SERUM INSULIN GLUCOCORTICOIDS AND ZINC AND ADRENAL CORTICAL FUNCTION IN STRESS SUCEPTIBLE AND STRESS RESISTANT PIGS

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ABSTRACT

SERUM INSULIN, GLUCOCORTICOIDS AND ZINC AND ADRENAL CORTICAL FUNCTION IN STRESS SUSCEPTIBLE AND STRESS RESISTANT PIGS

by D. Meiburg

Stress susceptible (SS) and stress resistant (SR) Hampshire and Hampshire x Yorkshire pigs previously determined to be either SS or SR were randomly and consecutively subjected to either 180 min. of a 37 C (heat stress) or 25 C (control) environment at 50 and 100 kg bodyweight in Experiment 1. Serum insulin was quantified by radioimmunoassay, serum zinc by atomic absorption spectrophotometry, and serum and in vitro corticoids were isolated on Sephadex LH-20 columns and quantified by protein binding assay. Serum insulin decreased from control levels at 50 and 100 kg during heat stress in both SS and SR pigs. During heat stress serum zinc was similar to control levels in SS and SR pigs at 50 kg but SS pigs at 100 kg had a significantly ($P<.05$) greater (86.5%) increase in serum zinc over that of controls at 1 1/2 hr. of heat stress than did SR pigs (13.6%). At 50 kg bodyweight SR pigs had a significantly ($P<.10$) greater increase in serum cortisol (213.8%) than SS pigs (38.1%) at the endpoint of heat stress compared to control conditions. The SR pigs also had a greater increase in cortisol after heat stress (200%) at 50 kg. However, at 100 kg SR pigs showed no change after heat stress and SS pigs had lower serum cortisol (-66%) compared to control conditions and all pigs had only small changes in serum cortisol during heat stress compared to the control environment.

In Experiment 2 the same SS and SR pigs used in Experiment 1 were injected I.M. with 80 IU of ACTH. Serum cortisol increased 50% over preinjection serum levels in SS pigs 1 hr. after injection while the

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SR pigs did not show the 50% increase in serum cortisol until 4 hr. after injection.

In Experiment 3, adrenal glands were removed immediately postexsanguination from the same pigs used in the previous experiments and small portions of the glands were either frozen immediately (A), incubated for 2 hr. at 37 C (B) and frozen or incubated for 2 hr. at 37 C with 1 IU ACTH (C) and frozen. Adrenal cortisol (97 and 92 ng/mg) and corticosterone (64 and 42 ng/mg) concentration (A) did not differ between SS and SR pigs, respectively. Likewise, cortisol (193 and 197 ng/mg) and corticosterone (27 and 32 ng/mg) synthesis *in vitro* (B) did not differ between SS and SR pigs, respectively. In addition, the cortisol (519 and 661 ng/mg) and corticosterone (46 and 50 ng/mg) synthetic response to ACTH (C) did not vary between SS and SR pigs, respectively.

No differences in morphology of the adrenal cortices between SS and SR pigs were apparent.

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by

D. Meiburg

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INTRODUCTION

The difference in generation interval and selection pressure have enabled producers to change the fat to lean ratio in pigs more than their contemporaries in the beef and sheep industries. Several problems, among them stress susceptibility, have accompanied the selection pressure for more muscle and less fat. An estimated three to five percent of all pigs die annually on the farm, in transit to market or during the handling associated with slaughter as a result of stress susceptibility. An additional 18% of all pigs exhibit some degree of pale, soft, exudative muscle (PSE) postmortem and the losses of pork products from excess shrinkage and lowered yields range from two to ten percent of total production. The overall adverse impact of stress susceptibility and PSE on the pork industry has been estimated to be 230 to 320 million dollars annually.

Recent investigations into the factors that lead to the development of PSE musculature have focused on the hormones of the adrenal cortex. Earlier reports suggested that stress susceptible (SS) pigs had lower adrenal function with a corresponding lower level of circulating adrenocorticosteroids than normal pigs. With lower circulating glucocorticoids, SS pigs often died when exposed to stressful conditions and usually had lower postmortem muscle quality.

Later studies have suggested that the SS pig has higher than normal circulating levels of ACTH, but the adrenal cortex has an impaired response to the ACTH and hence the SS pig is unable to cope with changes in his environment. Other investigators reported higher circulating

levels of plasma glucocorticoids in SS than in normal or stress resistant (SR) pigs. More recent results have implicated a normal release of cortisol in response to stress, but a several fold higher turnover rate of cortisol during stress.

Hence, the role of the adrenal cortex in stress adaptation and its overall effect on ultimate muscle quality is not completely resolved. The objectives of this study were as follows:

1. To monitor the changes in serum cortisol, insulin and zinc during heat stress of SS and SR pigs.
2. To measure the changes in serum cortisol, corticosterone, insulin and zinc in SS and SR pigs in response to exogenous ACTH.
3. To measure the cortisol and corticosterone content, synthesis and ACTH response of adrenal tissue from SS and SR pigs *in vitro*.

