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Specific plasma endocrine and protein changes in the conventionally reared and artificially reared neonatal pig from birth to six weeks of age

Shawn Frey Charles

To the Graduate Council:

I am submitting herewith a thesis written by Shawn Frey Charles entitled "Specific plasma endocrine and protein changes in the conventionally reared and artificially reared neonatal pig from birth to six weeks of age." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

H.G. Kattesh, Major Professor

We have read this thesis and recommend its acceptance:

G.A. Baumbach, F.B. Masincupp

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

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Henry G. Kattesh

H. G. Kattesh, Major Professor

We have read this thesis and recommend its acceptance:

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SPECIFIC PLASMA ENDOCRINE AND PROTEIN CHANGES IN THE CONVENTIONALLY REARED AND ARTIFICIALLY REARED NEONATAL PIG FROM BIRTH TO SIX WEEKS OF AGE

A Thesis

Presented for the

Master of Science

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Shawn Frey Charles

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ABSTRACT

Forty-eight artificially reared (AR) and twenty conventionally reared (CR) crossbred pigs were used to characterize specific endocrine and protein changes from birth to six weeks of age. AR pigs were delivered by cesarian section on day 114 of gestation, allowed to nurse for the first 48 hours following birth and then fed a commercial milk replacer diet. CR pigs were born naturally and maintained with the sow until six weeks of age. Blood samples (5 ml), hematocrits, and body weights were taken from all pigs on days 1, 3, 7, 14, 21, 28, 35, and 42 after birth. Total cortisol (TC), unbound cortisol (UB-C), cortisol bound to corticosteroid binding globulin (CBG-C), cortisol bound to albumin (ALB-C), total protein (TP), albumin, tyroxine (T4), and triiodothyronine (T3) were measured in plasma. Overall mean body weights of CR pigs were greater ($P < .01$) than AR pigs. Hematocrits in both groups of pigs were lowest ($P < .01$) on days 3 and 7. Hematocrits increased ($P < .01$) by day 14 to values similar to that observed on day 1. Concentrations of TC were higher at birth ($P < .01$) than on days 7 to 42 in both groups of pigs (55.6 vs. 19.2 ng/ml). Similarly, UB-C was highest at birth (29.9%) and decreased ($P < .01$) to its lowest value by day 42 (11.9%). A decline in UB-C for CR pigs began by day 21 in contrast to day 28 for AR pigs. Levels of CBG-C were lowest ($P < .01$) in both groups of pigs on days 7-21 with an overall mean of 33%. CBG-C for CR and AR pigs peaked on day 42 (54.1%) and day 35 (76.4%), respectively. ALB-C was correlated negatively with CBG-C ($r = -.87$; $P < .01$). Total plasma protein and albumin concentrations in CR and AR pigs were

lowest ($P < .01$) at birth and increased to their highest levels by day 14. Overall mean total protein and albumin concentrations for AR pigs (3.2 and 2.2 g/dl, respectively) were lower than for CR pigs (3.9 and 2.4 g/dl, respectively). For both CR and AR pigs T4 concentrations were highest ($P < .01$) at birth (7.8 µg/dl) and declined ($P < .01$) to basal levels by day 7 (4.6 µg/dl). CR pigs overall mean T4 concentration was higher ($P < .01$) than that of AR pigs. T3 concentrations were at or below the detectable limit of the T3 assay (0.5 ng/ml). These results suggest that plasma endocrine and protein changes in the neonatal pig can be influenced by method of rearing.

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CHAPTER I

INTRODUCTION

Despite improved rearing techniques there is still a high mortality rate (20-30%) in pigs during the period from birth to weaning. This is due primarily to the newborn pigs inability to adapt to its new extrauterine environment (Lecce, 1986). During this time the neonate must cope with a changing environment (i.e., temperature and humidity) as well as with imposed management practices (e.g., early weaning, artificial rearing, and castration). Whether or not the neonatal pig can adapt to any of these demands made upon it may dictate its ability to survive.

Artificial rearing is the act of weaning pigs at an early date (2-3 days postpartum) and raising them on a milk replacer diet until they are able to consume solid feed by approximately 21 to 28 days postpartum. Conventional rearing on the other hand is when the pig remains on the sow until time of weaning which may be anywhere from 21 to 35 days. The management technique of weaning pigs early (2-3 days postpartum) and raising them artificially has not reached its full potential because of the reduced ability of the neonatal pig to adapt and survive under the conditions imposed. The application of artificial rearing could enable the producer to utilize the sow and her litter more efficiently by increasing the number of litters delivered by a sow per year as well as increasing the survival rates at weaning. Artificial rearing has been an attractive management concept since 1949 but has

seen only limited application because of the high death losses associated with its use (Lecce, 1975). These death losses have been attributed to an increased susceptibility of the pig to cold environmental temperatures (compared to temperatures in utero) and disease (Curtis, 1970), suggesting that the artificially reared pig has a compromised ability to maintain homeostasis.

Hormones released from specific endocrine glands, along with many other physiological processes that occur after birth, play an important role in establishing and maintaining homeostasis in the young pig (Slebodzinski et al., 1981; McCauley and Hartmann, 1984). The ontogeny, or pattern of development, of the neonatal pigs endocrine system has been partially documented. For example, it is known that the hormone thyroxine (T4), which is released by the thyroid gland, plays a major role in regulating body temperature and the basal metabolic rate (Nowak and Slebodzinski, 1986). It has been suggested that the high concentration of T4 found in the neonatal pig may also serve to acclimate the newborn to its new environmental temperature (Fisher et al., 1977). Likewise, adrenal glucocorticoids, primarily cortisol, serve in making glucose and fatty acids available both for energy production and for mobilization of energy stores, such as carbohydrates, proteins and fats, needed by the various tissues of the body especially during times of stress (Moberg, 1987). In that the first month of a pig's life can certainly be considered stressful, period especially under conditions imposed through the use of artificial rearing, it would follow that dramatic changes in the ontogeny of cortisol release would be evident.

Considerable research has demonstrated that cortisol is present in the circulation in two forms, either bound to high molecular weight proteins or unbound. Unbound cortisol (UB-C) is active biologically, while cortisol bound to carrier proteins (corticosteroid binding globulin [CBG] also known as transcortin, albumin) is inactive. By measuring UB-C i.e., the biologically active portion of total cortisol (TC), one can assess how much cortisol is actually available to the animal. The distribution of cortisol among unbound and bound forms has not been investigated in the neonatal pig. The neonatal pig is vulnerable and susceptible to various factors in the environment, for example temperature and disease, as indicated by high mortality rates. In order for the neonatal pig to survive it must make certain endocrine adaptations. If in fact cortisol plays an important role in establishing and maintaining homeostasis in the neonatal pig, critical changes in the distribution of cortisol between unbound and bound forms should be evident. The deleterious effects of artificial rearing on the neonatal pig, as indicated by an even higher mortality rate than that observed in conventionally reared pigs, may suggest that artificial rearing is further compromising the animals ability to make specific endocrine changes needed for its survival.

The objectives of this study were to: (1) document selected plasma hormone and protein changes that occur in the pig from birth to six weeks of age and, (2) examine the influence that method of rearing (conventional vs. artificial) might have on these physiological changes.

CHAPTER II

LITERATURE REVIEW

Survival and Development of the Neonatal Pig

The birth process is an abrupt and profound event during which the newborn pig must undergo various physiological adjustments to adapt to its new environment (Curtis, 1970). A pig's overall capability to cope with its new environment will dictate its survival. An understanding of normal physiological patters of development during this period is a necessity before advances in management practices can occur which could lead to an increase in the survival rate of the neonatal pig.

The most vulnerable period of a pig's development is the time from birth to weaning (Curtis, 1970). This is when mortality rates reach their maximum, especially within the first 3 days (Dividich and Noblet, 1981). Roughly there is a 20-30% mortality rate between birth and weaning (Lecce, 1986). High mortality rates are attributed to low immunity and birth weights or crushing by the sow (Pomeroy, 1960) as well as inadequate thermoregulation (Dividich and Noblet, 1981).

Under conventional rearing systems pigs remain on the sow from birth until they are weaned at about 3-5 weeks of age. Pig birth weights, which normally range between 1.1 to 1.4 kg (Dividich and Noblet, 1981; Close and Stainer, 1984), are positively correlated with its survivability (Parker et al., 1980a). Himmelberg et al. (1985) reported three week weaning weights between 4.1 to 6.7 kg, while Close

and Stainer (1984) reported a mean body weight of 4.7 kg at 25 days of age. Average daily gains of 0.39 kg/day have been observed in pigs from 3 to 7 weeks of age (Dividich, 1981; Himmelberg et al., 1985). Brown et al. (1976) reported an average weight of 13.1 kg at 7 weeks of age.

Along with birth weight, the thermal environment has been shown to significantly affect pig survival rate, especially during the first 48 hours of life (Aumaitre and Seve, 1978). The neonatal pig is born with little hair, lack of subcutaneous fat and a high ratio between surface area and volume (Parker et al., 1980b). Energy reserves are rapidly depleted after birth and will only last for 12 to 24 hours (Bayley et al., 1980) until additional energy is supplied from the sows milk. Therefore, intake of colostrum is necessary to supply the needed energy to maintain thermogenesis (Dividich and Noblet, 1981). The thermoneutral temperature of the newborn pig is 34°C (Noblet and Dividich, 1981). The optimal temperature for normal growth of pigs at 3 weeks is reported to be 28°C during the first week after weaning with a 2°C drop with each following week up to 5 weeks after weaning (Dividich, 1981).

The pig is essentially an immunological virgin at birth. This is a result of the fetus being immunologically incompetent to acquire active immunity before birth (Lecce, 1986). Along with being immunologically naive, the pig is born without circulating immunoglobulins (Ig) (Lecce, 1986). A neonatal pig acquires immunity from the consumption of colostrum which is rich in immunoglobulins especially IgG and IgA (Owen et al., 1961). The neonatal pig relies on this passive immunity for

several weeks until the pig is able to produce its own antibodies (Owen et al., 1961). At the time of weaning the pig has become more mature and better able to maintain homeostasis. Even though weaning and post-weaning times are crucial periods, the pig is less vulnerable to temperature extremes and susceptibility to diseases and is more apt to survive from this point on (Owen et al., 1961).

Hematocrits, which are a measure of packed red blood cell volume, are used as an indicator of anemia. Swine reared under normal management conditions tend to develop anemia within three days after birth (Miller et al., 1961a). One factor that contributes to anemia is the increase in plasma volume as a result of the intake of colostrum which causes a dilution of the circulating cellular components (McCance and Widdowson, 1959; Miller et al., 1961b). Iron deficiency is also a major cause of pig anemia. A pig is born with only 50 mg of iron in its body. In order to meet the demands of rapid growth the neonatal pig requires 7 mg of iron per day from its diet during the first three weeks of its life (Miller et al., 1960). The sow can only provide 1 mg of iron through milk per day. Anemia lowers the body's defenses against disease, which can result in such conditions as scours and pneumonia. Over 90% of newborn pigs, which are not administered exogenous iron, will develop anemia within 3 days (Kernkamp, 1957; Miller et al., 1961a). Hematocrits for newborn pigs are relatively high at birth (38.4%) then decline through day 7 to values of 26.1% (Parker et al., 1980b). Hematocrits have been shown to increase from day 7 to 14 (34.4%) after which no further changes occur (Ramirez et al., 1963).

Ramirez et al. (1963) also found a negative relationship between blood volume and hematocrit level while a positive correlation was seen when hematocrits were compared to total proteins.

Specific Endocrine and Protein Changes in the Neonatal Pig

Specific endocrine changes have been documented in the pig from birth to six weeks of age (McCauley and Hartmann, 1984; Nowak and Slebodzinski, 1986). These changes in the circulating levels of hormones enable pigs to survive and cope with their new extrauterine environment. Most of these internal changes occur within the first few weeks of life, with the most dramatic occurring immediately after birth. In the pig, cortisol (Worsaae and Schmidt, 1980) and thyroxine (T4) and triiodotyronine (T3) (Slebodzinski et al., 1981) have been assigned major roles in supporting various bodily functions.

Total Cortisol

Cortisol, the major glucorticoid in swine, is produced and released from the adrenal cortex (Yousef, 1985) in response to adrenocorticotropic hormone (ACTH) from the anterior pituitary gland (Hafez, 1980; Frandson, 1981). The functions of cortisol are many and varied. They include the regulation of lactation, reproduction, inflammation, metabolism, immune response, hepatic gluconeogenesis and many other physiological mechanisms essential for development processes in both fetal and newborn life (Munck et al., 1984; Moberg, 1987). The broad range of effects of cortisol are due to its having receptors in nearly every

nucleated cell type in the body (Munck et al., 1984). Of major importance to the neonatal pig is the role that cortisol plays in promoting gluconeogenesis (McDonald, 1969). Gluconeogenesis is the production of glycogen, which in turn can be broken down into glucose. Glucose can then be used for muscular work and for liberating heat.

Cortisol concentrations in the pig have been shown to be at their highest levels at birth (McCauley and Hartmann, 1984). McCauley and Hartmann (1984) reported finding a mean cortisol level of 193 ± 11 μg/L in neonatal pigs which was 12 times higher than that found in adults (15.5 ± 1.2 μg/L). After birth, cortisol concentrations rapidly decline until day 5 postpartum at which time they are not higher than those of adults. These sharp declines from high concentrations at birth to adulthood concentrations within 3 days after birth suggests a profound change in the metabolism and/or production of plasma cortisol in the neonate during this time (McCauley and Hartmann, 1984).

