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## Inhibition of Lipolysis by Hypoxia in Puppies.* (32310)

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In the neonatal mammal, heat and energy requirements are large, met primarily by aerobic metabolism(1,8). Lipid is an important source of energy for these aerobic pathways in fasting mammals. Therefore, it might be anticipated that diminished mobilization and utilization of free fatty acid (FFA) might handicap the very young mammal.

Many cardiopulmonary disorders of the human neonate may result in acute, severe hypoxia. Heat(2) and energy(14) production are curtailed during acute hypoxia. Although defective oxidation of FFA could, in part, explain these difficulties, the role of FFA mobilization has not been investigated. It is the purpose of this report to describe experiments in puppies which demonstrate that inhibited FFA mobilization is associated with acute hypoxia.

*Methods and materials.* Ten puppies, 21-35 days of age and weighing 900-1200 g, were the subjects of this study. (Dogs of this age group were chosen because a) the neonate's ability to respond to hypothermia with an increase in oxygen consumption and heat production develops rapidly in the first week of life(5), b) inhibition of these metabolic responses to cold stress by acute hypoxia is not apparent until the responses mature(7), and c) increase in heat production and oxygen consumption occurring with cold stress has been inhibited up to 40 days of age in mammals similar to the dog(2).) The puppies were 3-5 hours post-prandial at the beginning of the study. Each animal was studied in the constant thermal environment that resulted in the lowest oxygen consumption for that specific animal (ambient temperature range 27-29°C). Although very young puppies are less likely to shiver, a neutral thermal environment was sought in order to further minimize this possibility. Innovar®-Vet† was administered in amounts sufficient to produce a degree of sedation that would allow only occasional limb movement, although permitting arousal on painful stimulation. This degree of sedation resulted in an arterial $PO_2$ of at least 75 mm Hg during the control and recovery periods when room air was breathed. A mixture of 8% oxygen and 92% nitrogen was administered for 45 minutes to produce hypoxia. A plastic hood was sealed over the animal's head and the respired gas mixture was pulled through at

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† Each ml of Innovar®-Vet contains .4 mg fertanyl citrate and 20 mg droperidol.

5 times his expected minute volume to prevent carbon dioxide accumulation. Microanalyses for glycerol(15), FFA(9), lactate (12), glucose(3) and pH, $PO_2$ and $PCO_2$‡ were performed on arterial blood. Blood loss (11-14 ml per animal) was replaced with 0.85% sodium chloride solution. Deep rectal and environmental temperatures were measured with a multi-channel telethermometer and appropriate probes.§

*Results.* Severe hypoxia and a partially compensated metabolic acidosis resulted from breathing 8% oxygen and 92% nitrogen (Table I). The severity of hypoxia is shown by decreases in arterial $PO_2$ to 15-36 mm Hg. All developed metabolic acidoses, but of varying degree (range of pH fall .00-.16). This was in part the result of differences in lactic acid accumulation (range of increases 27-130 mg%). The fall in arterial $PCO_2$ during hypoxia also varied (range of fall 4-17 mm Hg), indicating a spectrum in compensation of the metabolic acidosis.

There was a pronounced fall in plasma glycerol (.48 ± .16 μmole/ml, mean change ± S.D.) and FFA (.35 ± .15 μEq/ml) in all animals during acute hypoxia (Table I). The return of these metabolites toward control levels was erratic and usually slow after the return to room air. The course of plasma glycerol and FFA for all animals is shown graphically in Fig. 1.

Marked hyperglycemia (158 ± 71 mg%) occurred with hypoxia in 9 of 10 animals studied (Table I). Plasma glucose often did not fully return to control levels by the end of the 45-minute recovery period. Deep rectal temperature fell (1.0 ± 0.4°C, mean fall ± S.D.) in all puppies during hypoxia (Table I). Rectal temperature continued to fall for a short time during recovery, but the core temperature was increasing in all animals by the end of each experiment.