LITERATURE REVIEW

Porcine Stress Syndrome and Stress Susceptibility

The term stress is a general expression used to describe the physiological response of the body, which results during exposure of an animal to adverse conditions. Thus any disturbance of the homeostatic state may be described as stress and the specific factor causing the stress is referred to as the stressor. The physiological adjustments that occur during periods of stress are aided by many factors, including hormones.

Many and varied environmental factors may cause a stress reaction in an animal. It is well documented that swine from certain genetic strains are unable to withstand any of a number of stressors and may succumb to the stress (Aberle et al., 1973). When a stress prone pig is subjected to an environment where a stress condition can develop, it may not adjust physiologically. If this stress condition persists or increases in intensity, lactic acid accumulates in the blood resulting in an acidotic condition. Most stress prone pigs die a few minutes after they collapse; however, some may survive. Pigs which survive and are sacrificed within several hours after the stress conditions, have greatly reduced muscle glycogen stores and therefore reduced glycolysis post-mortem. This results in a high muscle pH and dark colored muscle. Pigs which die or are sacrificed in the late stages without much depletion of glycogen have pale, soft, exudative (PSE) muscle properties (Topel, 1969). In PSE muscle, postmortem glycolysis is accelerated leading to a buildup of muscle lactic acid. This accumulation of lactic acid occurs while muscle temperature is high or near normal body

temperature and consequently results in muscle protein denaturation and rapid development of rigor mortis. The denatured proteins exhibit the pale color, and soft, exudative characteristics typical of low quality or PSE musculature (Lister, 1969; McLaughlin and Tarrant, 1969).

Environmental Factors. Various environmental factors may cause a stress reaction in an animal. Attempts have been made to correlate response to stress with ultimate meat quality.

Heat is often used as the stressor in laboratory experiments, but in feedlot conditions normal handling and movement, transport, temperature changes, and fighting can cause death (Topel et al., 1968) or reduced postslaughter meat quality (Lendfers, 1971). Animals that are able to withstand these stressors are commonly referred to as stress resistant (SR) and those not able to withstand minor stress are termed stress susceptible (SS) (Judge et al., 1968a). Stress susceptibility may vary in its degree of expression. Topel (1968) has identified this condition as the porcine stress syndrome (PSS).

Porcine Stress Syndrome. The symptoms of PSS develop rapidly, with greater intensity as time progresses. The first indication of an impending stress syndrome is a rapid tremor of the tail when the pig is aroused or excited, but this clue requires close observation. Dyspnea occurs and becomes severe with the affected pigs generally developing openmouth breathing. Body temperature is elevated during this phase and irregularly shaped, alternating areas of blanching and erythema develop in the skin. Finally, the pig becomes reluctant to move and will collapse and die if the stress conditions are continued.

This sequence of events often occurs within a few minutes after the initial signs of stress are observed (Topel et al., 1968). Rigor mortis develops almost immediately postmortem among these pigs (Topel et al., 1968; Forrest et al., 1968).

Body Homeostasis. The changes in body homeostasis in SS and SR pigs in response to various stressors have been described. Forrest et al. (1968) observed that heart rate tended to rise in SS and SR animals during a 30 min. exposure to a warm environment, although the rate of increase was significantly greater in SS animals. Heart rate in SS pigs rose during the first 10 min. of exposure and then declined slightly, while SR pigs showed a gradual increase in heart rate during heat exposure (Forrest et al., 1968). Judge, Cassens and Briskey (1965) found that SS pigs restrained on their backs for periods of 3 to 5 hr. had more rapid and variable heart rates than SR pigs.

Respiration rate paralleled heart rate during heat treatment in the studies of Forrest et al. (1968). SS pigs increased respiration rate during the first 10 min. followed by a sharp decline during the 20 min. of heat treatment. SR pigs, however, showed continuous but moderate increases in respiration throughout the 30 min. of heat treatment. Esophageal temperature increased more rapidly in SS than SR pigs during heat treatment (Forrest et al., 1968).

By visual appearance, SS animals or animals with PSS tend to have less fat and to be more extreme in their muscularity, especially when comparisons are made within a particular breed. SS animals tend to be easily frightened and/or difficult to manage, have leg weakness and have higher rectal temperature, even after adaptation to a minor stress condition (Judge, 1972).

The consequences of continued selection pressure for increased muscle development and decreased carcass fat have been summarized by Vos and Sybesma (1971). They concluded that selection for lower backfat had less effect on meat quality than selection for quantity of muscle, which was generally associated with lower meat quality.

Glucocorticoids

Many stressors described in the PSS section affect the domestic pig, especially the stressors associated with handling and marketing and in the period immediately before slaughter. When an animal is subjected to a stressor, among the physiological responses are a discharge of hormones from its adrenal gland to help cope with the stress conditions (Briskey and Lister, 1968). These adrenal hormones (steroids) are secreted from the cortex of the adrenal gland, and their biosynthesis takes place in the cortical cells.

