS OLD ♂ PUP
WT. 438 GMS
BATH TEMPERATURE 20.2°C
10 MIN ANOXIA
FLUSH
FLUSH
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION AND ITS CALORIC VALUES
TIME IN MINUTES
HEAT (CAL/MIN/KG)
TEMPERATURE °C
OXYGEN CONSUMPTION - CC (STPD)/MIN/KG

Figure 15. Experiment 27-A. Relatively short anoxic episode with little decrease in heat production during the anoxia and a correspondingly larger oxygen debt pay off in the absence of a return to control rectal temperatures. In this experiment, oxygen consumption transiently exceeded pre-anoxic oxygen consumption. This increase would represent the conventional demonstration of an oxygen debt by indirect calorimetry.

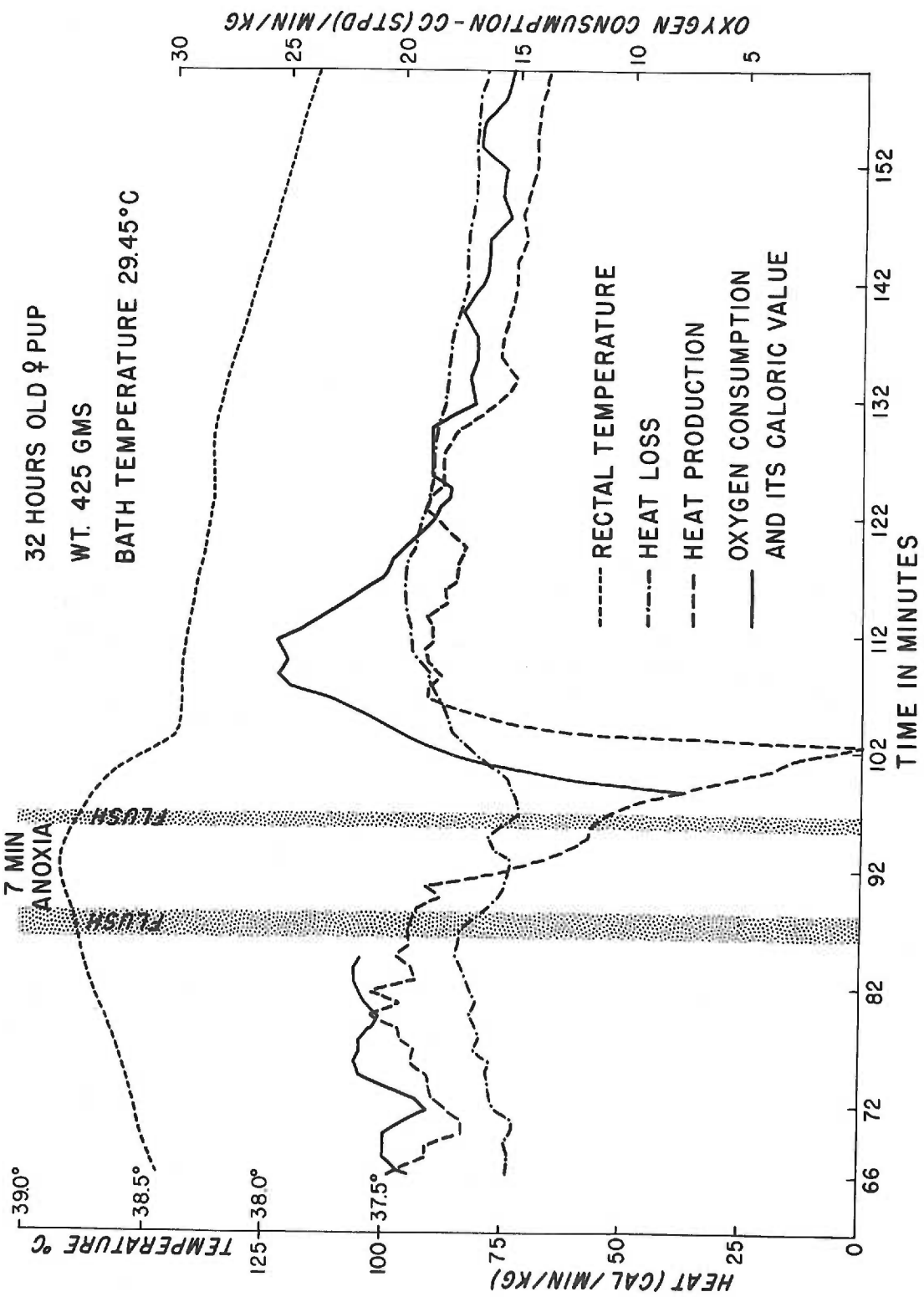
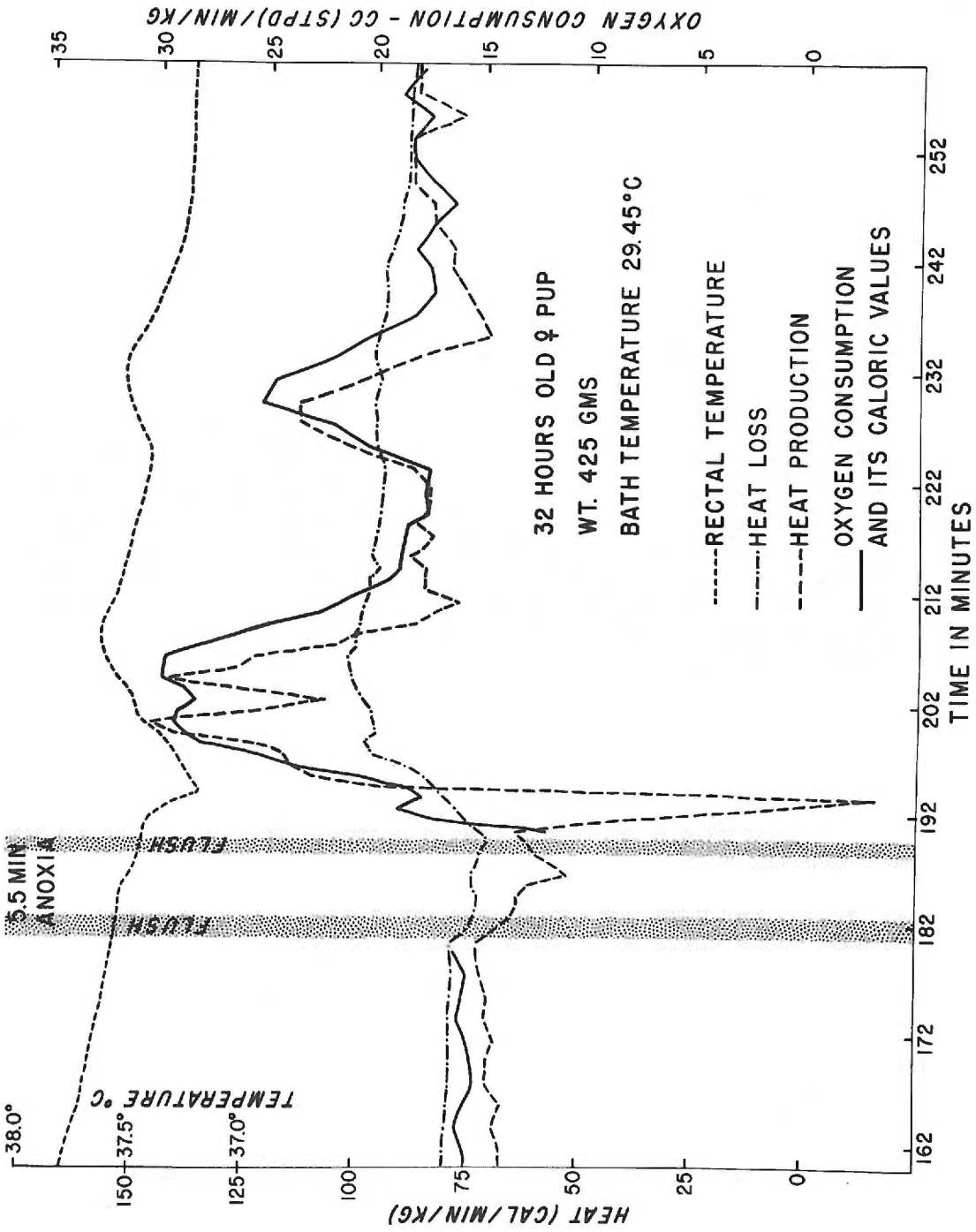