*Discussion.* Although it is unclear from the course of plasma FFA levels as to whether FFA utilization was increased or FFA mobilization diminished with acute, severe hypoxia,

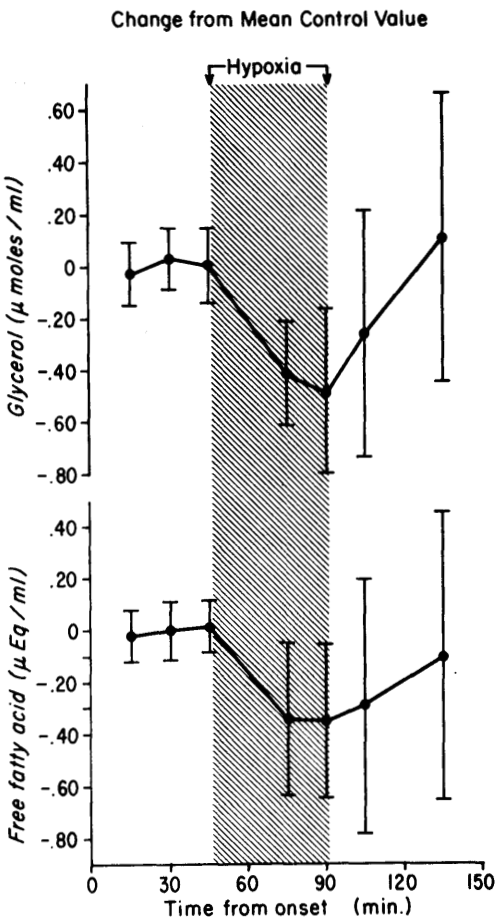


FIG. 1. Upper Graph-course of plasma glycerol in terms of change from mean control value. Lower Graph-course of plasma FFA in terms of change from mean control value. (Each point represents the mean value for 10 animals; brackets demonstrate ± 2 S. D.)

the parallel course of plasma glycerol clearly indicates an inhibition of lipolysis. Thus, the continual turnover of adipose tissue triglycerides has been partially interrupted.

Several mechanisms could explain diminished lipolysis during hypoxia. Recent observations in dogs indicated that lipolysis is inhibited by severe acidosis(11). *In vitro* studies suggest that this inhibition is mediated *via* diminished adenyl cyclase activity, thereby limiting cyclic 3′, 5′ AMP formation and hormone induced lipase activity(13). However, the degree of acidosis shown to be effective in this respect was not achieved in the puppies examined. FFA mobilization

‡ pH, $PO_2$ and $PCO_2$ meters made by Instrumentation Laboratories, Boston, Mass.

§ Made by Yellow Springs Instrument Co., Yellow Springs, Ohio.

TABLE I. Summary of Data from 10 Puppies.