Synthesis and Release. The adrenocortical steroids are of two structural types: those that have a 2-carbon side chain attached at carbon 17 of the main ring and contain 21 carbon atoms (C-21 steroids) and those that have a keto or hydroxyl group at position 17 and contain 19 carbon atoms (C-19 steroids). The C-21 steroids that have a hydroxyl group at the 17 position in addition to the side chain are often called 17-hydroxycorticosteroids (17-OHCS). The C-21 steroids are classified as either mineralocorticoids or glucocorticoids. All C-21 steroids have both mineralocorticoid and glucocorticoid activity. The mineralocorticoids influence sodium and potassium excretion, and the glucocorticoids influence the vascular system, glucose and protein metabolism. The only

glucocorticoids normally secreted in physiologically significant amounts are the 17-OHCS, cortisol and corticosterone (Ganong, 1971).

Adrenocorticotrophic hormone (ACTH) from the pituitary catalyzes a step in the conversion of cholesterol to pregnenolone, which is an intermediate steroid in the early stages of corticoid biosynthesis. ACTH is apparently not altered in the process of exerting its effect on adrenal tissue (Ganong, 1971). When a normal healthy animal is exposed to any of an immense variety of noxious or potentially noxious stimuli, there is an increased secretion of ACTH and a corresponding rise in the circulating glucocorticoid level. The rise in glucocorticoids is essential for survival.

The action of glucocorticoids on the intermediary metabolism of carbohydrate, protein and fat is not fully known. In general, they usually increase protein catabolism, hepatic glycogenesis, and gluconeogenesis. Seventeen-OHCS increase glucose-6-phosphate activity and cause blood glucose to rise (Topel, 1972). The liver is the principal site of glucocorticoid catabolism. Most of the cortisol and corticosterone is conjugated in the liver to become freely soluble in blood and then they are rapidly excreted in urine. In the domestic pig cortisol is the major glucocorticoid (Heap, Holzbauer and Newport, 1966).

Response to Stress. As noted in the review of PSS, various stimuli can be stressful to both SS and SR pigs. However, the change in circulating levels of glucocorticoids may be different between SS and SR pigs in response to the specific stressor.

Physical Restraint. A psychrometric chamber at ambient temperature and humidity provided stressful conditions to 60 to 70 kg pigs and resulted in differing plasma corticoid and ACTH levels in SS and SR pigs (Marple, Judge and Aberle, 1972). After a 4 to 7 day conditioning period of handling and blood sampling these same authors found that SS pigs had three to four fold higher saphenous artery or vein ACTH when compared to SR pigs in the chamber environment. However, there were no significant differences between plasma adrenal corticoid levels in the same pigs. The ratios of mean plasma adrenocorticoids levels/mean plasma ACTH levels for SS pigs were approximately 1/3 to 1/4 the ratios for the SR animals. Marple et al. (1972) concluded that SS pigs had either a lower level of response to endogenous ACTH or an increased rate of metabolism of adrenal corticoids. After measuring 17-OHCS metabolites in the urine of SS and SR pigs confined in metabolism cages, Judge et al. (1968b) concluded that muscular pigs with the PSE condition, tended to have deficiencies in adrenal steroid production.

Environmental Temperature. Elevated environmental temperature has often been used as a stressor when comparing responses of SS and SR pigs. Some reports have correlated increased environmental temperatures with changes in meat quality. Ludvigsen (1969) reported SR pigs easily adapted to 30 min. of high environmental temperature (43 to 45 C). He observed that SS pigs were unable to adapt to the experimental conditions and showed characteristic signs of stress syndrome. However, when the level of adrenal cortical steroids in the blood and body fluids was increased by injection of synthetic adrenal cortical steroids, the pigs easily adapted to the heat exposure, a reaction which

was not only reflected by the behavior of the pigs, but also in the postmortem characteristics of their skeletal muscles (Ludvigsen, 1969).

Kallweit (1969) demonstrated the effect of temperature on postmortem quality in randomly selected German Landrace pigs. Group I (controls) was kept in the holding pens of a slaughterhouse for 24 hr. prior to slaughter. Groups II and III were placed in an environmental chamber for 4 hr. at 37°C with 100% relative humidity. Group II was slaughtered in the chamber immediately after the 4 hr. heat stress. Group III was exercised by walking in the heated chamber for 10 min. before exsanguination in the environmental chamber.

The overall quality (color and expressible juice) of the M. longissimus of Group I was superior to groups II and III. In the M. semimembranosus, Group I had superior quality to Group II but lower quality than Group III. Kallweit (1969) suggested that glycogen stores were depleted and the muscle became dark, firm and dry (DFD) and concluded heat and exercise had marked effects on most meat quality characteristics.