Pigs, along with man and sheep, exhibit a circadian rhythm in plasma cortisol concentrations through a 24 hour period (Fulkerson and Tang, 1979). In adult swine, cortisol levels peak in the morning (0900-1000 hr.) and reach a nadir by early afternoon (Barnett et al., 1981a).

The percentage of free cortisol has not yet been documented in the neonatal pig. However, free cortisol has been measured in adult pigs and in the lamb. In mature, ovariectomized, nonparous pigs, concentrations of free cortisol ranged from 2 to 3.2 ng/ml (Barnett et al., 1981b). Barnett et al. (1981a) reported 5 ng/ml of unbound plasma

cortisol in 2 year old ovariectomized gilts. Ballard (1982) found that in the lamb, the percentage of free cortisol is highest at birth followed by a 50% decrease by day 14.

Thyroxine and Triiodothyronine

Thyroid hormones are essential to a neonatal pigs survival. The hypothalamus, in response to an appropriate stimulus (e.g., drop in body temperature), releases thyroid releasing hormone (TRH) which acts on the anterior pituitary gland to produce and release thyroid stimulating hormone (TSH). Thyroid stimulating hormone promotes the uptake of iodide by the thyroid gland and hydrolysis of thyroglobulin which causes the release of T4 and triiodothyronine (T3) from the colloid of the thyroid gland (Mason and Wilkinson, 1973).

Peripheral monodeiodination of T4 and T3 is a general phenomenon observed in mammals, birds and fish (Nowak, 1983). This extrathyroidal monodeiodination of T4 to T3 is the major source of the total daily production of T3 in man (72-76%; Chopra, 1975) and sheep (40-52%; Bianchi, 1983). T3 is secreted in small quantities (30%) by the thyroid gland, as evidenced in pigs (Nowak and Slebodzinski, 1986). Although T3 is more biologically active than T4, its half-life, in humans is approximately 1.5 days compared to 6.7 days for T4 (Chopra, 1975). This could possibly be due to the weak association of T3 to thyroxine binding globulin (TBG) as suggested by Mason (1975). The free fraction of T4 is less than .05% of the total circulating concentration of T4 (Nowak, 1983).

Thyroid hormones regulate metabolic processes in young developing animals. Both T4 and T3 serve to stimulate protein synthesis, mitochondrial activity, oxygen utilization, gluconeogenesis and synthesis of nuclear RNA (Mason and Wilkinson, 1973; Singh and Snyder, 1978). Circulating levels of T4 and T3 increase during cold exposure in most animals including pigs 3 days of age or older (Kaciuba-Uscilko, 1972). In view of the strong calorigenic potency of T3 and the rather inert state of T4, T3 may be acting as the major adaptive mechanism in the neonatal pig for thermogenesis (Nowak and Slebodzinski, 1986). This increased role of the thyroid gland in the neonatal pig is primarily due to a functionally and morphologically mature hypothalamo-pituitary-thyroid axis at birth (Slebodzinski, 1965). The newborn pig does not respond metabolically to noradrenalin which is important in thermogenic responses in other species (Swanson, 1957; Cassuto and Ammit, 1969). These above observations suggest that the thyroid is a major mediator of thermogenesis in the neonatal pig (Slebodzinski, 1965). MacDonald (1969) suggested that since growth hormone does not cross the placenta, T4 is perhaps the major fetal growth promoter making the thyroid gland an even more important factor in the early development of the pig (Fisher et al., 1977).

The concentrations of total T4 and T3 have been documented in the neonatal pig. Similar to cortisol, T4 values are highest within 24 hours after birth (8.7 µg/dl) than any other postnatal time (Parker et al., 1980a; Slebodzinski et al., 1981). From birth to 3 days of age T4 concentrations decline rapidly to 3.9 µg/dl at which point they

remain unchanged through day 42 (Nowak, 1983). T3 concentrations are low immediately after birth (0.97 ng/ml). Concentrations of T3 then increase sharply to reach a maximum concentration during the first 12 to 24 hours of life (Parker et al., 1980a; Slebodzinski et al., 1980). Concentrations of T3 then decline on day 3 to values similar to that at birth (Slebodzinski et al., 1980). A second increase in T3 levels is seen between days 3 and 7, at which point values again decline by day 10 and remain constant thereafter (Parker et al., 1980b).

Total Protein

Total serum protein levels in the neonatal pig, which include immunoglobulins, and albumin reach their maximum concentrations within 48 hours after birth (Ingvarsson et al., 1978). At birth total protein concentrations range from 2.2 to 2.4 gm/100 ml (Miller et al., 1961b; Ramirez et al., 19863; Ingvarsson et al., 1978). Within two days, values have been reported to increase to 5.3 to 7.2 gm/100 ml (Miller et al., 1961b; Ingvarsson et al., 1978). This rapid increase in total plasma protein levels from birth to 48 hours is apparently the result of the pig ingesting a high concentration of protein contained within the colostrum (Ingvarsson et al., 1978).

Albumin, which is produced in the live (Ruoslahti et al., 1974), constitutes approximately 40% of the total serum protein content in the adult (Ingvarsson et al., 1978). It has a molecular weight of 66,000 and an isoelectric point between 4.7 and 5.5 (Englebienne, 1984). The concentration of albumin is low at birth and steadily increases until reaching a peak at 3 weeks of age with concentrations of 2.9 to 3.3 g/dl

(Owen et al., 1961; Ingvarsson et al., 1978). Albumin has high capacity but low affinity for binding cortisol and is important in determining the free fraction of cortisol in plasma (Siiteri et al., 1982). Albumin has also been suggested as a factor that may influence fetal growth and survival of the neonatal pig (Stone, 1984). Stone (1984) found that there was a positive correlation between birth weight and albumin concentration in the pig. This researcher reported that genetically obese pigs had higher albumin values and a 20% increase in survival rates from birth to weaning than did lighter, genetically leaner pigs. Conventionally reared pigs were similar to these genetically lighter pigs, in regard to body weights and albumin concentrations. Therefore, Stone (1984) concluded that increased albumin concentrations in heavier pigs may be related to their degree of physiological maturity, which may explain the increase in survival rates in these pigs as compared to that of leaner, lighter pigs.

Alpha fetoprotein (AFP) is a major serum protein in mammalian fetuses (Adinolfi et al., 1975). The function of AFP is unknown. It has been used clinically as an indicator of maturity during the perinatal period (Stone, 1984). The concentration in the neonate however is low, with only a slight increase observed within the first week of life (Karlsson, 1970). AFP exhibits major similarities with albumin in its physiochemical characteristics (Carlsson et al., 1977) and is produced by the same cells (hepatocytes) in liver.

Corticosteroid binding globulin (CBG) is a glycoprotein with a molecular weight ranging from 43,000 in the guinea pig to 52,000 in man

(Westphal, 1983). Corticosteroid binding globulin is synthesized in the liver and plays an important role in regulating the free hormone concentration of corticosteriods in blood plasma (Englebienne, 1984). It has a high affinity and low capacity for corticosteroids with complexes of hydrophilic bonds in contrast to the hydrophobic binding typically of albumin (Westphal, 1983). Albumin and CBG bound corticosteriods provide a readily available pool of free hormone when needed. Corticosteroid binding globulin also acts to protect cortisol from rapid hepatic catabolism (Siiteri et al., 1982).

In early gestating sows mean binding capacities of CBG (CBC) were 18.0 µg cortisol bound/100 ml plasma as reported by Kattesh et al. (1980). Barnett et al. (1981a) also reported maximum corticosteroid binding capacity (MCBC) of 27.3 ng/ml in ovariectomized 2 year old gilts. Other researchers have reported MCBC values of 32 and 49 mg/ml in 2 year old ovariectomized gilts (Seal and Doe, 1963; Steeno and DeMoor, 1966). The binding affinity (strength of attachment) of cortisol to CBG was estimated to be $5.6 \times 10^{8}\ M^{-1}$ in early gestating gilts (Kattesh et al., 1980). Barnett et al. (1981a) found values of $3.3 \times 10^{7}\ M^{-1}L$ in 2 year old ovariectomized gilts.

In the newborn lamb CBG binding capacity decreases most dramatically between birth and 4 days of age (5.3 to 3.3 µg/dl), with a slower decline occurring between days 5 and 21 (<.61 µg/dl at day 21). Concentrations of albumin bound corticoids remained constant from days 4 to 14 after birth in lambs (Ballard et al., 1979). Unlike the lamb, rat CBG levels undergo dramatic changes throughout their postnatal life. At

birth, CBG concentrations are high, but then rapidly drop to undetectable levels by day 5 postpartum. By the second postnatal week, CBG concentrations increase sharply (Agostino and Henning, 1982). Ballard et al. (1979) concluded that decreasing concentrations of CBG are partly due to hemodilution and a decreased synthesis occurring after birth. They also proposed that corticosteroids, which are elevated at birth, may be inhibiting CBG production.

The concentration of free (biologically active) or unbound cortisol is known to be partially controlled by CBG (Ballard et al., 1983). Therefore, the level of production or synthesis of CBG within the liver ultimately controls the concentration of biologically active cortisol (Agostino and Henning, 1980). As demonstrated above, fluctuations in the concentration of CBG occur in the fetal and neonatal period in both the lamb (Ballard et al., 1982) and rat (Agostino and Henning, 1980), indicating that there may be changes occurring in the secretion of CBG during the neonatal period in these species. Agostino and Henning (1980) proposed that these changes may be hormonally controlled.

In a study of postnatal development of CBG in the rat, CBG was found to be independent of estrogen and primarily regulated by T4 (Agostino and Henning, 1980). The mechanism in which T4 acts is still uncertain. One hypothesis was that T4 could be activating previously masked molecular sites on liver cells (Agostino and Henning, 1980). Another possibility, as reported in a later paper by Agostino and Henning (1982) is that differences may exist in the maturity of the animals hepatic receptors for T4 and T3. In the rat, increasing

responsiveness to T4 occurred as the hepatic system matured. Binding capacity for T3 receptors in rat liver nuclei increased 1.7 to 3.6 fold from birth to 20 days of age (DeGroot et al., 1977). The evidence suggesting that thyroxine controls postnatal development of CBG is convincing in the rat, but still unknown in other animals such as the sheep, cow and pig.

Ballard et al. (1982) found that neither T4, estrogen, prolactin or the corticoids appeared to be controlling factors of CBG development in the lamb. It becomes quite clear that there are species differences not only in the developmental patterns of CBG but also in the factors controlling its production.

Effects of Artificial Rearing on Swine

Weaning pigs early and rearing them artificially has been an attractive management concept for years (Lecce, 1986). Researchers have long known the great potential that artificial rearing could have on the swine industry. Many attempts have been made to early wean/artificially rear pigs for various reasons. The two most recognized reasons are: (1) by weaning pigs early a sow can be used more efficiently by increasing the number of litters per sow per year and, (2) allows pigs that a sow could not raise effectively to be reared artificially, thereby decreasing overall mortality rates. On the average 20 to 30% of pigs farrowed die before weaning (Dividich and Noblet, 1981; Lecce, 1986), with the heaviest losses occurring within 3 days after birth (Fahmy, 1971; Dividich and Noblet, 1981). The majority of research dealing with

the comparison of artificial versus conventional rearing of pigs has involved such topics as weight gain, feed efficiency (Braude and Newport, 1977), crowding, optimal temperatures for maximal thermal stability (Dividich, 1981), colostrum and milk intake (Owen et al., 1961), plus susceptibility to enteropathogens resulting in diarrhea (Lecce, 1986). Little information is available on the hormonal differences of pigs subjected to these two types of rearing conditions.

Even though the potential of artificially rearing pigs is unlimited, it's practicality to the farmer has yet to be fulfilled. The artificially reared pig has a high susceptibility to cold, and lacks protection against diseases (Curtis, 1970), both of which contribute to high death losses (Mount, 1969). High death losses in the neonatal pig are mostly attributed to diarrhea resulting from an immature immune system (Bellis, 1975). The earlier a pig is weaned from the sow the more susceptible it becomes to enteropathogens (Kohler, 1972; Moon, 1975; Kirtsein et al., 1985). Enterotoxigenic Escherichia, transmissible gastroenteritis virus (TGE) and retrovirus are the major etiological agents that cause diarrhea (Lecce, 1986). Colostrum and milk from the sow protect the nursing piglet by neutralizing enteropathogens in the lumen of the gut (Lecce, 1986). Lecce (1986) studied possible mechanisms to artificially supply immunoglobulins through the feed to possibly prevent diarrhea in artificially reared pigs. Passive protection was obtained by feeding specific antibodies against the major etiological agents.

Thermoregulation by the neonatal pig can have significant effects on weight gain and mortality rate (McConnell et al., 1987). Low

resistance to cold temperatures is due to the lack of brown adipose tissue used for thermogenesis (Mount, 1969) and a lack of white adipose tissue used for insulation and energy (Manners et al., 1963). Cold temperatures were found to decrease colostrum intake needed for thermogenesis (Noblet and Dividich, 1981). Optimal temperatures ranging from 23 to 32°C are needed to maintain thermoneutral conditions (Noblet and Dividich, 1981) and to promote the most favorable conditions for growth, feed intake and efficiency of feed utilization.

Contrary to other researchers, Braude and Newport (1977) have reported advantages of artificial rearing over conventional rearing of pigs. Superior growth rates of artificially reared pigs were reported within the first month of age when feeding a liquid diet containing 125-200 g dry matter/L. Braude and Newport (1977) also stated that these superior growth rates in artificially reared pigs could not be achieved when pigs were weaned at 2 days of age and immediately put onto a pelleted creep feed diet.