32 HOURS OLD ♀ PUP
WT. 425 GMS
BATH TEMPERATURE 29.45°C
7 MIN ANOXIA
FLUSH
FLUSH
RECTAL TEMPERATURE
HEAT LOSS
HEAT PRODUCTION
OXYGEN CONSUMPTION
AND ITS CALORIC VALUE
TEMPERATURE °C
39.0°
38.5°
38.0°
37.5°
HEAT (CAL./MIN/KG)
125
100
75
50
25
0
OXYGEN CONSUMPTION - CC (STPD)/MIN/KG
30
25
20
15
10
5
66
72
82
92
102
112
122
132
142
152
TIME IN MINUTES

Figure 16. Experiment 27-B. Second shorter anoxic episode for the same animal as in figure 12. In contrast to the first episode, the observed rise in oxygen consumption above control levels was associated with a rapid increase in rectal temperature and a corresponding increase in heat production. Under these circumstances, the conventional measurement of an oxygen debt by indirect calorimetry would have come to the opposite conclusion, namely that the second shorter anoxic period had a larger oxygen debt pay off.


OXYGEN CONSUMPTION - CC (STPD)/MIN/KG
35
30
25
20
15
10
5
0
32 HOURS OLD ♀ PUP
WT. 425 GMS
BATH TEMPERATURE 29.45°C
-----RECTAL TEMPERATURE
-·-·-HEAT LOSS
- - - -HEAT PRODUCTION
OXYGEN CONSUMPTION
AND ITS CALORIC VALUES
TIME IN MINUTES
162
172
182
192
202
212
222
232
242
252
5.5 MIN ANOXIA
FLUSH
FLUSH
TEMPERATURE °C
38.0°
37.5°
37.0°
HEAT (CAL/MIN/KG)
150
125
100
75
50
25
0

DISCUSSION

DISCUSSION

In attempting to estimate the amount of anaerobic metabolism in anoxic newborn mammals it was found that the standard method of measuring an oxygen debt by indirect calorimetry alone was inadequate. The present experiments show conclusively that one cannot assume a constant rate of heat production as is implicit in the conventional approach. Instead, studies of the anoxic energy metabolism and associated oxygen debt must take into consideration the possibility of more than a 50% decrease in heat production during anoxia. Equally important are the persistence of a decrease in energy metabolism during the first part of the recovery period and the marked rises in heat production when rectal temperature returns towards control levels.