A number of investigators have measured the changes in plasma glucocorticoids in response to increased environmental temperature in comparison to ultimate meat quality. Judge et al. (1968a) studied pigs (SS and SR) which had been acclimated to a cool natural environment (-18 to 0 C) and were subsequently held in a moderate temperature (21 to 24 C) room. Blood samples were drawn from the anterior vena cava while the pigs were maintained in the control room for 15 days. The SS pigs which ultimately developed lower postmortem quality, had low mean levels of adrenal steroids excreted in the urine compared to the adrenal steroid levels in the urine of SR pigs (Judge et al., 1968a).

Aberle et al. (1973) also imposed heat as a stressor in SS and SR pigs weighing 55 to 75 kg and fitted with a catheter in the anterior vena cava. In a psychrometric chamber temperature of 35 C, plasma corticoid levels significantly increased in both SS and SR pigs in the first replication. In replicate 2, however, corticoids were high at the beginning of the sampling period, but decreased prior to and during the onset of heat stress and then showed only a slight increase later during the heat stress. Marple et al. (1972a) used various combinations of temperature and relative humidity (R.H.) as stressors. He used SS and SR gilts weighing 50 to 60 kg. The pigs were sampled from the saphenous artery or vein for 7 days and maintained at either cold temperature, low humidity (4 C, 46% R.H.), moderate temperature, high humidity (21 C, 97% R.H.), or high temperature, moderate humidity (32 C, 66% R.H.) conditions. Plasma ACTH levels were not influenced by exposure to a 4 C, 46% R.H. environment, but 21 C, 97% R.H. and 32 C, 66% R.H. induced increased plasma ACTH levels in both the SS and SR pigs as compared to controls at 21 C, 61% R.H. Plasma corticoid levels were depressed in both SS and SR pigs at all temperature humidity conditions with the greatest depression noted when the animals were exposed to the high temperature - moderate humidity environment (Marple et al., 1972a).

In a related experiment Marple et al. (1972b) examined the effect of high and low humidity at both high and low temperatures on 40 to 45 kg gilts. Plasma corticoids were significantly increased in response to low temperature, low humidity as compared to an ambient control environment, while plasma ACTH was not affected. When the pigs were exposed to low temperature, high humidity, plasma ACTH increased but

plasma corticoids fell compared to controls. Plasma ACTH was higher and corticoids lower at high temperature, low humidity compared to pigs in the control environment. ACTH decreased slightly in response to the higher temperature and humidity combination, while corticoids showed a corresponding increase. Marple et al. (1972b) concluded that stress may change the turnover rate of corticoids in the body and indicated that both plasma ACTH and corticoids should be determined to more accurately measure pituitary-adrenal responses to stress.

Fluctuating environmental temperatures have also been used as the stressor. In pigs classified as exhibiting either fast or slow glycolyzing muscle, none of the 4 treatments of high, low or two fluctuating temperatures produced a consistent alteration in plasma 17-OHCS levels (Topel et al., 1971). The pigs were consecutively exposed for 2 days to each of four environments. Samples were collected from the femoral artery and vein via a catheter only at the times of extreme temperature or once daily when the pigs were on the constant temperature treatment (Topel et al., 1971). A two-fold increase in plasma ACTH was noted among both SS and SR 80 to 90 kg pigs exposed to a daily one-cycle elevation in temperature for 7 days or a daily six-cycle elevation in temperature for 6 days (Marple, Judge and Aberle, 1972). Plasma corticoids were reduced when the pigs were exposed to the one cycle temperature fluctuation per day but approached that of controls when the frequency of fluctuation was increased to six cycles per day. They concluded that SS pigs do not suffer from a secondary type of adrenal insufficiency but may be affected with an acute form of primary adrenal insufficiency (Marple, Judge and Aberle, 1972).

Exogenous ACTH. ACTH directly stimulates the adrenal cortex to secrete glucocorticoids. Reichel and Braun (1962) found a three-fold increase in Porter Silber corticoids in blood collected by jugular puncture 5 hr. after an intramuscular injection of 0.5 IU ACTH/kg in pigs. Lister, Lucke and Perry (1971) reported a much more rapid response in pigs they described as mesomorphic (heavily muscled, Pietrain) and also controls (Large White). Resting cortisol was found to be 5 µg/100 ml of plasma and it rose gradually to 18 µg/100 ml 60 to 90 min. after an intramuscular injection of 250 µg tetracosactin and declined slowly to 10 µg/100 ml 2 hr. after the injection. The resting cortisol values, the peak and the time to peak responses were similar in the two groups of pigs. However, castrated mesomorphic males had a significantly prolonged response to ACTH with a longer period of elevated plasma cortisol than that observed in intact mesomorphic females, castrated normal males and intact normal females.