Effects of Stress in Swine

It is widely accepted that the vulnerability of the neonatal pig to adverse environmental conditions is greatest within the first few weeks of its life (Slebodzinski, 1965). Within this period it is critical that the newborn pig have the ability to cope and deal with its changing environment a process known as maintaining homeostasis. Homeostasis is the state of equilibrium of the internal environment which includes the blood components, tissue fluid and lymph. Homeostasis is controlled by

a number of factors including the nervous and endocrine systems (Yousef, 1985).

Since the welfare of an animal is difficult to ascertain, researchers have frequently used responses to stress as indicators of how an animal is coping and changing with its environment. The term stress implies any nonspecific response of the body to any specific demand (termed a stressor) made upon it. Yousef (1985) defines stress physiology as the study of the animals physiological, biochemical and behavioral responses to the various factors of the physical, chemical and biological environment.

An animal's physiological response to stress was first described by Selye (1956). According to Selye (1956) a response to a stressor is characterized in three phases: (1) emergency reaction, (2) stage of adaptation and (3) stage of exhaustion (Munck, 1984). The emergency reaction is characterized by stimulation of the sympathetic nervous system and release of catecholamines, epinephrine and norephinephrine from the adrenal medulla. In times of chronic stress the adrenal gland is stimulated by ACTH to secrete glucocorticoids as described earlier. Epinephrine, from the emergency reaction, potentiates the release of ACTH from the anterior pituitary gland. It is this secondary response (the release of glucocorticoids) to stress which enables animals to cope with their ever changing environment (Munck, 1984).

Total plasma cortisol changes have been reported in the neonatal pig in response to chronic stressors (Worsaae and Schmidt, 1980). In this study pigs were subjected to 45°C temperatures for 15 minutes, 0°C

for 1 hour, and chasing for 29 minutes. All of these forms of chronic stressors led to elevated plasma corticosteroid concentrations in the 4 to 8 week old pigs. Worsaae and Schmidt (1980) also stated that early weaning at 3 weeks postpartum was a form of long term stress, as indicated by increased concentrations of cortisol up to 1 week after weaning. Wild rainbow trout that were confined in small containers for 6 and 12 hours exhibited up to three times the concentration of total cortisol as compared to cortisol values of hatchery fish subjected to the same treatment (Woodward and Strange, 1987). These researchers concluded that wild fish were more susceptible to stress than hatchery-reared fish, as indicated by more pronounced changes in cortisol concentrations in the wild rainbow trout.

Circulating concentrations of thyroid hormones are also affected by stress (Parker et al., 1980b). Parker et al. (1980b) found T4 levels to be elevated at birth and even higher at 6 hours after birth. Slebodzinski (1972) stated that these high levels are probably associated with the stress that occurs at parturition. The thermogenic properties of T4 are apparently working at full capacity at the time of birth. T3, which is the most biologically active thyroid hormone, was also found to increase during periods of cold stress (Parker et al., 1980b).

The release of glucorticoids during stressful events acts to maintain physiological homeostasis. However, the high concentrations of cortisol also have deleterious effects on an animal. High levels of corticosteroids have been found to modify the secretions of gonadotrophins (Moberg, 1987). The preovulatory surge of LH has been shown to

be inhibited by corticosteroids (Moberg, 1987) and the administration of ACTH has been shown to decrease the production of testosterone in boars (Knight et al., 1982). Kattesh and coworkers (1980) reported a reduction in gestation length in stressed, crossbred sows. Prolonged secretion of corticosteroids has also been documented as causing the regression of lymphoid tissues, changes in protein metabolism, reduced growth and weight loss (Freeman, 1975).

The suppressive effects of corticosteroids on the immune response has been known for years (Kendall, 1971). It has been proposed that: (1) a stress-induced increase in glucocorticoid concentrations is not to protect against the stress itself but against the normal defense reactions, and (2) glucocorticoids accomplish this function by suppressing these defense reactions in order to prevent them from overshooting themselves and, therefore, disrupting homeostasis (Munck, 1984). However, this suppression of the immune response by high cortisol levels can possibly leave an animal susceptible to disease and infection which could lead to decreased survival (Mason, 1975).

CHAPTER III

MATERIALS AND METHODS

Animals and Treatments

A total of sixty-eight crossbred pigs (Landrace x Duroc x Hampshire) were either delivered by cesarean section on day 114 of gestation (day 1 = day of breeding) and reared artificially (AR; n = 48) or born naturally and reared conventionally (CR; n = 20). The AR pigs were kept with the sow for the first 48 hours after birth and then grouped in raised expanded metal wire cages (0.4 sq.m/pig). Pigs were provided a commercial milk diet (Littermilk, Land O Lakes Co., Minneapolis, MN) twice daily until day 28. The CR pigs were left with the sow in a conventional farrowing crate and weaned at six weeks of age. All pigs were provided an 18% crude protein pelleted creep feed beginning on day 21. All pigs were given iron dextran (2 ml, IM) on day 7 and all males were castrated on day 21.

Blood Sampling

Pigs were bled (5 ml) via puncture of the anterior vena cava on days 1, 3, 7, 14, 21, 28, 35, and 42. Day 1 is the day of parturition or cesarian delivery. At each sampling time, hematocrits and body weights were recorded. The heparinized blood was centrifuged at 3000 rpm for 15 minutes, plasma was removed and stored at -20°C.

Hormone Analysis

Plasma cortisol was measured by radioimmunoassay similar to the procedure reported by Woodward and Strange (1987) for unextracted plasma (Foster and Dunn, 1974). The assay procedure is outlined in Appendix A. Radiolabeled [1,2 ^{3}H] cortisol (53 Ci/mmol) was purchased from New England Nuclear, Boston, MA. Rabbit antiserum prepared against cortisol-3-bovine serum albumin was purchased from Cambridge Medical Diagnostics, Billerica, MA. Steroids having less than 1% cross reactivity with the cortisol antibody (measured at 50% displacement of bound [^{3}H]-cortisol) were β-estradiol, pregenolone, progesterone, B-estradiol 3-benzoate, 5α-androstane 17-β-3 one and estrone. The steroids having greater than 1% cross reactivity were 17-α hydroxyprogesterone (2%), corticosterone (8%), cortisone (38%), and hydrocortisone 21-acetate (100%). Intra- and interassay coefficients of variation were 5.1% and 15%, respectively.

Estimation of the percentage of free cortisol in undiluted plasma was determined using the procedure of Hammond et al. (1980) as outlined in Appendix B. Pig plasma samples (500 µl) were incubated with 3 x 10^{5} dpm of [1,2-^{3}H]-cortisol (53 Ci/mmol, New England Nuclear, Boston, MA) and 12 x 10^{3} dpm of [^{14}C]-glucose (271 mCi/mmol, ICN Radiochemicals, Irvine, CA). Duplicate aliquots (200 µl) of the mixture were subjected to centrifugal ultrafiltration through a dialysis membrane at 37°C. The percentage of free cortisol in plasma samples was estimated by comparing the ratio of [1,2-^{3}H]-cortisol to [^{14}C]-glucose in the ultrafiltrate with the corresponding ratio in the plasma retained by the

dialysis membrane. The intra- and interassay coefficients of variation of the percentage of free cortisol in a reference pig plasma sample (n = 22) was 7.0% and 13.9%, respectively.

The percentage of total cortisol bound to CBG was likewise determined for each plasma sample following prior heating at 60°C for 1 hour (Siiteri et al., 1982). The following calculations were performed to obtain the percentage of cortisol bound to CBG:

$$\text{Percentage of cortisol bound to CBG} = \frac{\text{\% free cortisol (heated plasma)} - \text{\% free cortisol (native plasma)}}{\text{\% free cortisol (heated plasma)}} \times 100$$

The percentage of cortisol bound to albumin was calculated as follows:

$$\text{Percentage of cortisol bound to albumin} = 100 - (\text{\% free cortisol} + \text{\% cortisol bound to CBG})$$

Plasma (T4) and (T3) concentrations were quantified using GAMMACOAT [^{125}I] competitive radioimmunoassay kits (Baxter Travanol Diagnostics, Inc., Cambridge, MA). Bound radioactivity was measured in a gamma counter and calculated as T4 in micrograms of T4 per decaliter and T3 in nanograms per milliliter by extrapolating from a standard curve. T4 intra- and interassay coefficients of variation for reference control were 7.1 and 9.6%, respectively. The intra- and interassay coefficients of variation for T3 were 7.3 and 8.4%, respectively.

Total protein concentrations were determined on unknown plasma samples (100 µl) using a dye-binding procedure (Pierce Chemical Company, Rockford, IL). An absorbance shift from 465 to 595 nm occurs when

Coomassie blue G-250 binds to proteins in an acidic solution. The assay procedure is outlined in Appendix C. Plasma was diluted with physiological saline (0.9% NaCl) to adjust for increasing levels of total protein occurring in pig plasma over the sampling period. Plasma dilutions, in the ratio of plasma to saline, and the corresponding bleeding days were as follows: 1:20 for day 1 and 3, 1:50 for days 7, 14 and 21, and 1:100 for days 28, 35 and 42. A standard curve of 25, 50, 100, and 200 mg/dl of albumin (bovine albumin Fraction V, Pierce Chemical Company, Rockford, IL) was prepared using physiological saline (0.9% NaCl) as the diluent. Protein concentrations of samples were calculated from a standard curve using absorbance values read on a spectrophotometer at 595 nm. The interassay coefficient of variation as determined in control porcine plasma, was 10%.

Albumin concentrations were determined using 10 µl of plasma with an assay (Sigma Diagnosistics, St. Louis, MO) based on the binding of endogenous albumin to bromcresol green to produce a blue green color with a maximum absorbance of 628 nm. The assay procedure is outlined in Appendix D. Samples were read on a spectrophotometer at an absorbance level of 628 nm. Calculations were as follows:

$$\frac{(\text{A sample} - \text{A blank})}{(\text{A standard} - \text{A blank})} \times \text{Concentration of standard (5 g/dl)} = \text{Albumin concentration [g/dl]}$$

The interassay coefficient of variation, as determined in control porcine plasma was 8%.

Statistical Analysis

The effects of treatment and day were tested by least-squares analysis of variance according to Goodnight (1979) for a randomized block design with unequal subclasses. The model fitted was as follows:

$$Y_{ijk} = T_i + D_j + TD_{ij} + e_{ijk}$$

where Y_{ijk} represents the dependent variable measured, T_i is treatment, D_j is day of sampling, TD_{ij} is the treatment x day interaction and e_{ijk} is the residual. The dependent variables were body weight, hematocrit, total cortisol concentration, percentage of unbound cortisol, percentage of cortisol bound to CBG, percentage of cortisol bound to albumin, T4 and T3 concentration, total plasma protein and albumin concentrations. Mean separation procedures were carried out on the main effects using Duncans multiple range test (1955). Mean separation on the interaction was carried out by running a second model which contained only treatments, and was run separately for each day. Another mean separation on the interaction was carried out by running a third model which contained only days, and was run separately for each treatment.

The mean square error (MSE) in the tables in the following chapter can be used to determine the standard error of each mean by using the following equation for unequal sample sizes:

$$S_{\bar{y}} = \sqrt{\frac{MSE}{n}}$$

where n_h = harmonic mean of the n's, and

$$n_h = \frac{\text{number of treatments}}{\text{No. of TRTS} \sum_{i=1} (\frac{1}{n_i})}$$

CHAPTER IV

RESULTS

Survival, Body Weights and Hematocrits

Of the sixty-eight pigs which were initially assigned to this study, eight of the twenty conventionally reared (CR) pigs (60%) and thirty-two of the forty-eight cesarean derived, artificially reared (AR) pigs (66%) were alive by six weeks of age. The majority of the deaths occurred within the first week postpartum for both groups of pigs (CR = 63%; AR = 81%).

Conventionally reared pigs gained a total of 8.8 kg from birth to six weeks of age, with the fastest ($P < .01$) gains occurring from day 21 to 42 (Table 1). The average daily gain over the six week period was 0.21 kg/day. Weights of AR pigs were lower than that of CR pigs from birth to four weeks of age. Although AR pigs gained the same amount of weight (8.8 kg) as the CR pigs over the six week period, 49% of their total weight gain, compared to 60% for the CR pigs, occurred over the first 28 days. In the last 2 weeks the AR pigs compensated by gaining 4.5 kg, which was over half their total gain during the entire six week period.

Hematocrits in CR pigs fell from day 3 to their lowest level on day 7 and then abruptly increased ($P < .01$) by 12.6% from day 7 to 14 where they remained unchanged through day 42 (Table 1). The initial hematocrit values taken on day 1 for AR pigs were highest ($P < .01$) at

Table 1. Body weights and hematocrits in conventionally reared (CR) and artificially reared (AR) pigs from birth to six weeks postpartum.

	Days Postpartum								
ITEM	1	3	7	14	21	28	35	42	MSE†
Body weight, kg									
CR	1.9^{a} (20)	2.3^{a} (18)	3.3^{b} (15)	4.1^{b} (15)	5.9^{c} (12)	7.2^{d} (12)	8.5^{e} (12)	19.7^{f} (12)	0.9
AR	1.3^{a} (38)	1.7^{ab} (40)	2.4^{b} (37)	3.3^{c} (35)	4.3^{d} (30)	5.6^{e} (33)	8.0^{f} (33)	10.1^{g} (33)	1.2
CR VS AR	*	*	*	*	*	*	NS	NS	
Hematocrit, %									
CR	--	24.3^{a} (17)	21.5^{a} (13)	34.1^{b} (14)	36.1^{b} (9)	36.1^{b} (12)	35.3^{b} (12)	33.5^{b} (10)	9.9
AR	33.6^{a} (16)	23.2^{b} (43)	24.7^{b} (36)	31.4^{a} (35)	32.1^{a} (29)	31.8^{a} (33)	32.6^{a} (30)	33.0^{a} (33)	20.9
CR VS AR		NS	*	*	*	*	*	NS	

a,b,c,d,e,f,g Numbers with different superscripts in the same row are different ($P < .01$).