In order to deal with the changes in the level of heat production the concept of an oxygen debt was modified to include both direct and indirect calorimetry. Thus, an oxygen debt incurred is equal to the total amount of anaerobic metabolism which was estimated from the heat produced during anoxia. The oxygen debt paid back corresponds to the replenishing of depleted energy stores and this is estimated from the excess of oxygen consumed over heat evolved during the recovery period. The advantage of this approach is illustrated by experiment 25 (fig 11) where there is a sizeable oxygen debt paid off although oxygen consumption remains below control levels. Alternatively, in experiment 27-B (fig 16) most of the rise in oxygen consumption above control levels

corresponds to a heat debt rather than an oxygen debt. If indirect calorimetry alone had been used, then the conclusion would be opposite from the fact, namely that the second much shorter anoxic exposure had the greater oxygen debt paid back.

The utilization of a rapidly responding gradient calorimeter in these experiments made possible the simultaneous measurements of heat loss and body temperature during anoxia for the first time. It is thus possible to demonstrate conclusively that in anoxia the newborn pup has a decreased rate of heat production and by inference, a decreased rate of energy exchange.

The magnitude of the decrease in heat production as a function of duration of anoxia remained somewhat uncertain because of an apparent disparity between changes in rectal temperature and changes in mean body temperature. There was, however, a trend toward decreasing heat production during the course of anoxia. After nine minutes of anoxia the rate of heat production was always at least 50% below control levels. The puppy with the longest anoxic exposure had an 80% decrease in the rate of heat production at the end of the anoxic period. These pronounced decreases in the rate of heat production are consistent with the previously reported findings relating to gross muscular inactivity, depressed cardio-respiratory function, carbohydrate depletion, and excess lactate accumulation.

The net heat production for anoxic episodes lasting nine minutes or longer was always below 67% of a corresponding control period. Anoxic exposures extending beyond 11 minutes had less than 50% of the

heat production of an equivalent control period.

The four pups with the greatest measured heat production during anoxia were in the cooler environment. It is possible that cold promotes survival by facilitating anaerobic metabolism. A more likely alternative explanation is that in the cold bath the disparity between rectal and mean body temperature becomes more pronounced and obscures what is actually a greater decrease in heat production.

The initial intent of the experimental design was to obtain an estimate of the anoxic energy metabolism by measuring the excess of oxygen consumption over heat production during the recovery period to compare with the directly measured energy exchange during anoxia. The two estimates of an oxygen debt show an overall agreement within 12% and have a correlation of r= .69 (table 4).

Although the anoxic heat production agrees fairly well with the subsequent excess of oxygen consumption over heat production, both estimates of an oxygen debt are probably high. The negative values of calculated heat production shortly after anoxia in experiment 28 illustrate the limitations of the method. These spurious values for heat production result probably from a redistribution of heat within the body during the early recovery period. This is evidenced by the rapid fall in rectal temperature after the anoxia. The probable sequence of events is that with the marked decrease in cardiac function during anoxia there is disproportionate cooling of superficial tissues compared to the core. Once cardiac output recovers, the stagnant cool

blood from the periphery is brought to the core where it causes a sudden decrease in rectal temperature even though the corresponding change in mean body temperature occurred during the anoxia. No allowance has been made in these experiments for the disparity between rectal temperature and mean body temperature. Consequently, both indices of the amount of anaerobic metabolism are too large to the extent that rectal temperature lags behind mean body temperature. In the light of of these considerations the values for the percent depression of metabolism must be taken as minimum values. Experiment 28 illustrates another much smaller factor which also shifts the anoxic heat production curve upwards. This is the abrupt increase in the rate of heat loss with the reflex urination and defecation which occurs in the latter half of the anoxic exposure.

The estimation of an oxygen debt pay off was subjected to the following sources of error:

1) Inability to measure oxygen consumption during the interval when the chamber is being flushed;
2) Inability to define the exact time when the period of excess oxygen consumption starts and ends;
3) Difficulty in estimating the point at which flushing is neither inadequate or too generous. An overflush elevates the effluent P_{O_2} and gives spuriously low values for oxygen consumption. This was the usual direction of error by intent to avoid over estimating the oxygen debt pay off;

4) The downward shift in the heat production often below zero from the decrease in rectal temperature secondary to a redistribution of body heat with recovery;

5) No allowance has been made for the depletion and replenishing of the animal's oxygen stores which could correspond to about 65 calories. This adjustment was omitted since the corresponding events probably occurred during the flush periods. It is to be noted that such a correction alone would not eliminate the oxygen debt pay off which was always at least three times as great and sometimes ten-fold larger than the estimated oxygen store.

Other sources of potential error include the possibility of a change in the caloric equivalent of oxygen during recovery and the wrong temporal relation between the curves describing oxygen consumption and heat production.

In view of the possible errors, little more than qualitative significance can be attached to the excess oxygen consumption of the recovery period. The observed excess in oxygen consumed over the heat production of the recovery period always disappeared by 20 to 30 minutes. This agrees well with the time of disappearance of "excess lactate" which Richabee (64) has investigated and correlated with an oxygen debt. Although the difference between oxygen consumption and heat production vanishes within 230 minutes of the anoxic period, it takes hours before anoxia tolerance returns to control levels.

The initial decrease in oxygen consumption to below 50% of control levels provided further evidence for a decrease in energy metabolism during anoxia. This finding cannot be attributed to overflushing of the chamber since it was present in experiment 29 (fig 12) where a short 1-minute flush was inadequate.

In general, the greater than two-fold variability in energy metabolism during the control period was consistent with the reported ontogeny of the homeothermic response. In the warm bath both one and three day old pups had relatively lower levels of energy turnover. At the colder temperature pups in the first day of life responded poikilothermically and had decreasing rates of heat production with a falling rectal temperature. Older pups also had a relative inability to restrict heat loss, but they maintained their rectal temperature with marked increase in heat production.

Most of the variability in anoxia tolerance between pups can be explained in terms of decreased anoxia endurance with increasing age, higher ambient temperatures, and with successive anoxic exposures.