Sebranek et al. (1973) reported a more immediate response to a 10 IU injection of ACTH through a catheter in an ear vein in 89 to 99 kg normal pigs previously injected with 4 mg dexamethasone to suppress adrenal activity. Plasma corticoids rose from 8 ng/ml of plasma before injection to 66 ng/ml of plasma 60 min. after injection and declined to 45 ng/ml of plasma by 90 min. after injection in normal pigs. When both SS and SR pigs were treated in a similar manner and sampled only at 60 min. after injection, ACTH levels of the SS pigs were not depressed due to the dexamethasone treatment and were found to be markedly increased at 60 min. post ACTH injection (Sebranek et al., 1973). There was a lower level of plasma corticosteroids in SS pigs than in SR pigs 60 min. after ACTH

injection even though the suppressed level (dexamethasone treatment) was higher than that in SR pigs. No significant differences in corticoid levels were found before dexamethasone treatment in SS and SR pigs. These authors concluded that when the adrenal is stimulated by ACTH, as in a stressful environment, the response is apparently not sufficient to maintain homeostasis in SS pigs (Sebranek et al., 1973). A decline in plasma corticoids was also found in adult male humans 4 to 8 hr. after a 44 IU injection of highly purified porcine ACTH (Brombacker, Buytendijk and Maesen, 1969).

Plasma cortisol responded much more dramatically than corticosterone to an intramuscular injection of 100 units of ACTH in 60 kg crossbred pigs. Plasma cortisol increased from 1.2 μg/100 ml (preinjection) to 2.3 μg/100 ml one hr. after injection and peaked at 4.2 μg/100 ml two hr. after injection. At 3 hr. postinjection, plasma cortisol had declined to 2.4 μg/100 ml and continued to fall until it reached the preinjection levels 24 hr. after the injection. On the other hand, preinjection plasma corticosterone was 0.3 μg/100 ml and rose to 0.9 μg/100 ml at one hr., 1.3 at two hr. and fell to .07 μg/100 ml three hr. after ACTH injection. Twenty four hr. after injection of 100 units ACTH plasma corticosterone was slightly below preinjection levels (Sebranek et al., 1973)

Induced Adrenal Insufficiency. Attempts to quantify the effect of induced adrenal insufficiency on muscle characteristics have been made. Prednisolone has been shown to suppress 17-OHCS in plasma of pigs (Topel and Merkel, 1967; Marple, Topel and Matsushima, 1969). Aberle and Merkel (1968) injected prednisolone for 10 days before slaughter and 10 min. prior to slaughter the pigs also received an I.M. epinephrine injection.

Prednisolone plus epinephrine did not result in lower pork quality than control pigs or pigs injected with prednisolone only (Aberle and Merkel, 1968). Two days after the last of 10 daily injections of prednisolone, 4 of 8 animals survived 5 min. of exercise but none of the surviving exercised or injected unexercised pigs produced PSE carcasses (Marple, Topel and Matsushima, 1969).

Using 80 to 90 kg pigs, Topel and Merkel (1968) were able to induce adrenal atrophy and to markedly reduce plasma 17-OHCS by injection or oral administration of prednisolone and methylprednisolone for periods of 7 to 21 days, but no PSE musculature developed in any of the pigs. In another experiment, Topel and Merkel (1966) were able to reduce adrenal gland weight in normal pigs, though not significantly, by feeding methylthiouracil for either 10 or 21 days. Some pigs in this group (methylthiouracil) had slight PSE musculature but there were no differences in circulating plasma 17-OHCS in blood collected at slaughter. Topel (1969) summarized these two studies and concluded that adrenal atrophy following thiouracil administration was caused by lowered ACTH titers.

Diurinal Variation. The diurinal variation in plasma glucocorticoids in all animals is clearly documented. The diurinal variation in total glucocorticoids in normal pigs has been reported by a number of researchers, although there is considerable variation in sampling interval within a day and length of sampling period. Total glucocorticoids have been found to be highest in the early daylight hours and lowest in the middle of the darkness period (Steinhauf, Weniger and Augustine, 1969; Topel et al., 1971). In samples from the aorta via the

right femoral artery in male first generation specific pathogen free pigs 3 to 6 months old, Whipp, Wood and Lyon (1970) found mean plasma cortisol concentrations of 2.4 µg/100 ml at 8:00 a.m., 0.8 and 0.6 µg/100 ml at 4:00 p.m. and 12:00 midnight, respectively. Bottoms et al. (1972) found a different circadian variation for cortisol but not corticosterone in 68 kg pigs sampled hourly for 24 hr. through jugular cannulae. The largest mean cortisol value was found at 10:00 a.m. (1.40 µg/100 ml) and the smallest at 2:00 p.m. (0.59 µg/100 ml). In contrast to cortisol, the high value of corticosterone (0.34 µg/100 ml) occurred at 6:00 p.m. and the low (0.17 µg/100 ml) occurred at 6:00 a.m.

Age and Sex Effects. Only scant information is available on the effect of age on plasma 17-OHCS in pigs. In 205 piglets and older pigs of the Large White breed, 17-OHCS were highest the first day post natally and decreased significantly at 10 day intervals through one month of age. The downward trend continued through maturity. However, 17-OHCS levels were not significantly different from one month of age until after weaning at 46 to 60 days of age. Thereafter a nonsignificant downward trend continued through maturity, the lowest levels being found in sows (Dvorak, 1972).