*Significantly different at $P < .01$.

NS = Not Significant.

Numbers in parenthesis represent number of pigs sampled.

†Mean square error of day within treatment.

birth and declined ($P < .01$) sharply to their lowest levels on day 3 and 7 (Table 1). Similar to CR pigs, hematocrits for AR pigs increased ($P < .01$) by day 14 and remained unchanged through day 42 (Table 1).

Total Cortisol

The concentration of plasma cortisol in CR pigs was highest at birth ($P < .01$) as compared to all other postnatal sampling times (Table 2; Figure 1). Within the first 3 days after birth, total cortisol concentrations dropped 24.5 ng/ml which was an approximate 50% decrease (Table 2). From days 3 through 42 cortisol levels remained unchanged. Unlike CR pigs, cortisol concentrations in AR pigs were highest ($P < .01$) on days 1 and 3 as compared to all other sampling days. A decrease of 38.2 ng/ml in total cortisol occurred from day 1 to day 7 in the AR pigs (Figure 1). Like CR pigs, total cortisol for AR pigs remained unchanged from day 7 to 42 (Table 2).

Unbound Cortisol

The percent unbound cortisol (UB-C) in the CR pigs was highest and not different ($P < .05$) between days 1 and 14 (Table 2). Levels then declined from 22.9% (day 14) to 18.4% (day 21) as illustrated in Figure 1. UB-C levels were similar from days 21 to 42. UB-C levels for AR pigs measured at birth were higher ($P < .01$) than that measured for CR pigs (33.3 vs 24.3%). Unlike CR pigs, UB-C levels for AR pigs decreased ($P < .01$) sharply by 10.2 percentage points from birth to day 3 and values remained unchanged from day 3 to 21 (Table 2). Between days 21

Table 2. Total plasma cortisol concentration and percent distribution of cortisol among unbound, corticosteroid binding globulin bound and albumin bound forms in conventionally reared (CR) and artificially reared (AR) pigs from birth until six weeks postpartum.

ITEM	Days Postpartum 1	3	7	14	21	28	35	42	MSE†
Total cortisol, ng/ml									
CR	50.1^{a} (20)	26.6^{b} (16)	21.9^{b} (15)	16.9^{b} (12)	16.9^{b} (12)	18.1^{b} (12)	13.2^{b} (13)	10.9^{b} (12)	177
AR	59.1^{a} (32)	48.2^{a} (32)	20.9^{b} (31)	22.2^{b} (33)	16.7^{b} (25)	18.2^{b} (31)	21.5^{b} (32)	21.8^{b} (32)	634
CR VS AR	NS	*	NS	NS	NS	NS	*	*	
Unbound cortisol, %									
CR	24.3^{ab} (19)	26.3^{a} (16)	26.1^{a} (15)	22.8^{abc} (13)	18.4^{bcd} (12)	13.8^{d} (12)	16.5^{cd} (13)	14.9^{d} (12)	45
AR	33.3^{a} (32)	23.1^{bc} (28)	22.2^{bc} (22)	25.8^{ba} (11)	29.4^{ba} (11)	13.7^{dc} (11)	7.1^{d} (11)	8.8^{d} (11)	74
CR VS AR	*	NS	*	NS	*	NS	*	*	
CBG bound cortisol, %									
CR	52.3^{ab} (19)	42.3^{ab} (14)	32.2^{a} (15)	35.5^{ab} (12)	35.3^{ab} (11)	46.8^{ab} (12)	42.1^{ab} (13)	54.1^{b} (12)	331
AR	45.6^{ab} (32)	43.1^{ab} (20)	35.6^{a} (15)	31.7^{a} (11)	28.2^{a} (11)	67.1^{bc} (11)	76.4^{c} (11)	71.6^{c} (11)	305
CR VS AR	NS	NS	NS	NS	NS	*	*	*	
Albumin bound cortisol, %									
CR	23.4^{a} (19)	32.3^{ab} (14)	41.7^{b} (15)	42.2^{b} (12)	46.7^{b} (11)	39.4^{b} (12)	41.4^{b} (13)	31.1^{ab} (12)	190
AR	22.7^{a} (32)	31.1^{ab} (20)	40.8^{b} (11)	42.6^{b} (11)	43.2^{b} (11)	19.1^{a} (11)	16.5^{a} (11)	19.6^{a} (11)	165
CR VS AR	NS	NS	NS	NS	NS	*	*	*	

^{a,b,c,d}Numbers with different superscripts in the same row are different ($P < .01$).

*Significantly different at $P < .01$.

NS = Not Significant.

Numbers in parenthesis represent number of pigs sampled.

†Overall mean square error as determined for days within treatment.

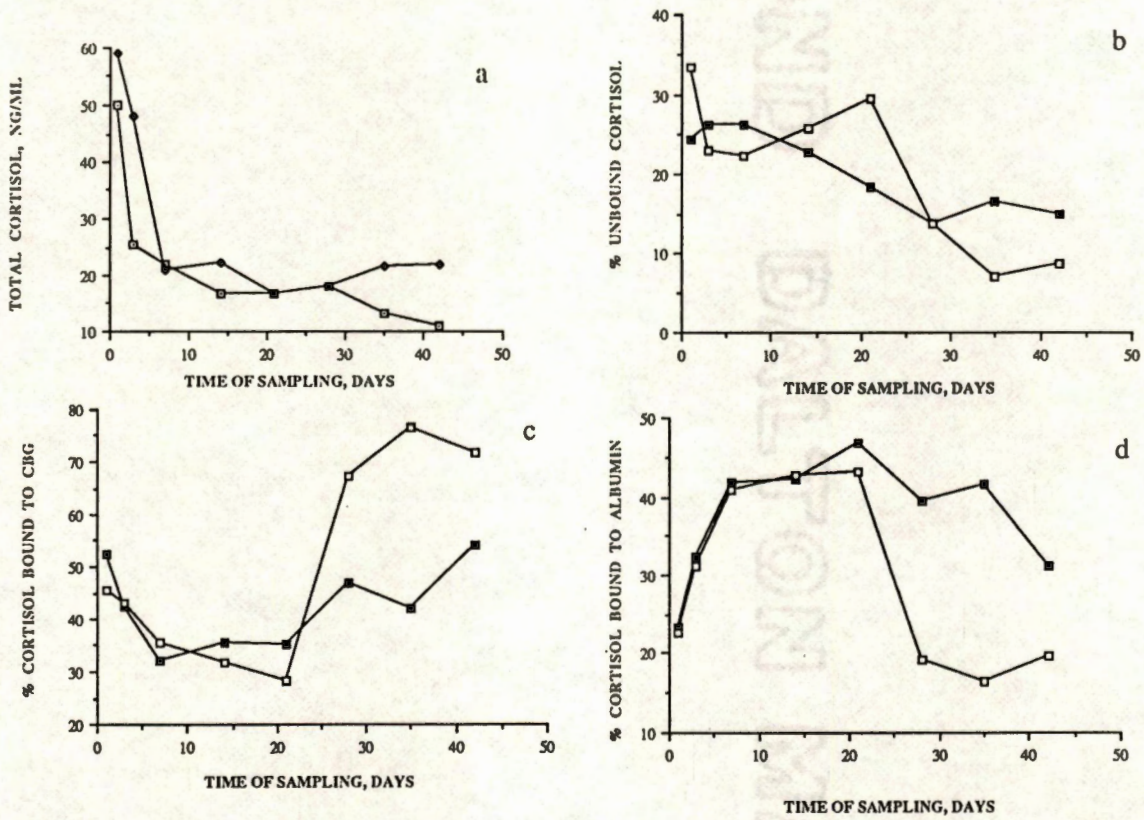

Figure 1. Changes in the concentration of plasma (A) total cortisol, (B) percent unbound cortisol, (C) percent corticosteroid binding globulin bound cortisol, and (D) percent albumin bound cortisol in the conventionally reared (-●-) and artificially reared (-○-) pigs from birth to six weeks postpartum.

and 28 UB-C values for AR pigs declined ($P < .01$) from 29.4 to 13.7% and continued to be lower throughout the sampling period. The ratio of total cortisol to UB-C in the CR and AR pigs on day 1 was 3.2 and 2.8 to 1, respectively. The unbound to total cortisol ratio changed slightly in both groups if pigs by day 42.

CBG-Bound Cortisol

The percent cortisol bound to CBG (CBG-C) in CR pigs was elevated but not different ($P < .05$) at birth as compared to all other postnatal sampling times (Table 2). CBG-C was lowest ($P < .01$) on day 7 when compared to the highest value seen on day 42 (Figure 1). CBG-C for AR pigs was similarly higher at birth and levels were not different through day 21 (Figure 1). However, in contrast to CR pigs, CBG-C for AR pigs increased from day 21 (28.2%) to 28 (67.1%). Thereafter, concentrations on days 28 through 42 remained elevated but not different (Table 2).

Albumin Bound Cortisol

The percent cortisol bound to albumin (ALB-C) is derived from the difference of 100% minus the sum of CBG-C and UB-C. Hence, ALB-C values were found to be highly correlated ($P < .01$) with those of CBG-C in CR ($r = -.91$) and AR ($r = -.84$) pigs, respectively. ALB-C values in CR pigs were low at birth (23.4%) and then increased to 46.7% by day 21 (Table 2; Figure 1). Similarly, ALB-C in AR pigs increased ($P < .01$) from 27.7% at birth to 43.2% on day 21, with the greatest increase ($P < .01$) of 18.1 percentage points occurring between days 1 and 7

(Table 2). Concentrations then decreased sharply ($P < .01$) from day 21 to 42 reaching values similar to that found on day 1 (Figure 1). These values were considerably lower ($P < .01$) than those measured for CR pigs.

Thyroxine

Concentrations of T4 in CR pigs were higher ($P < .01$) at birth than any other postnatal sampling time (Table 3). T4 concentrations steadily decreased by 52% during the first 3 days after birth and remained constant through day 42 (Figure 2). Similar to CR pigs, T4 concentrations of AR pigs were at their highest levels at birth (Table 3). Concentrations then declined ($P < .01$) by 20% and 23% on days 3 and 7, respectively (Figure 2). T4 values remained unchanged on days 14 through 28 followed by a further decline ($P < .01$) on days 35 and 42.

Triiodothyronine

Triiodothyronine concentrations of CR pigs were near or below detectable limits of the assay (Table 3). Concentrations of T3 were relatively unchanged throughout the 6 week period except on day 21 when there was a sharp increase (Figure 2). Concentrations remained high on day 28 but returned to below detectable limits of the assay (0.5 ng/ml) on days 35 and 42. Similar to that of CR pigs, T3 concentrations of AR pigs were near or below the limit of the assay (Table 3). Unlike CR pigs, however, there were no significant changes in T3 for the AR pigs between days 1 and 42 (Figure 2).

Table 3. Plasma concentrations of thyroxine (T4) and triiodthyronine (T3) in conventionally reared (CR) and artificially reared (AR) pigs from birth to six weeks postpartum.

ITEM	Days Postpartum 1	3	7	14	21	28	35	42	MSE†
T4, µg/dl									
CR	9.0^{a} (19)	4.3^{b} (16)	5.1^{b} (14)	5.0^{b} (13)	4.9^{b} (12)	5.3^{b} (12)	4.6^{b} (12)	4.3^{b} (12)	1.9
AR	6.6^{a} (22)	5.3^{b} (22)	4.1^{cd} (20)	4.0^{cd} (19)	4.3^{c} (17)	4.5^{bc} (17)	3.2^{d} (19)	3.3^{d} (18)	1.5
CR VS AR	*	*	*	*	NS	NS	*	*	
T3, ng/ml									
CR	$.78^{ab}$ (20)	$.75^{a}$ (16)	$.73^{a}$ (15)	$.67^{a}$ (13)	1.1^{b} (12)	$.81^{ab}$ (12)	$.56^{a}$ (13)	$.57^{a}$ (12)	.09
AR	$.59^{ab}$ (32)	$.72^{a}$ (29)	$.62^{ab}$ (25)	$.50^{b}$ (29)	$.58^{ab}$ (25)	$.57^{ab}$ (25)	$.57^{ab}$ (27)	$.60^{ab}$ (19)	.04
CR VS AR	NS	NS	NS	*	*	*	NS	NS	

^{a,b,c,d}Numbers with different superscripts in the same row are different ($P < .01$).

*Significantly different at $P < .01$.

NS = Not Significant.

Numbers in parenthesis represent number of pigs sampled.

†Overall mean square error as determined for day within treatment.