SUMMARY

SUMMARY

The energy metabolism of newborn pups subjected to anoxia was investigated by direct and indirect calorimetry before, during, and after anoxic episodes. In every experiment the decrease in respiratory rate, rectal temperature, heat loss, calculated heat production, and post anoxic oxygen consumption provided incontrovertible evidence for a significant decrease in the rate of energy turnover during anoxia. Because of this the concept of an anoxic oxygen debt was extended to encompass situations with changing rates of heat production by defining an oxygen debt in terms of the difference between oxygen consumed and heat production. Because of a disparity between changes in rectal temperature and mean body temperature, it was impossible to give more than a qualitative description of the heat production. This would indicate that with nine or more minutes of anoxia there is at least a 50% decrease in the rate of heat production. The experiments also showed a gross correlation between the amount of anoxic heat production (oxygen debt incurred) and the subsequent excess of indirect calorimetry over direct calorimetry (oxygen debt paid back) during the early recovery period.

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APPENDIX

TABLES 5 - 16

Excerpted data for experiments discussed in text of thesis.

TABLE 5

Experiment 22

349 gram male pup. Approximately 30 hours old.
Bath temperature 29.45°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
0	35.37	50.5	———	72.4
10	.49	51.8	61.8	72.4
20	.61	54.0	62.3	74.8
30	.71	55.3	63.6	———
40	.82	56.6	65.7	73.6
42	.87	57.8	70.2	73.6
44	.86	57.9	49.6	73.6
46	.86	58.2	58.2	72.4
48	.87	58.4	70.8	———
Anoxic Period				
49	.88	57.9	66.2	———
50	.90	57.5	61.6	———
51	.88	56.6	56.6	———
52	.89	57.1	48.8	———
53	.88	56.7	44.2	———
54	.85	55.9	39.3	———
55	.81	56.4	35.6	———
56	.80	56.4	31.5	———
57	.78	56.3	27.2	———
58	.74	56.7	23.5	———
59	.70	56.6	19.2	———
60	.66	55.9	22.7	———
61	.62	55.9	22.7	———
62	.56	55.0	21.8	———
Recovery Period				
63	.51	52.9	15.6	———
64	.46	53.7	20.5	———
65	.46	54.4	21.2	24.9
66	.42	54.8	17.4	35.9
67	.37	55.4	18.0	45.8
68	.33	55.9	22.7	51.6
69	.27	56.6	23.4	60.8
70	.24	57.3	36.6	69.0
71	.22	57.7	49.4	73.6

TABLE 5 Continued

Time (min)	Rectal Temp. °C	Heat loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
72	35.18	58.2	58.2	77.1
73	.22	58.4	62.6	76.8
74	.22	58.9	63.0	76.8
75	.25	59.5	67.8	76.2
76	.26	60.4	72.8	77.7
77	.26	70.9	73.4	77.1
78	.28	61.0	73.4	76.5
79	.30	61.1	69.4	75.3
80	.28	61.1	73.6	74.2
82	.31	60.4	68.7	73.6
84	.34	60.0	68.3	71.9
86	.36	61.1	71.5	75.3
88	.40	62.1	78.7	77.1
92	.48	64.1	80.7	78.8
96	.52	63.7	76.1	78.2
100	.60	64.7	73.0	75.9
104	.65	66.1	72.3	73.6
108	.66	64.8	64.8	77.1
112	.66	65.1	65.1	70.7
116	.63	65.1	56.8	63.2
120	.61	64.6	64.6	58.5
124	.62	64.6	70.8	67.8
128	.66	64.8	----	67.8

TABLE 6

Experiment 23

330 gram female pup. Approximately two days of age.
Bath temperature 29.45°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
100	37.39	77.95	-----	84.0
105	.39	78.89	78.89	81.5
110	.45	77.95	86.25	87.8
115	.45	80.31	80.31	83.4
120	.47	80.31	83.63	81.5
125	.50	80.42	85.40	86.6
130	.50	81.60	81.60	84.7
135	.50	82.07	82.07	84.0
140	.46	80.78	74.14	82.8
141	.46	81.36	81.36	82.8
142	.47	81.72	94.17	83.4
143	.49	81.83	94.28	85.3
144	.50	80.89	89.19	85.9
145	.51	81.08	85.16	86.6
146	.51	81.48	77.33	85.9
147	.50	81.48	73.18	-----
Anoxic Period				
148	.49	81.13	76.98	-----
149	.49	79.13	79.13	-----
150	.49	79.36	87.66	-----
151	.52	77.60	98.35	-----
152	.53	79.48	79.48	-----
153	.52	79.95	67.50	-----
154	.49	78.66	62.06	-----
155	.47	77.95	61.35	-----
156	.43	77.13	48.08	-----
157	.39	75.48	42.28	-----
158	.36	73.49	36.14	-----
159	.33	72.19	34.84	-----