The sex effect on circulating plasma glucocorticoids is clearly documented. Dvorak (1972) found that boars and gilts up to 90 days of age did not differ in plasma levels of total corticoids. Others have shown barrows and gilts to have similar circulating levels of 17-OHCS (Topel, Merkel, Wismer-Pederson, 1967; Marple and Cassens, 1973). Castrated SS males had higher circulating corticoids for a longer period of time after an I.M. injection of ACTH, but their peak response was not different from intact SS or SR females or SS castrated males.

Turnover of Cortisol. Early experiments implicated an adrenal insufficiency and lower plasma 17-OHCS in SS pigs while recent reports indicate that resting SS pigs have higher circulating levels of plasma glucocorticoids. Marple and Cassens (1973) reported that the metabolic clearance rate of cortisol (volume of plasma that is completely and irreversibly cleared of cortisol per unit of time) was 5 times higher in SS compared to normal pigs weighing 100 to 110 kg when labeled cortisol was infused into the vena cava and blood samples were taken through an ear vein. Additionally, cortisol turnover rate (product of the metabolic clearance rate and the total cortisol concentration) in the same SS pigs was 3 times greater than that of normal pigs.

In Vitro Response to ACTH. Dvorak (1972) observed that adrenal glands incubated with 0.5 units ACTH/50 mg adrenal tissue showed highest production of 17-OHCS at birth. Activity decreased with advancing age of piglets and the lowest activity was observed in sows. Mean production of 17-OHCS during 2 hr. of incubation was 8.6 μg/100 mg of adrenal tissue in pigs from 107 to 289 days of age. The adrenal tissue was preincubated for one-half hr., then the original medium was discarded and incubated for an additional 2 hr. with fresh medium containing ACTH (Dvorak, 1972).

Adrenal Cortex Morphology

In adult mammals, the adrenal cortex has 3 zones of variable distinctness. The outer zone, zona glomerulosa, is made up of whorls of large cells which rest on the columns of cells that form the zona fasciculata (middle zone). The columns of cells are separated by venous sinuses. The inner portion of zona fasciculata merges into the inner

zone, zona reticularis, where the cell columns become interlaced in a network. The cells contain abundant lipid, especially in the outer portion of the zona fasciculata. All 3 cortical zones secrete corticosterone and the enzymatic mechanism for forming cortisol and the sex hormones is found in the inner 2 zones. Injections of ACTH and stimuli which cause endogenous ACTH secretion produce hypertrophy of the zona fasciculata and zona reticularis but do not increase the size of the zona glomerulosa. The cholesterol in the adrenal is presumably the store from which steroids are synthesized (Ganong, 1971). Hematoxylin and eosin (H & E) stains the nuclear structures of the adrenal gland dark purple or blue and practically all cytoplasmic structures and intracellular substances are stained varying shades of pink. Sudan black B stains lipid materials black and other structures light gray (Blom and Faiucett, 1970; Disbrey and Rack, 1970).

Differences in adrenal cortex size in various breeds of pigs have been reported. Ludvigsen (1968) reported that the adrenal cortex of Chester White pigs is thicker than the adrenal cortex of either Hampshire or crossbred pigs and Poland China pigs had the thickest adrenal cortex of all breeds sampled.

Some environmental factors have been correlated with adrenal weight and adrenal lipid deposits. Dvorak (1972) found that the ratio of adrenal gland weight to body weight decreased with slight fluctuation until maturity when sampling pigs before and after weaning, at 4 to 9 months of age and at maturity. Addis et al. (1965) found no effect of SS pigs raised in four types of housing on the weight of the right adrenal gland in the SS pigs at slaughter. Howe et al. (1969) studied

the left adrenal gland from pigs from 8 weeks of age to market weight that were exposed to two ambient temperatures alternated every three days in combination with varying relative humidity. The variable temperature environments consistently increased the abundance of lipid masses in the zona reticularis. Low humidity resulted in adrenal lipid accumulation that was more extensive than that resulting from the variable temperature using Sudan black B stain. Weight of the adrenal glands did not vary significantly among the treatments. Howe et al. (1969) concluded that adrenocortical alterations may be induced in pigs believed to be SS when subjected to variable temperature or low humidity.

Cassens et al. (1965) stained left adrenal glands with Sudan black B from pigs described as having fast or slow glycolyzing muscle while maintained at ambient temperature. Sudanophilic material appeared as two general types: fine granules and large masses. Cortices of both porcine types had varying amounts of fine sudanophilic granules throughout the zona fasciculata and zona reticularis. However, large masses of sudanophilic material were localized in the zona reticularis of adrenal glands from the fast glycolyzing group. These large lipid masses were seen very infrequently in the adrenal glands of the slow glycolyzing group. Although the border between the zona fasciculata and zona reticularis did not appear to be clearly defined, the large masses of sudanophilic material, when present, always extended outward from the clearly defined border of the medulla. Cassens et al. (1965) suggested that the heavy lipid masses may be indicative of a degenerative process, since there is predisposition of the fast glycolyzing type pigs to be susceptible to stressful conditions and develop lower muscle quality postmortem.