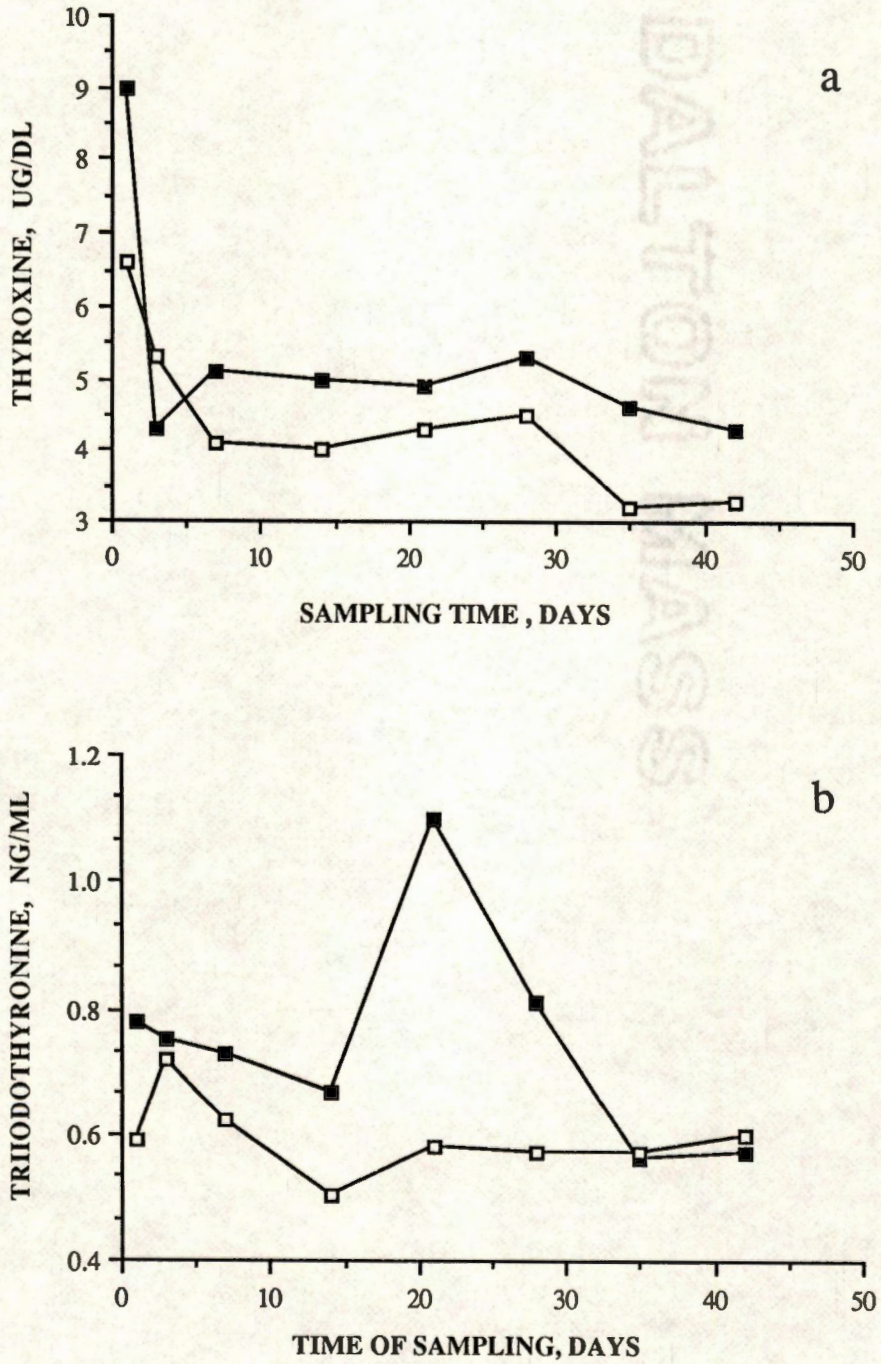

Figure 2. Concentrations of (A) thyroxine and (B) triiodothyronine in conventionally reared (●) and artificially reared (○) pigs from birth to six weeks postpartum.

Total Plasma Protein

From their lowest values at birth (1.6 g/dl) total plasma protein concentrations of CR pigs increased ($P < .01$) three-fold by day 14 (Table 4; Figure 3). Total plasma protein remained unchanged from day 7 to day 42 with an average value of 4.7 g/dl. As with CR pigs total plasma protein concentration in AR pigs was lowest ($P < .01$) at birth (Table 4). Levels increased ($P < .01$) 3.3 g/dl between days 1 and 14 and remained unchanged through day 28. Total plasma protein for AR pigs declined on days 35 and 42 and were lower ($P < .01$) than that measured for CR pigs (Table 4).

Albumin

Plasma albumin concentrations for CR pigs increased a total of 2.3 g/dl from birth to six weeks of age, with 86% of that occurring within the first two weeks (Table 4, Figure 3). Albumin concentrations in AR pigs were lowest at birth, followed by a rapid increase until day 14. Unlike CR pigs, albumin levels in AR pigs reached a plateau of 2.6 g/dl from day 14 to 42 which was less ($P < .01$) than that found for CR pigs over the last two weeks of sampling (Table 4). At birth, albumin concentrations comprised 57.5 and 68.2% of the total protein concentration in CR and AR pigs, respectively. By day 42 values increased to 68.7% (CR) and 81.8% (AR) of the total protein concentration (Table 4).

Table 4. Total protein and albumin concentrations in conventionally reared (CR) and artificially reared (AR) pigs from birth to six weeks postpartum.

ITEM	Days Postpartum 1	3	7	14	21	28	35	42	MSE†
Total protein, g/dl									
CR	1.6^{a} (18)	3.2^{b} (16)	4.5^{c} (15)	4.7^{c} (12)	4.5^{c} (12)	4.5^{c} (12)	5.0^{c} (12)	4.7^{c} (12)	562
AR	1.1^{a} (21)	2.6^{b} (15)	3.0^{bc} (17)	4.4^{d} (18)	4.1^{de} (15)	4.2^{d} (15)	3.6^{ce} (14)	3.3^{c} (16)	362
CR VS AR	*	*	*	NS	NS	NS	*	*	
Albumin, g/dl									
CR	0.92^{a} (20)	1.9^{b} (16)	2.4^{c} (15)	2.9^{d} (12)	2.8^{d} (12)	3.4^{e} (12)	3.4^{e} (12)	3.2^{e} (12)	0.11
AR	0.75^{a} (32)	1.8^{b} (30)	2.1^{c} (29)	2.7^{d} (28)	2.6^{d} (24)	2.6^{d} (26)	2.6^{d} (25)	2.7^{d} (27)	0.16
CR VS AR	*	NS	NS	NS	NS	*	*	*	

^{a,b,c,d,e}Numbers with different superscripts in the same row are different ($P < .01$).

*Significantly different at $P < .01$.

NS = Not Significant.

Numbers in parenthesis represent number of pigs sampled.

†Overall mean square error as determined for days within treatment.

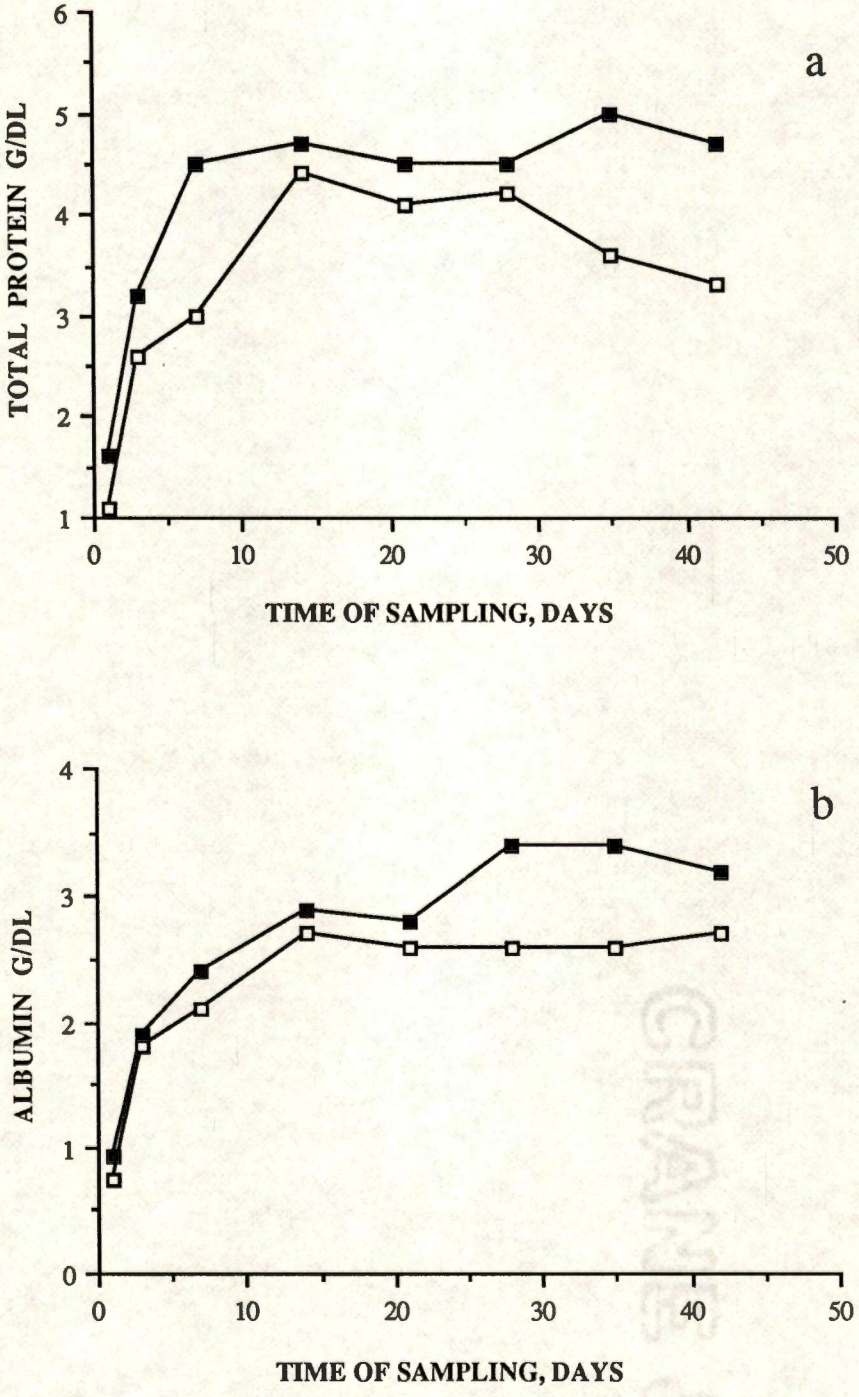

Figure 3. Comparison of plasma changes in (A) total protein and (B) albumin concentrations in conventionally reared (●) and artificially reared (○) pigs from birth to six weeks post-partum.

CHAPTER V

DISCUSSION

The high mortality rate observed in conventionally reared pigs is misleading. One of two sows in the CR group lost up to 70% of her litter due to agalactia. This apparent reduction in milk production may have been the result of high environmental temperature evident at this time. The mortality rate within the litter of the remaining sow was 10% which is still well below that found in AR pigs (33%). The 33% mortality rate is also higher than that reported by Bille et al. (1978) and Lecce (1986) in CR pigs (15-20% and 20-30%, respectively). The results of the present study provide additional evidence that artificial rearing has higher death losses associated with its use as compared to conventional rearing. Higher mortality rates in artificially reared pigs as compared to conventionally reared pigs have been reported by other researchers as well (Lecce, 1975; Dividich, 1981).

Body weights of CR pigs throughout the six week period were similar to that found by Close and Stainer (1984). The greatest increases in body weight for CR pigs occurred between days 21 and 42 which corresponds to the time period during which creep feed was provided. The lower initial weights of AR pigs could be a result of premature birth weights due to early delivery by cesarean section and/or inopportunity to nurse the sow prior to being weighed. Lower body weights of AR pigs during the first 28 days could be attributed to the environment imposed on the pig by artificial rearing. However, AR pigs seemed to have

experienced compensatory gains (during days 28-42) as a result of being weaned off the milk replacer diet and onto creep feed. This suggests that a liquid milk replacer diet may not be adequate to sustain normal average daily gains. Possibly the necessary nutrients are not being supplied by the milk replacer in adequate amounts or that the pig itself is not able to consume enough of the milk replacer to meet its nutritional requirements. Seve (1980) determined that variations in dietary nutrient intake caused changes in fat deposition of early weaned pigs. Decreased fat deposition would ultimately result in lower body weights. If AR pigs were not consuming enough dietary fat, their fat deposition would then be decreased, resulting in lower body weights as compared to CR pigs. Close and Stainer (1984) also reported that a diet low in fat, and thus lower in energy, resulted in decreased body weight.

Hematocrits in the AR pigs at birth were similar to those found by Miller et al. (1961). The lower values observed by day 3 and 7 in both CR and AR pigs has been well documented (Miller et al., 1961a; Ramirez, 1963; Parker, 1980b). The 30% reduction in hematocrits in AR pigs by 3 days of life is similar to the 25% reduction found by Miller et al. (1961a). This decrease has been attributed to a corresponding increase in plasma volume (20-30%) following colostrum intake by the pig (Parker, 1980a). The large increase in hematocrits from day 7 to 14 in CR and AR pigs were probably the result of erythropoiesis which is sustained by the administration of iron dextran on or before day 7. The dramatic increase in hematocrit after the administration of supplemental iron has been documented previously (Miller et al., 1961a).

The high concentrations of plasma cortisol at birth for both groups of pigs is comparable to that found by other researchers (Herbein et al., 1977; McCauley and Hartmann, 1984). The peak in the concentration of cortisol that is observed at birth is due to its role in the initiation of parturition (First and Bosc, 1979). The periparturient peak in cortisol has also been associated with the corresponding induction of hepatic gluconeogenic enzymes (Herbein et al., 1977). As a pig matures, gluconeogenic pathways provide a source of glucose (Herbein et al., 1977) which is the primary energy source of the neonatal pig (Mount, 1959). The rapid postnatal decline in cortisol concentrations by day 3 is in agreement with that reported by Herbein et al. (1977) and McCauley and Hartmann (1984). The rapid drop in cortisol concentrations from birth to day 3 may suggest possible changes in the metabolism and/or synthesis of cortisol in the neonatal pig (McCauley and Hartmann, 1984). Similar to our results, McCauley and Hartmann (1984) found total cortisol concentrations to remain unchanged from day 3 to 42. Worrsaae and Schmidt (1980) reported a slight increase in cortisol concentrations at three weeks of age subsequent to pigs being weaned.