TABLE 6 Continued

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Recovery Period				
160	37.28	68.20	22.55	----
161	.20	68.20	22.55	----
162	.14	69.37	2.97	28.4
163	.05	69.96	3.56	39.8
164	36.98	71.14	13.04	53.7
165	.92	72.78	22.89	62.6
166	.87	73.49	44.44	68.9
167	.84	74.90	58.30	77.7
168	.83	75.60	75.60	84.7
169	.84	76.07	80.22	88.5
170	.84	76.07	76.04	89.1
171	.84	76.54	76.54	89.7
172	.84	77.37	77.37	91.0
173	.84	76.66	76.66	92.3
174	.84	76.78	76.68	91.6
175	.84	78.78	78.78	91.6
176	.84	77.25	77.25	92.3
177	.84	76.90	76.90	92.9
178	.84	76.43	84.73	91.6
179	.85	76.43	84.73	91.0
180	.87	76.54	84.84	89.1
181	.89	75.96	84.26	87.8
182	.90	75.72	92.32	87.8
183	.91	75.96	88.41	87.2
184	.93	76.19	92.79	86.6
185	.95	75.96	92.56	87.2
186	.96	75.80	92.20	87.2
187	.98	75.49	96.24	86.6
188	37.00	75.72	96.47	87.2
189	.02	75.84	96.59	89.1
190	.03	79.25	104.15	94.8
195	.22	81.95	98.55	99.8
200	.28	81.83	90.13	95.4
205	.28	85.13	85.13	89.1
210	.32	85.48	85.48	89.1

TABLE 7

Experiment 13 - A
587 gram male pup. Approximately three days old.
Bath temperature 20.3°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
144	36.96	115.2	———	117.9
148	36.98	113.8	171.9	117.8
152	37.13	114.0	118.2	127.5
156	37.02	113.5	96.9	121.0
158	37.04	112.5	104.2	———
Anoxic Period				
160	36.94	115.2	101.0	———
162	36.93	114.5	95.4	———
164	36.86	109.6	83.0	———
166	36.80	104.6	72.3	———
168	36.69	114.5	67.2	———
170	36.56	109.2	42.8	———
Recovery Period				
172	36.39	105.0	+ 1.2	———
174	36.01	106.3	-43.2	30.9
176	35.76	107.5	+16.2	52.7
178	35.50	108.6	33.9	61.1
180	35.30	109.9	39.4	71.2
182	35.21	110.9	56.9	76.2
184	35.11	112.2	62.4	79.4
186	35.02	114.5	78.8	94.4
188	34.90	116.8	83.6	97.5
190	34.82	117.5	85.9	99.8
194	34.76	127.3	121.5	114.3
198	34.63	129.6	92.3	118.4
202	34.43	129.9	96.7	104.5
206	34.29	129.6	104.7	113.3
210	34.16	133.6	117.0	121.4
214	34.12	134.2	130.1	127.5
218	34.14	134.5	149.5	134.9
222	34.28	134.3	162.5	142.2
226	34.38	130.9	151.7	162.5
230	34.49	130.9	155.8	152.1
234	34.62	131.2	150.3	158.7

TABLE 7 Continued

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
238	34.71	133.6	150.2	161.0
242	34.78	134.5	146.2	172.3
245	34.84	134.2	159.1	167.5
250	34.97	135.5	158.8	168.5
255	35.10	131.9	150.2	155.0
260	35.19	134.5	152.8	164.3
265	35.31	137.0	154.4	162.8
270	35.40	136.8	151.8	167.5
275	35.46	141.0	154.3	165.5
280	35.54	140.1	152.6	163.8
285	35.60	140.8	153.2	------
290	35.69	140.1	150.9	171.2
295	35.77	138.5	149.3	153.5
300	35.80	137.8	147.8	156.5
305	35.80	137.3	147.2	160.1
310	35.91	142.7	151.9	158.4
315	35.97	142.4	150.7	160.1
320	36.02	144.4	152.7	171.2

TABLE 8

Experiment 13 - B

587 gram male pup. Approximately three days old.
Bath temperature 29.6°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
400	37.08	71.1	76.1	81.6
420	37.12	71.5	72.3	75.2
440	37.15	72.7	75.2	74.9
460	37.23	75.0	75.8	81.4
480	37.24	74.9	74.9	83.7
500	37.24	75.9	75.9	79.4
520	37.24	77.2	77.2	81.3
540	37.24	75.9	75.9	----
Anoxic Period				
542	37.25	76.4	76.4	----
544	37.26	74.1	69.1	----
546	37.23	71.1	54.5	----
548	37.18	72.8	39.6	----
550	37.06	70.8	25.2	----
Recovery Period				
552	36.94	65.2	3.0	----
554	36.80	66.2	12.3	43.0
556	36.70	66.6	25.0	71.2
558	36.58	68.2	51.6	86.3
560	36.58	71.5	71.5	90.9
562	36.58	73.4	73.4	94.2
564	36.58	74.1	82.4	94.5
566	36.61	78.4	96.6	97.1
568	36.74	81.3	109.6	113.0
570	36.74	----	----	----
572	36.89	87.6	119.1	117.0
574	36.89	86.2	114.5	118.6
576	36.97	----	----	107.3
578	36.97	87.2	87.2	104.4
580	36.97	86.6	86.6	91.0
582	36.97	85.9	85.9	85.1
584	36.97	85.6	85.6	83.4
586	36.97	84.9	84.9	83.4
588	36.97	84.3	84.3	77.6
590	36.97	83.3	83.3	76.7
594	36.97	84.9	84.9	90.9
598	36.97	84.9	84.9	81.5
602	36.97	87.6	87.6	96.2

TABLE 8 Continued

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
606	36.97	87.2	87.2	90.0
610	36.97	87.6	87.6	91.8
614	36.97	90.3	90.3	92.7
618	36.97	86.6	86.6	81.5
622	36.94	86.9	86.9	89.1