Zinc

In humans low serum zinc has been implicated as limiting adrenal cortex output of 17-OHCS. Fifteen patients in Egypt, believed to be zinc deficient showed varying, but generally lower than normal excretion of 17-OHCS when given 40 units of ACTH on 3 successive days after periods of zinc supplementation, had increased 17-OHCS in response to the same dose of ACTH in 5 patients. Zinc supplementation also increased growth in zinc deficient children (Sandstead et al., 1966).

Solutions prepared from pig pituitaries with either zinc phosphate or zinc hydroxide had increased corticotropin activity when measured by the liver glycogen test in hypophysectomized rats, the eosinophil test in dogs and the thymus-involution test (Homan et al., 1954) in rats. The combination of corticotropin with zinc phosphate caused increased urinary 17-OHCS (Greene and Vaughn-Morgan, 1954). Corticotropin zinc phosphate also caused a decrease in the number of eosinophils and increased excretion of 17-OHCS (den Oudsten, VanLeewen and Coers, 1954; Ferriman, Anderson and Turner, 1954).

When using oligemic hypotension for 1 hr. as the stressor in rats, Flynn et al. (1971) showed that serum ACTH, corticosterone and zinc paralleled each other during periods of stress and stress recovery. During the 1 hr. stress, serum ACTH levels rose to twice control values and then quickly dropped to below control levels during the recovery period. Serum zinc showed the same significant percentage change as ACTH during both stress and stress recovery. Corticosterone followed the same pattern as zinc and ACTH, but the change was not as dramatic

and recovery serum levels were higher than control levels. These authors also reported a relatively high zinc level in two commercial ACTH preparations.

The Effect of Blood Insulin Levels on Body Homeostasis

Insulin has several effects on body metabolism, such as: (1) The increase in the entry of glucose into muscle fibers and the cells of certain other tissues. (2) The inhibition of hormone-sensitive lipase in adipose tissue. (3) The stimulation of protein synthesis, an effect that can occur in the absence of extracellular glucose. (4) The increase in the transport of amino acids into cells. (5) The increase in membrane potential of cells in skeletal muscle and adipose tissue (Ganong, 1971). Serum insulin may possibly regulate substrate supply during stress conditions. Serum and plasma levels of insulin in the domestic pig in response to circulating levels of exogenous glucose is well documented. Plasma insulin also responds to various exogenous amino acids and fatty acids. However, the role of insulin in maintaining body homeostasis in response to external stimuli has not been discussed.

Grigsby et al. (1972) found constant serum insulin levels during a 36 hr. fast and then a 20 fold increase in serum insulin one hr. after feeding. Romsos, Leveille and Allee (1971) recorded a 50% drop in plasma immuno-reactive-insulin (IRI) in alloxan diabetic pigs compared to controls. In pigs fasted 19 hr. and weighing 25 to 50 kg, Hertelendy et al. (1970) found significant increases in plasma insulin after arginine treatment in 7 of 9 pigs when blood samples were obtained from a tail vein. The two pigs that did not respond had baseline plasma levels 3 times higher

than the seven that did respond. Swaitek et al. (1968) sampled fed and fasted pigs at birth, 4 days, 7 days and 21 days of age. They found similar plasma insulin levels in both fed and fasted pigs at each age, except those pigs fasted from birth, which showed a decrease in plasma insulin after 6 hr. of fast compared to those fed from birth. Data obtained by Machlin et al. (1968) suggested that insulin responded more to long term doses of glucose than single massive doses. They used pigs fasted 36 hr. that weighed 40 to 50 kg and they injected glucose into the anterior vena cava over a 3 min. period to a level of 0.75 g/kg body weight. The glucose injection initially tripled plasma insulin over saline injected controls. Plasma insulin remained at double control levels to the end of their sampling period. In a second experiment Machlin et al. (1968) infused 100 mg glucose/kg/hr. during a 1 hr. period through an ear vein catheter in 30 to 35 kg pigs. During the 1 hr. infusion of glucose, plasma insulin rose steadily to 5 times the preinjection levels at the end of the one hr. period and then fell steadily to preinjection levels one and one half hr. later. In both experiments, plasma samples were taken from a tail vein. Similar experiments with miniature pigs led Stoll et al. (1971) to label the pig as being mildly diabetic.

Antigenicity of Insulin and Related Proteins. The immediate precursor of the double chain insulin molecule is proinsulin, a single chain molecule. Antibodies produced in the guinea pig and used in the radioimmunoassay for insulin are not specific for the double chain insulin molecule. Kitabachi et al. (1972) summarized other researchers work and concluded that serum proinsulin replaced 25 to 50 percent of the bound insulin in a normal double antibody radioimmunoassay.

Using the in vitro conversion of glucose to carbon dioxide in the isolated fat cell, Kitabachi et al. (1972) found proinsulin to have one-tenth the biological activity of insulin. In 65 to 78 kg female miniature pigs Stoll et al. (1971) reported the half life of proinsulin to be 20 min. and insulin to be 6 minutes.