The distribution of cortisol among its free and bound forms has not been documented previously in the neonatal pig. The high concentrations of UB-C found at birth, and up to 14 and 21 days in CR and AR pigs, respectively, suggests that there may be a need by the animal for biologically active cortisol for the performance of certain physiological processes during this time. The reduction in levels of UB-C by day 21 in CR pigs implies that these animals have developed the necessary

systems to maintain homeostasis. The 7 day delay in the decline of UB-C values of AR pigs may suggest that these pigs are under more stress and thus have a greater requirement for biologically active cortisol. These results may help explain the reduced performance and increased mortality rate seen in pigs weaned earlier than 3 weeks of age as observed by Lecce (1975). The high UB-C levels observed over the first 2 to 3 weeks in both groups of pigs may be accounted for by the high levels of ALB-C. Cortisol bound to albumin can readily dissociate to form a pool of unbound cortisol when needed by the animal (Englebienne, 1984). The decline in CBG-C levels from birth to day 21 in both groups of pigs may explain the increased UB-C levels seen at this time. This decline of CBG-C after birth is characteristic of that found in the lamb (Ballard, 1983) and in the rat (Agostino, 1980).

The reduction of UB-C levels observed on day 21 and 28 for CR and AR pigs, respectively, is related conversely to increasing levels of CBG-C ($r = .-64$; $P < .01$). The increase in CBG-C values during this time could possible be due to increased synthesis of CBG corresponding to maturation of the liver. In the rat, a surge of CBG was found to occur in the second and third postnatal weeks (Agostino and Henning, 1980). If CBG-C is directly related to increased CBG levels, the decrease in UB-C on day 21 and 28 may be the result of more cortisol becoming bound to CBG. The extremely high CBG-C, and consequently low UB-C values found on day 28, 35 and 42 in AR pig are unexplainable at this time.

The high concentrations of T4 in both groups of pigs observed at birth and, the subsequent decrease occurring within 3 days postpartum,

is in accordance with other research (Parker, 1980a; Slebodzinski et al., 1981). This initial postnatal peak in T4 concentrations that occurs within the first 24 hours after birth has been attributed to fetal reserves of T4 being released at birth or shortly thereafter (Slebodzinski et al., 1981). They also suggested that increases in T4 and T3 at birth are due to the stress of parturition.

Increased concentrations of thyroid hormones at birth are utilized for thermogenesis and as mediators of the basal metabolic rate in the neonatal pig (Slebodzinski et al., 1981). Unlike other species which are dependent upon noradrenaline for thermogenesis, pigs are dependent upon the thyroid hormones. A neonatal pig's peripheral tissue is unresponsive to noradrenaline because neonatal pigs are born with little or no brown adipose tissue (Parker, 1980b). Even though neonatal pigs are primarily unresponsive to noradrenaline, the calorigenic action of adrenaline is potentiated by T4 in the neonatal pig (Kaciuba and Uscilko, 1971). Slebodzinski (1965) also stated that a pig is born with a mature thyroid gland which may explain why the thyroid hormones are mediators of thermeogenesis in the neonatal pig.

The gradual fall of T4 concentration, to their lowest levels on day 3 and 7 in the CR and AR pigs, respectively, supports the findings of Parker et al. (1980) and Slebodzinski et al. (1981). This postnatal decline in T4 concentrations has been attributed to hemodilution (Slebodzinski et al., 1981).

The concentrations of T3 found in this study are lower than that reported elsewhere. The low concentration of T3 in the present study

may be accounted for by either the samples being unusually low in T3 or, as a result of a procedural problem in the assay itself. The manufacturer of the T3 kit (Sigma Chemical Co.) suggests that the plasma level of T3 may be reduced in the presence of silicone. The syringes used for blood collection were not marked as being silicone coated but, a silicone-like substance present on the rubber stopper could have interfered with the measure of T3.

There are alternative explanations for the low T3 concentrations other than mishandled blood plasma. For instance, Nowak and Slebodzinski (1986) reported a 43% decrease in the secretion of T3 when pigs were deprived of food and were fed only milk and water. This in some way may help explain the low T3 concentrations in the AR pigs, which were fed a milk replacer and water diet up to 28 days of age.

Parker et al. (1980a) also suggested how decreases in circulating T3 concentrations might possibly occur. Since T3 acts intercellularly in most tissues, low concentrations of T3 in the peripheral circulation could be the result of movement of T3 from the blood stream into the cells of thermogenic tissue. Increased blood flow of thermogenic tissue (i.e., kidneys, skeletal muscle and liver) facilitates the absorption of T3 instead of allowing it to remain in the general circulation, thereby causing decreased plasma levels of T3 (Parker et al., 1980b). In contrast, other researchers have found that T3 values parallel T4 values (Slebodzinski et al., 1981).

The increasing concentrations of total protein immediately after birth are the result of massive amounts of proteins being absorbed by

the gut from the intake of colostrum by the neonatal pig in the first 24 to 36 hours (Miller et al., 1961a). The proteins are primarily in the form of γ and β globulins (Ramirez, 1963). The uptake of these globulins in an intact form becomes diminished after 24-36 hours (Ingvarsson et al., 1978).

The increased synthesis of serum protein by the neonatal pig is responsible for the continued increase in total protein concentration after uptake by the gut has decreased (Ingvarsson et al., 1978). The stability of total protein concentration by day 14 may suggest that synthesis has reached its basal level of production. The lower concentrations of total protein in AR pigs during the first week of life could be the result of a decreased state of physiological development. A highly significant correlation ($r = .40$; $P < .01$) was found between body weight and the level of total protein in the plasma. This might be interpreted as indicating suppressed development, since the synthesis of total protein begins a few days after birth (Owen et al., 1961).

The synthesis and the increased concentration of plasma albumin is closely related to neonatal development (Ingvarsson et al., 1978). The low concentrations of albumin at birth are attributed to minimal synthesis by the liver. The sharp increase in albumin concentration in the neonate is the result of colostral albumin uptake within the gut during the first 24 to 36 hours (Miller et al., 1961b). Increased hepatic synthesis of albumin in the pig after 2 days of age is the result of increasing concentrations of liver albumin mRNA (Ingvarsson et al., 1978). The percentage of total protein consisting of albumin in both

the CR and AR pigs throughout this study were considerably higher than that reported by Owen et al. (1961). These higher values are possibly from the lower total protein concentrations reported in this study as a result of a different method of determining total protein than that used by Owen et al. (1961). The increasing percentage that occurred in this study from birth to six weeks of age has been suggested to be a result of an increase in the synthesis of albumin during this time (Owen et al., 1961). The lower overall levels of total protein, albumin, and body weight in the AR pigs may further indicate that normal growth and developmental processes were suppressed by the imposed artificial rearing environment.

The results of this experiment suggest that the neonatal pig undergoes explicit endocrine and protein changes from birth to six weeks of age, and that method of rearing has an influence on these changes. Following the first 4 weeks of life these dramatic physiological changes become stable and resemble levels similar to those observed by others in the adult pig (McCauley and Hartmann, 1984; Nowak and Slebodzinski, 1986). This may indicate that the vulnerability of the neonatal pig is greatest during the first 4 weeks of its life and that mechanisms controlling homeostasis may not be fully established until approximately day 28. Further, it appears that the added stress of artificial rearing suppresses the normal physiological development of the neonatal pig, as indicated by lower body weights, total protein and albumin concentrations as well as a seven day delay in the decrease of UB-C values seen in this study.

The results of this study have provided new information on the percent distribution of cortisol in the neonatal pig. Because of its undisputed role in controlling the level of free cortisol present in the blood stream, further research in the quantification of CBG during the postpartum period is needed. Likewise, future studies should be conducted in order to determine the controlling factor of CBG synthesis during the neonatal period, first *in vitro* and then ultimately *in vivo*.

CHAPTER VI

SUMMARY

Specific endocrine and protein changes were evaluated in conventionally reared (CR) pigs from birth to six weeks of age. The study also examined the influence that method of rearing (conventional versus artificial) might have on these physiological changes. Artificially reared (AR) pigs were derived by cesarean section on day 114 gestation and allowed to nurse for 48 hours following birth. Pigs were then fed a commercial milk replacer diet. CR pigs were born naturally and maintained with the sow until six weeks of age. Blood samples were collected via the anterior vena cava on days 1, 3, 7, 14, 21, 28, 35, and 42. At each sampling hematocrits and body weights were recorded. The specific parameters measured in both groups of pigs included: total cortisol, percent unbound cortisol (UB-C), percent cortisol bound to corticosteroid binding globulin (CBG-C), percent cortisol bound to albumin (ALB-C), thyroxine (T4), triiodothyronine (T3), total protein (TP), and albumin.

Pig mortality rates in the CR and AR litters were 10 and 33%, respectively. The results suggest that artificial rearing has higher death losses associated with it as compared to conventional rearing.

Overall mean body weights of the CR pigs were greater ($P < .01$) than the AR pigs. The lower weights in the AR pigs may indicate that the milk replacer diet may be inadequate to sustain normal average daily gains. Hematocrit levels in both groups of pigs fell to their lowest

($P < .01$) values on day 3 and 7. Values then increased ($P < .01$) by day 14 to values similar to that observed on day 1. The low hematocrit values seen on day 3 and 7 are attributed to an increase in plasma volume as a result of colostrum intake by the pig.

Concentrations of total cortisol were higher at birth ($P < .01$) than that measured on day 7 to 42 in both groups of pigs (55.6 vs 19.2 ng/ml). The peak in the concentration of cortisol observed at birth is due to its role in the initiation of parturition. Decreasing concentrations may suggest changes in the metabolism and/or synthesis of cortisol in the neonatal pig. Similarly, UB-C was highest at birth (29.9%) and decreased ($P < .01$) to its lowest value by day 42 (11.9%). A decline in UB-C for CR pigs began by day 21 in contrast to day 28 for AR pigs. Elevated levels of UB-C suggests an increased need for biologically active cortisol for the performance of certain physiological processes during this time. Levels of CBG-C were lowest ($P < .01$) in both groups of pigs on days 7-21 with an average mean of 33%. CBG-C for CR and AR pigs peaked on day 42 (54.1%) and day 35 (76.4%), respectively. The increase in CBG-C on day 21 through 42 in both groups of pigs may represent an increase in the production of CBG. ALB-C was negatively correlated with CBG-G ($r = .-87$).

For both the CR and AR pigs T4 concentrations were highest ($P < .01$) at birth (7.8 µg/dl) and declined ($P < .01$) to basal levels by day 7 (4.6 µg/dl). CR pigs overall mean T4 concentration was higher ($P < .01$) than that of the AR pigs. The high concentrations of T4 at birth are associated with the stress at parturition, and as a result of

fetal reserves being released at birth. The elevated T4 levels at birth are utilized for thermogenesis and as mediators of the basal metabolic rate. Even though there were significant differences between AR and CR pigs in T3 values, the exceptionally low concentrations found here, as compared to that of other researchers, prohibit any further inferences from being made of these results.

Overall mean total protein and albumin concentrations for AR pigs (3.2 and 2.2 g/dl, respectively) were lower than that of the CR pigs (3.9 and 2.4 g/dl, respectively). Total plasma protein and albumin concentrations in CR and AR pigs were lowest ($P < .01$) at birth and increased to their highest values by day 14. The postnatal increase in both total protein and albumin concentration is apparently the result of massive amounts of proteins being absorbed by the gut from the intake of colostrum by the neonatal pig in the first 24 to 36 hours.

The results of this study suggest that the neonatal pig undergoes explicit endocrine and protein changes from birth to six weeks of age. This study also provides evidence that artificial rearing does have an effect on the hormonal and protein changes that occur from birth to six weeks of age in the neonatal pig.

LITERATURE CITED

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Adinolfi, A., M. Adinolfi and M. Lessof. 1975. Alpha-fetoprotein during development and in disease. J. Med. Genet. 12:138.

Agostino, J. D. and S. J. Henning. 1980. Hormonal control of postnatal development of corticosteroid-binding globulin. Amer. J. Physiol. 240:402.

Agostino, J. D. and S. J. Henning. 1982. Postnatal development of corticosteroid-binding globulin. Effects of thyroxine. Endocrinology 111:1476.

Aumaitre, A. and B. Seve. 1978. Nutritional importance of colostrum in the piglet. Ann. Rech. Vet. 9:181.

Ballard, P. L. 1979. Delivery and transport of glucocorticoids to target cells. In: J. D. Baxter and G. G. Rousseau (Eds.) Glucocorticoid Hormone Action. pp. 25. Springer-Verlag, Berlin.

Ballard, P. L., J. A. Kitterman, R. D. Platzker, S. L. Kaplan and M. M. Grumbach. 1982. Ontogeny and regulation of corticosteroid binding globulin capacity in plasma of fetal and newborn lambs. Endocrinology 110:359.

Ballard, P. L., A. H. Klein and D. A. Fisher. 1983. Thyroid hormones and plasma corticosteroid binding globulin capacity in the fetal and newborn lambs. Endocrinology 113:1197.

Barnett, J. L., G. M. Cronin and C. G. Winfield. 1981a. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. General and Comp. Endocrinology 44:219.