TABLE 9

Experiment 21

354 gram female pup. Approximately 24 hours old.
Bath temperature 20.2°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Calorie Value of Oxygen Consumed cal/min/kg
Control Period				
30	32.55	86.39	69.79	69.38
60	31.89	81.49	60.74	66.78
90	31.02	79.31	60.22	56.06
120	30.44	71.68	53.42	56.49
150	29.82	67.33	50.73	61.24
180	29.24	64.71	48.11	52.91
210	28.70	61.12	47.01	56.68
240	28.17	56.11	42.83	41.51
270	27.82	55.46	42.18	48.17
300	———	52.41	42.45	46.76
330	———	52.84	44.54	51.46
360	28.68	50.99	36.88	41.31
390	26.30	45.87	36.74	39.34
420	26.02	47.50	39.62	43.52
450	25.61	47.50	36.70	42.78
461	25.56	48.27	34.16	———
Anoxic Period				
462	25.55	47.72	33.61	———
464	25.50	48.05	28.96	———
466	25.43	46.74	25.99	———
468	25.37	46.63	21.73	———
470	25.33	45.65	20.75	———
472	25.27	44.02	14.97	———
474	25.20	43.47	10.27	———
476	25.12	42.71	7.85	———
478	25.02	42.06	8.86	———
480	24.95	41.51	6.65	———
Recovery Period				
482	24.86	40.97	6.11	———
484	24.78	41.08	7.88	26.58
486	24.68	42.06	12.18	34.52
488	24.64	42.60	14.38	41.51
490	24.56	43.04	18.97	42.47
492	24.51	42.93	23.01	42.06
494	24.47	42.71	26.11	40.14
496	24.45	42.39	32.43	39.18
498	24.42	41.84	36.86	37.40
500	24.41	42.49	35.85	36.85
502	24.38	42.28	33.98	36.44
504	24.35	41.62	29.17	36.44
506	24.32	41.08	———	36.44

TABLE 10

Experiment 24
387 gram female pup. Approximately three days old.
Bath temperature 29.45°C.

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
70	39.07	88.3	88.3	96.0
74	39.05	92.6	82.6	97.5
78	38.98	92.7	86.9	92.7
82	.96	93.0	84.7	96.0
84	.93	89.3	77.7	----
Anoxic Period				
86	.92	87.3	70.7	----
88	.88	88.3	64.2	----
90	.80	86.0	59.4	----
92	.73	83.5	49.5	----
94	.64	80.2	38.7	----
Recovery Period				
96	.53	74.2	24.4	----
98	.40	73.9	34.1	27.6
100	.34	72.8	37.9	43.9
102	.25	72.8	39.6	71.6
104	.18	73.6	42.1	86.0
106	.10	74.8	48.3	94.1
108	.06	76.6	70.8	96.8
110	.06	78.2	78.2	101.9
112	.07	79.4	82.7	107.4
114	.14	79.5	87.0	114.0
116	.11	79.9	89.0	109.0
118	.12	80.7	93.2	111.9
120	.14	79.3	93.4	106.3
124	.24	82.0	101.9	104.9
128	.33	82.0	103.6	98.5
132	.38	82.3	80.6	95.2
136	.35	82.8	75.3	89.6
140	.33	82.6	79.3	87.1
144	.32	82.8	82.8	96.0
148	.30	82.4	78.2	----
Anoxic Period				
150	.27	83.1	74.8	----
152	.26	79.8	67.4	----
154	.26	80.6	64.0	----
156	.21	77.0	56.3	----
158	.14	76.7	51.8	----

TABLE 10 Continued

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
End of Anoxia				
160	38.07	71.5	36.2	----
172	37.32	64.0	3.8	----
184	36.47	56.7	4.8	----
196	35.76	50.8	3.1	----
208	35.09	44.4	.8	----
220	34.55	39.6	4.3	----
232	34.04	35.5	2.3	----
244	33.61	32.1	4.7	----
256	33.25	27.9	2.4	----
268	32.91	24.9	4.8	----
280	32.63	22.4	2.7	----
292	32.35	20.3	2.7	----
304	32.11	18.6	2.8	----
316	31.90	15.9	1.3	----
328	31.71	14.5	2.1	----
340	31.59	13.2	2.2	----
352	31.45	11.9	1.8	----
364	31.31	11.0	1.7	----
376	31.17	10.0	1.1	----
388	31.03	9.3	2.0	----
400	30.90	8.4	2.0	----

TABLE 11

Experiment 25

376 gram male pup. Approximately 10 hours old.
Bath temperature 20.2°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
140	30.89	74.36	63.98	73.14
160	.64	76.72	66.34	75.68
165	.58	83.38	73.42	84.77
166	.58	84.51	96.96	89.03
167	.61	86.77	95.07	93.08
168	.61	86.46	90.61	97.77
169	.60	84.62	80.47	95.64
170	.59	84.92	76.62	93.08
171	.58	83.90	75.60	94.35
172	.54	82.87	70.42	———
Anoxic Period				
173	.54	84.41	71.96	———
174	.51	84.10	71.65	———
175	.53	82.38	66.78	———
177	.49	81.44	56.54	———
178	.47	80.31	55.41	———
179	.43	78.77	45.57	———
180	.39	77.02	39.67	———
181	.34	76.10	34.60	———
182	.28	75.08	29.43	———
183	.21	74.26	24.46	———
184	.15	73.33	23.53	———
185	.09	72.31	22.51	———
Recovery Period				
186	.03	71.49	17.54	———
187	29.97	69.44	3.04	———
188	.91	69.75	- 0.80	———
189	.81	71.39	-11.61	25.99
190	.71	73.64	- 9.36	42.20
191	.60	72.62	-22.83	54.70
192	.43	73.03	-26.57	59.21
193	.30	73.13	-14.02	58.58
194	.20	73.13	- 5.72	56.44
195	.12	73.03	+ 6.63	53.68
196	.07	72.82	+10.57	52.40
197	.01	72.51	+22.71	50.91
198	28.96	72.21	26.56	50.29