| Puppy No. | Time (min) | Temp (°C) | $pO_2$ (mm Hg) | $pCO_2$ (mm Hg) | pH | Glycerol ($\mu$moles/ml) | FFA ($\mu$eq/ml) | Lactate (mg %) | Glucose (mg %) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 15 | 36.0 | 84 | 39 | 7.36 | .078 | .55 | 14 | 109 |
| | 30 | 36.0 | 90 | 39 | 7.36 | .078 | .59 | 10 | 108 |
| | 45 | 36.0 | 94 | 36 | 7.37 | .089 | .54 | 13 | 106 |
| | 75 | 34.5 | 20 | 29 | 7.29 | .055 | .17 | 67 | 285 |
| | 90 | 34.2 | 20 | 31 | 7.26 | .048 | .10 | 83 | 300 |
| | 105 | 34.3 | 84 | 31 | 7.29 | .071 | .19 | 43 | 246 |
| | 135 | 34.8 | 86 | 27 | 7.38 | .083 | .38 | 18 | 202 |
| 2 | 15 | 36.6 | 83 | 38 | 7.36 | .092 | .67 | 13 | 147 |
| | 30 | 36.5 | 75 | 38 | 7.36 | .101 | .78 | 15 | 154 |
| | 45 | 36.6 | 86 | 40 | 7.35 | .105 | .66 | 15 | 151 |
| | 75 | 36.1 | 26 | 24 | 7.30 | .052 | .12 | 81 | 360 |
| | 90 | 35.7 | 25 | 24 | 7.29 | .038 | .12 | 91 | 370 |
| | 105 | 36.1 | 86 | 26 | 7.30 | .068 | .14 | 79 | 341 |
| | 135 | 36.6 | 83 | 29 | 7.35 | .096 | .31 | 53 | 305 |
| 3 | 15 | 36.2 | 77 | 35 | 7.36 | .113 | .57 | 16 | 148 |
| | 30 | 36.2 | 80 | 34 | 7.37 | .119 | .54 | 14 | 142 |
| | 45 | 36.1 | 75 | 33 | 7.37 | .095 | .57 | 16 | 142 |
| | 75 | 34.8 | 21 | 31 | 7.26 | .050 | .22 | 91 | 312 |
| | 90 | 34.7 | 22 | 30 | 7.24 | .042 | .26 | 111 | 337 |
| | 105 | 35.0 | 94 | 26 | 7.31 | .072 | .24 | 91 | 318 |
| | 135 | 35.2 | 78 | 32 | 7.33 | .136 | .23 | 48 | 260 |
| 4 | 15 | 36.8 | 79 | 38 | 7.40 | .075 | .57 | 14 | 125 |
| | 30 | 36.8 | 76 | 39 | 7.40 | .070 | .65 | 13 | 120 |
| | 45 | 36.9 | 76 | 38 | 7.40 | .082 | .68 | 14 | 128 |
| | 75 | 36.4 | 22 | 30 | 7.35 | .040 | .12 | 72 | 260 |
| | 90 | 36.3 | 24 | 26 | 7.34 | .011 | .14 | 84 | 270 |
| | 105 | 36.5 | 75 | 25 | 7.37 | .034 | .10 | 41 | 224 |
| | 135 | 36.9 | 75 | 37 | 7.39 | .135 | .74 | 14 | 188 |
| 5 | 15 | 36.6 | 80 | 36 | 7.35 | .085 | .54 | 13 | 123 |
| | 30 | 36.6 | 75 | 35 | 7.35 | .091 | .54 | 12 | 120 |
| | 45 | 36.7 | 81 | 35 | 7.35 | .098 | .62 | 13 | 123 |
| | 75 | 36.2 | 27 | 28 | 7.36 | .061 | .37 | 36 | 224 |
| | 90 | 36.2 | 26 | 29 | 7.35 | .067 | .41 | 40 | 262 |
| | 105 | 36.3 | 75 | 32 | 7.34 | .121 | .77 | 27 | 244 |
| | 135 | 36.7 | 88 | 33 | 7.36 | .109 | .63 | 11 | 220 |
| 6 | 15 | 36.3 | 87 | 36 | 7.36 | .073 | .37 | 19 | 154 |
| | 30 | 36.1 | 83 | 36 | 7.36 | .072 | .42 | 17 | 154 |
| | 45 | 36.3 | 95 | 37 | 7.36 | .060 | .39 | 18 | 156 |
| | 75 | 35.8 | 36 | 25 | 7.31 | .037 | .33 | 67 | 228 |
| | 90 | 35.6 | 35 | 24 | 7.30 | .045 | .30 | 80 | 274 |
| | 105 | 35.4 | 82 | 29 | 7.32 | .062 | .45 | 58 | 279 |
| | 135 | 35.6 | 80 | 30 | 7.36 | .082 | .43 | 32 | 179 |
| 7 | 15 | 37.3 | 84 | 37 | 7.38 | .076 | .54 | 18 | 106 |
| | 30 | 37.4 | 84 | 36 | 7.39 | .072 | .44 | 19 | 101 |
| | 45 | 37.3 | 81 | 37 | 7.38 | .078 | .45 | 17 | 106 |
| | 75 | 36.9 | 36 | 25 | 7.36 | .023 | .14 | 45 | 108 |
| | 90 | 36.3 | 28 | 33 | 7.32 | .014 | .15 | 55 | 117 |
| | 105 | 36.4 | 88 | 33 | 7.36 | .026 | .26 | 24 | 117 |
| | 135 | 36.5 | 82 | 34 | 7.39 | .099 | .99 | 18 | 113 |
| 8 | 15 | 37.6 | 75 | 39 | 7.37 | .099 | .58 | 14 | 171 |
| | 30 | 37.6 | 94 | 39 | 7.37 | .109 | .60 | 15 | 171 |
| | 45 | 37.6 | 87 | 34 | 7.39 | .089 | .50 | 19 | 173 |
| | 75 | 37.2 | 24 | 24 | 7.27 | .055 | .34 | 102 | 280 |
| | 90 | 36.8 | 24 | 22 | 7.25 | .048 | .29 | 115 | 294 |
| | 105 | 36.9 | 89 | 28 | 7.32 | .048 | .20 | 84 | 250 |
| | 135 | 37.4 | 82 | 30 | 7.37 | .137 | .40 | 34 | 210 |
| 9 | 15 | 37.6 | 95 | 35 | 7.36 | .061 | .44 | 13 | 136 |
| | 30 | 37.6 | 92 | 36 | 7.36 | .090 | .66 | 13 | 135 |
| | 45 | 37.6 | 84 | 40 | 7.38 | .082 | .68 | 13 | 137 |
| | 75 | 36.9 | 17 | 27 | 7.24 | .033 | .13 | 129 | 390 |
| | 90 | 36.3 | 22 | 23 | 7.21 | .032 | .12 | 143 | 420 |
| | 105 | 36.6 | 99 | 29 | 7.26 | .038 | .14 | 108 | 338 |
| | 135 | 37.5 | 81 | 35 | 7.31 | .053 | .15 | 61 | 319 |