Barnett, J. L., C. G. Winfield, G. M. Cronin and A. W. Makin. 1981b. Effects of photoperiod and feeding on plasma corticosteroid concentrations and maximum corticosteroid-binding capacity in pigs. Aust. J. Bio. Sci. 34:577.

Bayley, H. S., G. L. Crozier and J. L. Atkinson. 1980. Energy metabolism, substrate reserves and glucose utilization in the piglet. Anim. Prod. 26:439.

Bellis, D. B. 1957. Some problems in the nutrition of the baby pig. Proc. Nutr. Soc. 16:98.

Bianchi, R., G. Mariani, N. Molea, F. Vitek, F. Cazyuloa, A. Carpi, N. Mazzuia and M. G. Toni. 1983. Peripheral metabolism of thyroid hormones in man. J. Clin. Endocrinol. Metab. 56:152.

Bille, N., N. C. Neilsen, J. L. Larsen and J. Svendsen. 1974. Preweaning mortality in pigs. Nord. Vet. Med. 26:294.

Braude, R. and M. J. Newport. 1977. A note on a comparison of two systems for rearing pigs weaned at 2 days of age, involving either a liquid or a pelleted diet. Anim. Prod. 24:271.

Brown, D. E., R. R. Hacker and G. J. King. 1976. Growth and ACTH responses to cold stress of young pigs fed ad libitum. Can. J. Anim. Sci. 56:365.

Carlsson, R., B. Ingvarsson and B. Karlsson. 1977. Isolation and characterization of albumin from porcine serum, colostrum and urine. Int. J. Biochem. 8:285.

Cassuto, Y. and Y. Amit. 1969. Thyroxine and norepinephrine effects on metabolic rates of heat acclimated hamsters. Endocrinology 82:17.

Chopra, I. J., J. Sack and D. A. Fisher. 1975. 3,3',5-triiodothronine (rT3) and 3,3',5-triiodothyronine (T3) in fetal and adult sheep: studies of metabolic clearence rates, production rates, serum binding and thyroidal content relative to thyroxine. Endocrinology 97:1080.

Close, W. H. and M. W. Stainer. 1984. Effect of plane of nutrition and environmental temperature on the growth and development of the early weaned piglet. Anim. Prod. 38:211.

Curtis, S. 1970. Environmental-thermoregulatory interaction and neonatal pig survival. J. Anim. Sci. 31:576.

DeGroot, L. J., M. Robertson and P. A. Rue. 1977. Triiodothyronine receptors during maturation. Endocrinology 100:1511.

Dividich, J. L. and J. Noblet. 1981. Colostrum intake and thermo-regulation in the neonatal pig in relation to environmental temperature. Biol. Neonate. 40:167.

Dividich, J. L. 1981. Effects of environmental temperature on the growth rates of early-weaned piglets. Livestock Prod. Sci. 8:75.

Englebienne, P. 1984. The serum steroid transport proteins: biochemistry and clinical significance. Molec. Aspects Med. 7:313.

Fahmy, M. H. and C. Bernard. 1971. Causes of mortality in yorkshire pigs from birth to 20 weeks of age. Can. J. Anim. Sci. 51:351.

Fisher, D. A., J. H. Dusault, J. Sack and I. J. Chopra. 1977. Ontogenesis of hypothalmic-pituitary-thyroid function and metabolism in man, sheep and rat. Recent Prog. Horm. Res. 33:59.

Fisrt, N. L. and M. J. Bosc. 1979. Proposed mechanisms controlling parturition and the induction of parturition in swine. J. Anim. Sci. 48:1407.

Foster, L. B. and R. T. Dunn. 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clin. Chem. 20:365.

Frandson, R. D. 1981. Anatomy and physiology of farm animals. (3rd Ed.). Lea and Febiger, Philadelphia, PA.

Freeman, B. M. 1975. Physiological basis of stress. Proc. Prog. Soc. Med. 68:427.

Fujieda, K., A. K. Goff, M. Pugeat and C. A. Strott. 1982. Regulation of the pituitary-adrenal axis and corticosteroid binding globulin-cortisol interaction in the guinea pig. Endocrinology 111:1944.

Fulkerson, W. J. and B. Y. Tang. 1979. Ultradian and circadian rhythms in the plasma concentration of cortisol in sheep. J. Endocrinol. 81:135.

Hafez, E. S. E. 1980. Reporduction in farm animals. (4th Ed.). Lea and Febiger, Philadelphia, PA.

Hammond, G. L., J. A. Nisker, L. A. Jones and P. K. Siiteri. 1980. Estimation of the percentage of free steroid in undiluted serum by centrifugal ultrafiltration-dialysis. J. Biol. Chem. 255:5023.

Herbein, J. H., R. J. Martin, L. C. Griel and J. F. Kavanaugh. 1977. Serum hormones in the perinatal pig and the effect of exogenous insulin on blood sugars. Growth 41:277.

Himmelberg, L. V., E. R. Peo, A. J. Lewis and J. D. Crenshaw. 1985. Weaning weight response of pigs to simple and complex diets. J. Anim. Sci. 61:18.

HSU, B. R. S. and Kuhn. 1988. The role of the adrenal in generating the diurnal variation in circulating levels of corticosteroid-binding globulin in the rat. Endocrinology 122:421.

Ingvarsson, B. I., R. N. K. Carlsson and B. W. Karlsson. 1978. Synthesis of Alpha-fetoprotein, albumin and total serum protein in neonatal pigs. Biol. Neonate. 34:259.

Kaciuba-Uscilko, H. 1972. Hormonal regulation of thermogenesis in the newborn pig. Biol. Neonate. 21:245.

Karlsson, B. 1970. Fetoprotein and albumin levels in the blood serum of developing neonatal pigs. Comp. Biochem. Physiol. 34:535.

Kattesh, H. G., E. T. Kornegay, F. G. Gwazdauskas, H. R. Thomas and D. R. Notter. 1980. Glucocorticoid concentrations, corticosteroid binding protein characteristics and reproduction performance of sows and gilts subjected to applied stress during mid-gestation. J. Anim. Sci. 50:897.

Kendall, E. C. 1971. Cortisone. pp. 136. Scribners, New York.

Kernkamp, H. C. L. 1957. A parenteral hematinic for the control of iron-deficiency anemia in baby pigs. N. Am. Vet. 38:6.

Kirstein, C. G., D. A. Clare and J. G. Lecce. 1985. Development of resistance of enterocytes to rotavirus in neonatal agammaglobulinemic piglets. J. Virol. 55:567.

Knight, J. W., H. G. Kattesh, F. C. Gwazdauskas, H. R. Thomas and E. T. Kornegay. 1982. Peripheral testosterone in boars after administration of hCG, ACTH and testosterone at three ages. Theriogenology 17:383.

Kohler, E. M. 1972. Pathogenesis of neonatal enteric colibacillosis of pigs. J. Amer. Vet. Med. Assoc. 160:574.

Lecce, J. G. 1975. Rearing piglets artificially in a farm environment a practice unfulfilled. J. Anim. Sci. 41:659.

Lecce, J. G. 1986. Diarrhea: The nemesis of the artificially reared, early weaned piglet and a strategy for defense. J. Anim. Sci. 63:1307.

Manners, M. J. and M. R. McCrea. 1963. Changes in the chemical composition of sow reared piglets during the first month of life. Brit. J. Nutr. 17:495.

Mason, R. and J. S. Wilkson. 1973. The thyroid gland--a review. Aust. Vet. J. 49:44.

Mason, J. W. 1975. Emotion as reflected in patterns of endocrine integration. In: L. Leu (Ed.) Emotions--Their Parameters and Measurements. Raven Press, New York.

McCance, R. A. and E. M. Widdowson. 1959. The effect of colostrum on the consumption and volume of the plasma of newborn piglets. J. Physiol. 145:547.

McCauley, I. and D. E. Hartmann. 1984. Changes in the pig leucocytes, B lymphocytes and plasma cortisol from birth to three weeks after weaning. Res. Vet. Sci. 37:234.

McConnell, J. C., J. C. Eargle and R. C. Waldorf. 1987. Effects of weaning weight, co-mingling, group size and room temperature on pig performance. J. Anim. Sci. 65:1201.

McDonald, L. E. 1969. Veterinary Endocrinology and Reproduction. Lea and Febiger, Philadelphia, PA.

Miller, E. R., D. E. Ullrey, R. W. Luecke and J. A. Hoefer. 1960. The use of injectable iron preparations in preventing baby pig anemia. 5th. Ann. Mich. Swine Day report, Mimeo. A-H-53.

Miller, E. R., D. E. Ullrey, I. Ackerman, D. A. Schimidt, R. W. Luecke and J. A. Hoefer. 1961a. Swine hematology from birth to maturity. II. Erythrocyte population, size and hemoglobulin concentration. J. Anim. Sci. 20:890.

Miller, E. R., D. E. Ullrey, I. Ackerman, D. A. Schimidt, J. A. Hoefer and R. W. Luecke. 1961b. Swine hematology from birth to maturity. I. Serum proteins. J. Anim. Sci. 20:31.

Moberg, G. P. 1987. Influence of the adrenal axis upon the gonads. pp. 456. In: Oxford Reviews of Reproductive Biology. J. R. Clarke (Ed.) Vol. 9. Clarendon Press, Oxford.

Moon, H. W., L. J. Kemeny, G. L. Lambert, S. L. Stark and G. D. Booth. 1975. Age-dependent resistance to transmissible gastroenteritis of swine. III. Effects of epithelial cell kinetics on coronavirus production and on atrophy of intestinal villi. Vet. Path. 12:434.

Mount, L. E. 1959. The influence of huddling and body size on the metabolic rate of the young pig. J. Physiol. 147:13.

Mount, L. E. 1969. The respiratory quotient in the newborn pig. Br. J. Nutr. 23:407.

Munck, A., P. M. Guyre and N. J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmcological actions. Endocrine Reviews 5:25.

Noblet, J. and J. L. Dividich. 1981. Energy metabolism of the newborn pig during the first 24 hours of life. Biol. Neonate. 40:175.

Nowak, G. 1983. Free thyroid hormone levels during the postnatal period in the pig. Biol. Neonate. 43:164.

Nowak, G. and A. B. Slebodzinski. 1986. Extrathyroidal conversion of thyroxine to 3,5,3'-triiodothyronine (T3) and 3,3',5-triiodothyronine (rT3) and its contribution to total triiodothyronines production rates in fed and food restricted piglets. J. Vet. Med. Assoc. 33:337.

Owen, B. D., J. M. Bell, C. M. Williams and R. G. Oakes. 1961. Effects of porcine immune globulin administration on the survival and serum protein consumption of colostrum-deprived pigs reared in a nonisolated environment. Can. J. Anim. Sci. 41:236.

Parker, R. O., P. E. V. Williams, F. X. Aherne and B. A. Younge. 1980a. Postnatal changes in concentrations of serum and urinary thyroxine and 3,3',5-triiodothyronine in the pig. J. Anim. Sci. 51:137.

Parker, R. O., P. E. V. Williams, F. X. Aherne and B. A. Younge. 1980b. Serum concentration changes in protein, glucose, urea, thyroxine and triiodothyronine and thermostability of neonatal pigs farrowed at 25 and 10°C. Can. J. Anim. Sci. 60:503.

Pomeroy, R. W. 1960. Infertility and neonatal mortality in the sow. III. Neonatal mortality and fetal development. J. Agric. Sci. 54:31.

Ramirez, C. G., E. R. Miller, D. E. Ullrey and J. A. Hoefer. 1963. Swine hematology from birth to maturity. II. Blood volume of the nursing pig. J. Anim. Sci. 22:1068.

Ruoslahti, E., H. Pihko and M. Seppala. 1974. Alpha-fetoprotein. Immunochemical purification and chemical properties. Expression in normal state and in malignant and nonmalignant liver disease. Transplantation Rev. 20:38.

Seal, U. S. and R. P. Doe. 1963. Corticosteroid binding globulin: species distribution and small scale purification. Endocrinology 73:371.

Selye, H. 1956. Stress of Live. McGraw-Hill Book Co., NY.

Seve, B. 1980. Age at weaning and development of body components of piglets from 3 to 25 kg. live weight. 31 st. Mtg. Eur. Assoc. Anim. Prod. Munich, 5/6:27.

Singh, S. P. and Synyder. 1978. Effect of thyrotoxicosis on gluconeogenesis from alanine in the perfused rat liver. Endocrinology 102:182.

Siiteri, P. K., J. T. Murai, G. L. Hammond, J. A. Nieker, W. J. Raymoure and R. W. Kuhn. 1982. The serum transport of steroid hormones. Rec. Prog. Horm. Res. 38:457.

Slebodzinski, A. B. 1965. Interaction between thyroid hormone and thyroxine binding proteins in the early neonatal period. J. Endocrinol. 32:45.

Slebodzinski, A. B. 1972. Acute depletion of the hormonal iodine stores from the thyroidal gland after birth in lambs. J. Endoc. 53:195.

Slebodzinski, A. B., G. Nowak and H. Zamyslowska. 1980. Serum thyroxine, triiodothyronine and reverse triidothyronine in early postnatal period in the pig. Endocrinologia Experimentalis 14:73.

Slebodzinski, A. B., G. Nowak and H. Zamyslowska. 1981. Sequential observation of changes in thyroxine, triiodothyronine and reverse triiodothyronine during the postnatal adaption of the pig. Biol. Neonate 39:191.

Steeno, O. and P. DeMoor. 1966. The corticosteroid binding capacity of plasma transcortin in mammals and aves. Acta. Zool. Pathol. Antverp. 38:3.

Stone, R. T. 1984. Relationship of alpha-fetoprotein and albumin in fetuses and neonates from genetically lean and obese swine. Biol. Neonate 46:122.