TABLE II Continued

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Calorie Value of Oxygen Consumed cal/min/kg
199	28.90	71.00	30.30	49.42
200	.86	71.28	29.78	46.86
201	.81	70.57	33.22	46.01
202	.77	70.05	32.70	46.01
203	.72	69.54	28.04	46.01
204	.66	69.74	32.39	44.73
205	.62	69.54	36.34	44.73
206	.58	69.23	36.03	45.37
207	.53	69.13	35.93	46.01
208	.50	68.82	39.77	46.01
209	.47	68.31	43.41	46.86
210	.44	67.80	42.90	46.86
220	.37	69.54	71.61	68.80
230	.50	72.51	80.81	86.69
240	.57	75.28	81.92	78.38
250	.65	77.29	83.87	95.00
260	.69	76.28	82.02	91.59
270	.79	76.00	82.64	95.00
280	.89	75.90	84.20	91.59
290	.88	68.31	60.01	68.80
300	.69	65.03	55.07	57.72
310	.73	67.80	71.95	79.24
320	.82	70.75	71.40	86.69
330	.86	66.67	66.67	72.21

TABLE 12

Experiment 29

535 gram female pup. Three days old. Bath temperature 20.2°C.

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
20	34.94	99.3	119.2	141.1
40	35.40	98.0	108.1	117.8
60	35.84	106.5	140.6	142.2
80	36.28	108.7	120.3	141.6
100	36.24	104.4	106.8	105.3
120	———	105.4	107.9	96.4
140	36.62	110.5	100.5	130.1
142	36.54	108.0	83.1	110.7
144	36.50	106.5	89.9	———
Anoxic Period				
146	36.47	106.5	94.1	———
148	36.44	106.9	96.9	———
150	36.39	106.7	88.4	———
152	36.35	105.2	77.8	———
154	36.27	———	———	———
156	36.15	98.8	44.9	———
Recovery Period				
158	36.03	95.2	27.2	———
160	35.83	94.6	15.8	44.3
162	35.66	94.1	19.4	32.0
164	35.50	93.4	31.2	33.6
166	35.32	93.6	10.6	42.2
168	35.06	94.7	-13.2	54.5
170	34.85	96.2	+13.2	75.0
172	34.71	95.9	33.7	74.2
174	34.54	100.0	54.4	86.2
176	34.44	102.9	69.7	97.6
178	34.40	105.1	84.3	105.0
180	34.38	104.0	95.7	107.0
196	34.42	108.0	128.7	125.6
200	34.70	111.6	141.4	131.9
210	34.96	110.8	135.8	129.9
220	35.23	113.0	132.1	———
230	35.45	114.1	131.5	———
240	35.67	114.1	133.2	———
250	35.86	115.5	128.0	———
260	35.89	108.7	109.5	———
262	35.90	108.8	108.8	———
264	35.90	108.8	108.8	———
266	35.92	108.8	108.8	———

TABLE 12 Continued

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Anoxic Period				
268	35.92	109.5	109.5	———
270	35.85	105.2	88.6	———
272	35.82	———	———	———
274	35.98	121.5	92.5	———
276	35.65	115.3	77.9	———
End of Anoxic Period				
278	35.56	110.2	68.7	———
280	35.42	107.3	49.2	———
290	34.47	94.6	11.6	———
300	33.46	85.0	+ 2.0	———
310	32.35	76.3	-10.8	———
320	31.51	69.8	- 9.0	———
330	30.50	63.5	-11.5	———
340	29.80	58.3	- 6.0	———
360	28.30	49.4	- 4.6	———
380	27.04	42.1	+ 0.6	———
400	26.20	36.2	+ 7.2	———

TABLE 13

Experiment 28 - A
438 gram black male pup. Approximately two days old.
Bath temperature 20.2°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Calorie Value of Oxygen Consumed cal/min/kg
Control Period				
0	33.76	99.3	———	121.1
15	34.14	101.5	124.8	134.6
30	34.58	101.5	127.2	128.1
45	35.01	102.4	126.5	126.7
60	35.52	107.7	132.6	138.1
75	35.82	109.4	134.3	129.9
90	36.26	116.0	134.3	130.7
105	36.58	112.7	123.5	138.6
120	36.72	119.6	126.2	123.3
130	36.81	123.1	135.5	———
Anoxic Period				
132	36.79	122.2	118.0	———
134	36.81	124.0	111.5	———
136	36.77	123.5	93.3	———
138	36.62	120.2	62.1	———
140	36.50	129.2	62.8	———
142	36.29	120.8	42.0	———
Recovery Period				
144	36.09	114.5	14.9	———
146	35.84	114.1	27.0	41.7
148	35.65	112.5	12.9	50.4
150	35.32	112.8	-28.3	62.8
152	35.05	132.6	5.7	68.5
154	34.79	114.3	- 1.9	72.0
156	34.56	114.7	35.9	78.9
158	34.40	114.3	64.5	82.8
160	34.31	115.3	86.3	89.8
162	34.28	117.5	117.5	102.4
164	34.28	119.1	115.0	109.5
170	34.29	126.2	126.2	114.0
185	34.40	128.8	147.1	127.0
200	34.75	132.8	151.0	151.7
215	34.96	131.0	143.4	134.2
230	35.23	128.1	142.2	134.7
245	35.44	123.5	135.1	135.1
260	35.65	122.6	132.6	123.4
275	35.79	124.8	132.3	123.0
290	35.90	124.2	130.0	130.9
305	35.97	120.4	127.1	125.2
320	36.17	127.2	138.8	126.9