TABLE I (continued)

| Puppy No. | Time (min) | Temp (°C) | $pO_2$ (mm Hg) | $pCO_2$ (mm Hg) | pH | Glycerol ($\mu$moles/ml) | FFA ($\mu$eq/ml) | Lactate (mg %) | Glucose (mg %) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 15 | 37.6 | 89 | 35 | 7.37 | .105 | .52 | 19 | 135 |
| | 30 | 37.6 | 94 | 37 | 7.36 | .112 | .39 | 17 | 125 |
| | 45 | 37.6 | 87 | 37 | 7.36 | .108 | .65 | 14 | 136 |
| | 75 | 37.2 | 15 | 26 | 7.33 | .072 | .28 | 59 | 246 |
| | 90 | 36.9 | 17 | 19 | 7.29 | .062 | .18 | 99 | 282 |
| | 105 | 37.2 | 113 | 24 | 7.32 | .071 | .17 | 75 | 248 |
| | 135 | 37.5 | 90 | 31 | 7.33 | .072 | .27 | 41 | 172 |

has been inhibited in pancreatectomized dogs with infusions of l-lactate sodium(6), which may be due to the formation of alpha glycerol phosphate from lactate with the subsequent resynthesis of triglyceride. Lactate levels in the hypoxic puppies were well within the range found to inhibit FFA mobilization. Furthermore, high tissue concentrations of alpha glycerol phosphate are present with hypoxia(10), so that the potential for triglyceride resynthesis is further enhanced provided that ATP required for resynthesis is available. Thus, the inference that anaerobic glycolysis may be involved is suggested, but not substantiated. Another possibility stems from the observed hyperglycemia with hypoxemia. If insulin levels are elevated, as often occurs with hyperglycemia, then it may be that this hormone, a known inhibitor of lipolysis(16), is responsible for the limited FFA mobilization in the experiments reported here. However, the effect of hypoxia upon the insulin response to hyperglycemia is unknown. Lastly, the lack of oxygen *per se*, or other as yet undefined factors, must be taken into account as possibly playing a role in the observed inhibition.