Swanson, H. E. 1957. The effect on the potentiation of adrenaline by thyroxine in the albino rat. Endocrinology 60:205.

Yousef, M. K. 1985. Stree Physiology of Livestock. Vol. I and II. CRC Press, Inc., Florida.

Westphal, U. 1983. Corticosteroid binding globuilin. Molec. Cell. Biochem. 55:145.

Woodward, C. C. and R. J. Strange. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Trans. Am. Fish. Soc. 116:574.

Worsaae, H. and M. Schmidt. 1980. Plasma cortisol and behavior in early weaned piglets. Acta. Vet. Scand. 21:640.

APPENDIXES

APPENDIX A

PROCEDURE FOR CORTISOL RADIOIMMUNOASSAY

Procedure for Cortisol Radioimmunoassay

I. Antibody Preparation--Cambridge Medical Diagnostics (Rabbit-anticortisol Cat No. 299-206R Lot No. M8521)

A. Antibody (Ab) is stored as neat sera at -80°C. To prepare a 1:100 dilution of Ab from neat sear, take 0.05 µl of neat sera (prepacked by distributor) and add 5.0 ml of PBS-gel. Aliquot 300 µl, 100 µl, and 50 µl into tubes and store at -80°C.

B. On day of assay a 1/3000 dilution of antibody is made with PBS-gel.

Calculations:

$$\frac{\text{Volume of 1:100 Ab needed (x)}}{\text{Dilution of Ab 1:100 (100)}} = \frac{\text{total volume (PBS and Ab) needed for assay}}{\text{dilution of antibody in assay (3000)}}$$

II. Reagents

A. Solution A--(0.2 M)

Dissolve 27.8 g monobasic sodium phosphate in 1000 ml of distilled water.

B. Solution B--(0.2 M)

Dissolve 28.4 g anhydrous dibasic sodium phosphate in 1000 ml of distilled water

C. PBS

Add 95 ml of solution A to 405 ml of solution B. Then dissolve 9 g of sodium chloride and 1 g of sodium azide. Adjust

pH to 7.4 with appropriate solution. Add distilled water to make a final concentration of 1 L. Store at 4°C.

D. PBS with gel (PBS-g)

1. To 500 ml of PBS, add 500 mg of Knox gelatin
2. Heat to dissolve Knox gelatin
3. Store at 4°C

E. Dextran Coated Charcoal

1. Dissolve 0.0625 g of dextran T70 (Pharmacia Fine Chemicals) in 100 ml of PBX, then add 0.625 g of activated charcoal powder (MCB Manufacturing Chemist, Inc.)
2. Mix well, and refrigerate at 4°C

III. Preparation for standard curve

Cortisol standard prepared from Hydrocortisone (Sigma Chemicals No. 4001)

A. Solution A: Dissolve 5 mg of hydrocortisone in 5 ml of freshly distilled ethanol, making a solution of 1 mg/ml.

B. Solution B: 100 µl of solution A is added to 9.9 ml of distilled ethanol, making a solution containing 10 µg/ml.

C. Solution C: Add 100 µl of solution C to 9.9 ml of distilled ethanol, making a concentration of 100 ng/ml.

D. Solution D: (working solution)

Take 1 ml of solution C and add to 9 ml of distilled ethanol. Final concentration is 10 ng/ml.

E. Standard curve protocol

Tube	Contents of Tube
TCT	Total Counts Tube
NSB	Nonspecific Binding Tube
0.0	Zero Tube
0.01 ng/ml	1.0 µl Solution D
0.025 ng/ml	2.5 µl Solution D
0.05 ng/ml	5.0 µl Solution D
0.10 ng/ml	10.0 µl Solution D
0.25 ng/ml	25.0 µl Solution D
0.50 ng/ml	50.0 µl Solution D
1.0 ng/ml	100.0 µl Solution D

IV. ASSAY PROCEDURE

A. Preparation of tubes and standard curve

1. For a 50 tube assay, tubes are labeled

   1-10 TCT, NSB Standard curve tubes

   11-40 Samples in duplicate

   41-50 TCT, NSB, Standard curve tubes

2. Dry down standard curve in a vacuum oven to remove ethanol

3. After drying, add 500 µl of PBS-gel and an additional 100 µl to the NSB tubes

4. Vortex

B. Preparation of plasma samples and controls

1. Dilute 10 µl of plasma in 2.5 ml of PBS-g. Vortex.

2. Take two 500 µl aliquots of above dilution and put into appropriate duplicate tubes.
3. Heat all tubes (including the standard curve tubes) in a water bath at 70°C for one hour.
4. Allow tubes to return to room temperature. Add 100 µl of [^{3}H] cortisol (5,000 cpm/100 µl) to all tubes. Add 100 µl of 1/3000 anticortisol to all tubes, except NSB tubes, vortex and incubate for 16 hours at 4°C.
5. Transfer tubes to an ice water bath. Add 200 µl of dextran coated charcoal (DCC) to all tubes except TCT. To TCT tubes add 200 µl of PBS. Vortex.
6. Incubate for 20 minutes (while still remaining in ice water bath.
7. Remove DCC by centrifugation at 1600 x g for 15 minutes.
8. Pour off supernatant into vials, add 4 ml of Scintiverse II, vortex, and count on liquid beta scintillation counter for 1 minute.

APPENDIX B

PROCEDURE FOR DETERMINATION OF PERCENT DISTRIBUTION OF BOUND AND UNBOUND CORTISOL IN PLASMA

Procedure for Determination of Percent Distribution of Bound and Unbound Cortisol in Plasma

I. HANDLING OF PLASMA

A. Store at -20°C, and minimize freezing and thawing.

B. Thawed plasma should be centrifuged before each assay and any precipitate discarded.

II. MEMBRANE PREPARATION

A. Dialysis tubing was purchased from Fisher Scientific spectra/por, with a molecular weight cut off at 12,000-14,000 daltons. Size: 25 mm x 15.9 mm dia. 50 ft./roll.

B. Boil sections of dialysis tubing in 95% ethanol in the hood for 30 to 45 minutes. Tubing is rinsed extensively with distilled water to remove the ethanol.

C. The membrane is boiled twice for 15-20 minutes in a 2 to 3 L solution containing 5-10 mg of disodium EDTA and 5-10 g of sodium carbonate with a distilled water rinse between boilings.

D. The membranes are extensively washed with distilled water to remove the sodium carbonate and EDTA. Store in a .02% sodium azide (0.1 g/500 ml) solution at 4°C for up to 1 month.

E. Care should be taken to properly rinse membrane thoroughly to remove EDTA, because it can interfere with the assay.

F. A thin membrane should be used versus a thick one.

III. CAPSULE PREPARATION

A. Capsules are prepared in 4 cm sections from 12 x 75 mm borosilicate disposable test tubes. The cut end is fire polished.

B. The rubber used in making the rubber bands are made from Natural Latex tubing purchased from Pimeline Industries, Inc. (1/3" x 1/32").

C. Dialysis tubing is placed in distilled water before each assay to remove any EDTA residue and sodium azide.

D. Tubing is cut into small patches and fitted over the fire polished end of the capsule and held in place by the rubber band. An acrylic cone is used to expand the rubber band.

E. Capsules are stored in a 0.02% sodium azide solution for no more than 5 days prior to assay.

IV. CLEANING GLASS CAPSULES FOR REUSE

A. Remove membrane and rubber bands and rinse capsules, once in distilled water, and twice in methanol.

B. Heat capsules in a .01 N solution of sodium hydroxide for 2 hours.

C. Pour off 0.01 N sodium hydroxide and rinse twice with 0.01 N hydrochloric acid.

D. Rinse once with distilled water and twice with methanol.

E. Take an aliquot of the methanol and count in a Beta counter to see if there is any residual radioactivity. If radioactivity is present, then rinse with methanol again.

V. EXPERIMENTAL PROCEDURE

A. In preparation for an assay of 10 samples:

1. In 10 disposable test tubes add [1,2-^{3}H] cortisol (3 x 10^{5} dpm) dissolved in ethanol.
2. Evaporate ethanol, and add 5 µl of [^{14}C] glucose (12 x 10^{3} dpm) to each tube.
3. Add 500 µl of plasma to each tube. Vortex.
4. Tubes are then incubated at 37°C for 30 minutes. Vortex, and incubate for an additional 30 minutes at room temperature.

B. Preparation of scintillation vials for plasma equilibrated with radiolabeled cortisol and glucose.

1. Scintillation vials labeled 1-10 are prepared by numbering them 1a 1b, 2a 2b, 3a 3b, etc.
2. Add 3 paper filter discs to each vial (dia. 13 mm; Whatman No. 1).
3. Insert isodialysis capsules into the labeled scintillation vials.
4. Pipette duplicate aliquots (200 µl) of the plasma incubations into the isodialysis capsules.
5. The vials are then subjected to centrifugation at 1700 x g for 1 hour at 37-39°C.

6. A second set of scintillation vials are prepared by labeling them 1ap 1bp, 2ap 2bp, 3ap, etc. One filter disc is added to each scintillation vial.
7. After centrifugation, isodialysis capsules are carefully removed from the scintillation vials and 30 µl of the remaining ultrafiltrate are pipetted off and placed onto the filter disc of its respective scintillation vial. (Ultrafiltrate from isodialysis capsule 1a is placed onto filter disc of vial 1ap, and so on.)
8. Scintillation vials should then be in arranged in this order: 1a 1ap, 1b 1bp, 2a 2ap, 2b 2bp, etc.
9. Add 350 µl of distilled water into all scintillation vials; Vortex.
10. Add 3 ml of scintillation fluid (Scintiverse II), vortex and count on a beta scintillation counter, on the $^{14}C/^{3}H$ channel.

C. Determination of the percent distribution of bound steroid

1. Prior to the assay, heat an additional 600 µl of the same 10 samples at 60°C for one hour in a water bath.
2. Proceed with the same procedure for the determination of plasma free cortisol and distribution of bound cortisol.

APPENDIX C

PROCEDURE FOR QUANTIFICATION OF TOTAL PLASMA PROTEIN CONCENTRATION

Procedure for Quantification of Total Plasma Protein Concentration

I. Reagents

A. Coomassie Blue G-250 Protein Reagent (Peirce Chemical Company, Rockford, IL)

B. Standard BSA reagent (bovine albumin Fractain V, Peirce Chemical Company, Rockford, IL, 200 mg/dl of total protein/100 µl)

C. Physiological Saline 0.01% NaCl.

II. Standard curve preparation

200 mg/dl = 100 µl Std. BSA + 0 µl saline

100 mg/dl = 50 µl Std. BSA + 50 µl saline

50 mg/dl = 25 µl Std. BSA + 75 µl saline

25 mg/ml = 12.5 µl Std. BSA + 87.5 µl saline

0 mg/ml = 5 ml of protein reagent

III. Preparation of plasma dilutions

A. Dilutions used for appropriate sampling days

Days 1 and 3: 1:20 = 20 µl of plasma + 380 µl saline

Days 7, 14, and 21: 1:50 = 10 µl of plasma + 490 µl saline

Days 28, 35, and 42: 1:100 = 10 µl of plasma + 990 µl saline

IV. Assay Procedure

A. Prepare standard curve in duplicate in 13 x 100 mm test tubes.

B. Prepare proper dilution ratios for all samples in labeled 12 x 75 mm test tubes.

C. In a new set of 13 x 100 mm test tubes, add 5.0 ml of protin assay reagent. Mix protein reagent well before using.

D. Pipet 100 µl of unknown plasma dilution into the 13 x 100 mm test tubes already containing the 5 ml of protein assay reagent. Vortex.

E. Read absorbance against a blank of deionized water at 595 nm.

V. Preparation of spectrophotometer

A. Set the spectrophotometer wavelength to 628 nm, then "go to λ.

B. Blank machine with deionized water in both the front and back holder.

C. Push "Auto abs" to blank, should get a reading between -.005 - +.005.

D. Ready for samples, leave water in back holder.

VI. Calculations

(unknown plasma sample absorbance - blank absorbance) x dilution factor = Net absorbance of unknown sample

Protein concentrations in g/dl were then derived by plotting the net absorbance against the standard curve developed within the assay.

APPENDIX D

PROCEDURE FOR QUANTIFICATION OF ALBUMIN IN PLASMA

Procedure for Quantification of Albumin in Plasma

I. Sample preparation

A. Label tubes

1. reagent blank
2. standard
3. control
4. samples

II. Experimental Procedure

A. Pipet 1.0 ml of Albumin reagent [BCG] (Sigma, Diagnostics) into each tube.

B. Add 10 µl of deionized water, protein standard solution (5 g/dl), control and unknown samples to appropriately labeled tubes. Mix well by gentle inversion.

C. Read absorbance (A) at ambient temperature (18-26°C) at 628 nm.

III. Calculations:

$$\text{Albumin conc. (g/dl)} = \frac{\text{A sample - A blank}}{\text{A standard - A blank}} \times 5 \text{ g/dl}$$

VITA

Shawn Frey Charles was born in Lancaster, Pennsylvania, on July 7, 1964. He was graduated in 1982 from Penn Manor High in Millersville, Pennsylvania. In the fall of 1982, he matriculated at Delaware Valley College of Science and Agriculture, where he majored in animal husbandry and minored in business. While there, he was elected to membership in the Block and Bridle national animal science organization. He was awarded the Bachelor of Science degree in animal husbandry in 1986. In fall of 1986, he was admitted as a graduate student in the Department of Animal Science at the University of Tennessee, Knoxville. He was awarded the Master of Science degree in August of 1988.