TABLE 14

Experiment 28 - B

438 gram male pup. Approximately two days old.
Bath temperature 20.2°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
320	36.17	127.2	138.8	126.9
322	36.23	127.5	144.1	137.4
324	36.26	127.9	127.9	141.2
326	36.25	129.4	128.4	127.1
328	36.22	124.4	124.4	129.7
330	36.25	122.6	120.6	123.6
Anoxic Period				
332	36.22	122.0	107.5	———
334	36.19	122.2	97.3	———
336	36.10	124.8	95.8	———
338	36.01	123.4	77.7	———
340	35.91	137.9	88.1	———
342	35.70	131.0	39.7	———
Recovery Period				
344	35.47	124.0	11.9	———
346	35.14	124.0	- 4.7	37.6
348	34.84	124.4	-25.0	57.1
350	34.30	126.2	-73.0	70.2
352	33.98	127.6	28.0	73.0
354	33.78	126.8	60.4	78.2
356	33.66	121.3	79.8	82.6
358	33.61	117.5	96.8	83.6
360	33.56	123.1	114.8	90.6
364	33.62	128.5	142.6	120.9
368	33.66	130.0	144.1	124.4
372	33.75	131.0	145.1	132.4
376	33.81	129.4	143.5	143.2
380	33.87	131.0	145.1	130.8
384	33.94	129.7	143.8	132.8
388	34.00	127.7	135.1	125.8

TABLE 15

Experiment 27 - A
425 gram female pup. Approximately 32 hours old.
Bath temperature 29.45°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
65	38.39	72.9	85.3	89.0
70	38.52	72.7	83.1	99.3
75	38.58	77.9	90.3	104.5
80	.67	81.5	102.2	100.6
85	.77	84.7	97.1	104.5
86	.77	83.8	94.1	———
Anoxic Period				
88	.79	81.3	93.8	———
90	.82	75.1	87.6	———
92	.87	73.8	75.8	———
94	.84	76.5	62.0	———
96	.79	75.1	56.5	———
Recovery Period				
98	.74	72.2	47.3	23.5
100	.66	74.4	32.9	59.5
102	.53	80.1	13.7	85.2
104	.47	85.3	60.4	96.8
106	.36	87.1	85.0	105.7
108	.35	90.4	90.4	119.9
110	.34	92.8	90.8	119.9
112	.34	94.0	89.9	122.5
114	.31	95.4	91.2	112.2
116	.30	95.6	87.2	104.5
118	.28	95.4	85.0	99.3
120	.25	93.7	83.4	95.5
124	.23	91.0	91.0	86.5
128	.24	89.9	87.8	90.3
132	.20	87.7	77.3	81.3
136	.15	86.9	76.6	81.3
140	.11	84.7	74.3	84.5
144	.04	83.8	73.4	78.8
148	37.98	82.4	72.0	74.3
152	37.93	81.9	69.5	74.9
156	37.88	81.2	68.8	80.0
160	37.81	79.7	67.2	74.3

TABLE 16

Experiment 27 - B

425 gram female pup. Approximately 32 hours old.
Bath temperature 29.45°C

Time (min)	Rectal Temp. °C	Heat Loss cal/min/kg	Heat Production cal/min/kg	Caloric Value of Oxygen Consumed cal/min/kg
Control Period				
160	37.81	79.7	67.2	74.3
164	.76	69.0	68.7	76.8
168	.71	78.8	70.5	73.0
172	.66	78.8	68.4	74.9
176	.62	78.3	70.0	75.6
180	.60	78.8	72.6	77.5
Anoxic Period				
182	.60	74.7	68.5	----
184	.55	72.6	64.3	----
186	.54	73.6	61.2	----
188	.47	72.0	55.4	----
Recovery Period				
190	.45	69.5	61.2	27.4
192	.43	75.1	41.9	81.3
194	.25	89.1	-15.3	84.5
196	.23	84.7	109.6	99.3
198	.29	90.6	115.6	121.2
200	.38	95.1	140.7	137.9
202	.48	96.0	120.9	139.2
204	.50	99.6	124.5	137.9
206	.58	101.4	126.4	142.4
208	.63	99.9	104.1	135.3
210	.62	98.9	86.5	118.6
212	.57	97.8	77.1	103.2
214	.54	97.1	84.6	92.3
216	.52	96.6	88.3	89.7
218	.50	95.1	82.6	89.0
220	.47	94.4	84.0	83.9
222	.44	94.2	83.8	84.5
224	.41	93.5	87.2	83.6
226	.41	95.3	101.5	96.8
228	.46	95.4	112.3	105.1
230	.49	96.0	112.6	121.2
232	.55	94.7	98.9	118.0
234	.48	96.5	88.2	105.1
236	.48	95.2	70.3	96.1
238	.42	93.7	73.0	86.5
240	.32	93.2	74.5	82.6
244	.29	92.5	78.0	87.1
248	.24	90.1	83.9	78.1
252	.24	88.8	87.5	87.1
256	.24	88.3	75.8	83.3
260	.22	87.4	86.1	85.2

Figure 17. Diagramatic longitudinal section of the calorimeter.

1. Thin inner copper cylinder
2. Heavier outer copper cylinder
3. Rubber "O" rings
4. Copper-constantan ribbon 0.0025 inches thick
5. Longitudinal strips of dielecto plastic 0.919 inches thick
6. Clear seal electric insulation
7. Plastic seal
8. Removable lid
9. Opening for rectal thermistor
10. Plastic end
11. Plastic tube for gas inlet
12. Stainless steel tubing for gas outlet
13. Wire leads from the thermopile
14. Reflecting surfaces
15. Grated metal animal tray

CALORIMETER DIAGRAM