The fall in deep rectal temperature noted during hypoxia despite a neutral thermal environment suggests that heat production was curtailed during this period. Inasmuch as lipid metabolism has been implicated as a source of heat production(4), it is possible that decreased FFA mobilization as well as decreased FFA oxidation may have been involved. Along with the apparent fall in heat production during hypoxia, there is a fall in the production of high energy phosphate from lipid(14). This has been thought to be due to decreased FFA oxidation resulting from the limited oxygen supply. The demonstration that diminished FFA mobilization occurs during hypoxia no longer allows that diminished FFA oxidation be accepted as the sole explanation for the lowered energy yield from lipid.

*Summary and conclusions.* Upon breathing 8% oxygen and 92% nitrogen, 10 puppies, 21-35 days of age, became severely hypoxic and developed partially compensated metabolic acidosis. Marked falls in plasma glycerol (.48 $\pm$ .16 $\mu$mole/ml, mean change $\pm$ S.D.) and FFA (.35 $\pm$ .15 $\mu$eq/ml) indicate that lipolysis was inhibited with hypoxia. It is suggested that products of anaerobic glycolysis contribute to the decreased FFA mobilization. Evidence implying that insulin is also involved is incomplete. Although diminished lipolysis may play a role in decreased heat and energy production in the severely hypoxic mammal, its relative importance needs to be resolved.

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## Severe Metabolic Acidosis in the Rat Induced by Toxic Doses Of Tetracycline.* (32311)

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Adverse reactions to the tetracycline group of antibiotics have been described in increasing numbers in recent years(1). In particular, serious and frequently fatal illnesses have been reported after intravenous administration of excessive amounts of tetracycline(2-5). In most cases a typical syndrome developed, characterized by jaundice, metabolic acidosis, and azotemia. These reports have again emphasized that tetracycline may have metabolic effects which are potentially deleterious to patients. In this regard, it is not generally appreciated that tetracycline antibiotics can cause inhibition of protein synthesis in mammalian cells(6,7).

In earlier toxicology studies(8,9), the mechanism of death resulting from large doses of tetracycline was not clearly defined. Therefore, in the present investigation, studies were carried out in an attempt to delineate further the toxic effects of tetracycline and determine the mechanism of death. It was found in the rat that intraperitoneal administration of tetracycline in massive doses resulted in a severe and overwhelming metabolic acidosis and marked hyperkalemia.

*Materials and methods.* Female Wistar rats (Harlan Farms, Cumberland, Ind.) weighing 140-160 g were fasted 16 hours prior to study. A single injection of tetracycline hydrochloride was given, intraperitoneally, in doses of 20, 200, 300, or 400 mg/kg; rats that lived for 14 days were considered to be survivors. In other studies animals were sacrificed at 4, 8, and 16 hours after intraperitoneal injection of tetracycline and serum electrolytes determined using the Technicon Autoanalyzer. Arterial pH, carbon dioxide tension, and bicarbonate concentrations were determined by the Astrup method using a revised nomogram(10). The tissue and plasma levels of tetracycline were determined by a bio-assay method employing *Bacillus cereus* as the test organism(11). Sections of small intestine, kidney, and liver obtained from tetracycline-treated rats at necropsy were examined by light microscopy. Statistical analysis was performed using the Student's t test(12).

*Results.* Survival rates of rats after intraperitoneal injection of tetracycline in various dosages are shown in Table I. All of 10 rats given 20 mg/kg and 7 of 10 rats (70%) that received 200 mg/kg survived 14 days. The survival rate decreased to 44% and 38% in rats injected with doses of 300 and 400 mg/kg, respectively. Most deaths occurred within 16-24 hours after injection of tetracycline. In preliminary studies in this laboratory, survival rates were similar in nonfasted rats and in male rats.

Data on changes in serum electrolytes and blood gases after administration of 400 mg/kg of tetracycline are shown in Table II. Hematocrits and serum sodium concentrations were not affected during an 8-hour period. Blood urea nitrogen (BUN) increased from 15 $\pm$ 3 (1 S.D.) to 41 $\pm$ 7 mg/100 ml at 8 hours ($p<0.001$). Serum potassium increased from 4.8 $\pm$ 0.4 to 7.6 $\pm$ 2.4

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