Baseline Levels of Insulin. Widely varying plasma levels of insulin have been reported when sampling untreated control pigs or to establish a baseline in untreated pigs. Hertelendy et al. (1970) observed that means ranged from 4 to 17 μU/ml of plasma in one group of pigs and in another two groups the means were 30 and 39 μU/ml of plasma, respectively. Other researchers reported values over a considerable range as follows: 26 to 27 μU/ml of plasma (Romsos, Leveille and Allee, 1971), 30 μU/ml of plasma in young pigs (Swaitek et al., 1968), 5 μU/ml of plasma in 35 to 50 kg pigs (Machlin et al., 1968) and 132 μU/ml of plasma in pigs with no weight or age given (Wood, Whipp and Wetzel, 1971).

No significant diurinal variation was found in plasma insulin levels when sampling from the aorta through a vinyl catheter placed in the right femoral artery (Wood, Whipp and Wetzel, 1971) of pigs. In their study blood samples were taken at 8 a.m., 4 p.m. and 12 p.m. over a 5 day period.

MATERIALS AND METHODS

This study consisted of 3 separate experiments, designed so that the same pigs would be involved in each of the experiments. In experiment 1, the effects of environmental temperature on some serum hormone and zinc levels in SS and SR pigs were determined prior to and at various time periods during and following exposure to heat stress at 50 kg and again at 100 kg live weight. Experiment 2 was designed to study serum glucocorticoids, insulin and zinc in SS and SR pigs following an IM injection of ACTH. Adrenal tissue from SS and SR pigs was studied in experiment 3 to observe cortisol and corticosterone content before and after *in vitro* incubation and when incubated with ACTH.

Experimental Animals

Sixteen pigs (8 SS and 8 SR) were initially selected for these studies. Seven pigs died during the course of the study, either from exposure to the heat stress or from pneumonia. The pigs used in this study were either purebred Hampshire or Yorkshire, or Hampshire-Yorkshire first generation crossbreds. The pigs were maintained in an enclosed building on a concrete floor and were fed a normal 14% protein growing ration throughout the experiments. Once catheterized, they were maintained in individual pens. At all other times the SS and SR pigs were kept in two pens by group. Pigs were determined to be either SS or SR as described by Topel (1968).

Catheterization

Pigs were anesthetized with sodium pentobarbitol and an experimental drug (747) obtained from the MSU Veterinary Clinic. Pigs were placed in dorsal recumbency on a table and a 12 gauge thin walled needle three inches long was inserted into the anterior vena cava through a point one half inch anterior to the junction of the first rib and sternum. Entry into the vena cava was about 9 cm anterior to the heart.

Twenty five cm of sterile silastic tubing (0.102 cm inside diameter; Piling Co.) was inserted through the needle into the vena cava. The needle was removed and the catheter was sutured to the interior portion of the skin. A trocar was inserted through an incision made 4 cm anterior to the scapulae and forced through the subcutaneous fat to approximately 1 cm from the sutured catheter. The catheter was then threaded through the trocar and the trocar was removed. A small loop in the catheter was sutured to the outside of the skin and the remaining 50 cm of catheter were coiled in a tape patch which was secured to the dorsal neck region with branding cement. The catheter was filled with 3.5% sodium citrate and fitted with an 18 gauge needle which was sealed with a 1 ml tuberculin syringe. The catheterization procedure was completed 4 to 5 days before any blood samples were collected.

Serum Collection

In all three experiments, 15 ml blood were collected from the catheter, transferred to 15 ml centrifuge tubes and allowed to stand at room temperature for 1 hour. Then the blood was stored for 24 hr., loosened from the walls of the centrifuge tube and the serum separated

by centrifugation. Serum was divided as follows: 1 ml for zinc assay and 2 ml each for insulin and steroid assays. An additional 2 ml was stored for possible growth hormone determination. Individual vials were thawed at room temperature just prior to analytical determination. Samples were refrozen and used for reruns if needed.

Environmental Chamber

A controlled temperature and humidity environmental chamber 180 cm long, 90 cm wide and 150 cm high was used as the heat stress chamber in experiment 1. Chamber temperature could be changed at the rate of one half degree centigrade per minute and relative humidity (R.H.) remained at 65% from one half hour after the first sample was taken until the end of the sampling period.

A SS and a SR pig were paired and randomly assigned to a control environment (22 C, 65% R.H.) or a stressful environment (35 C, 65% R.H. followed by 22 C and 65% R.H.) separated by 4 days. Pigs were placed in the environmental chamber at 2300 to 2400 hr. on the day before exposure to either the control or stressful chamber environment.

The percentage change in each blood parameter measured during the stressful environment was compared with that of the control environment at the corresponding time period (e.g. sample 1 compared with sample 7, sample 21 compared with 27 etc., table 1) to determine the chamber stress affect. The serum samples were numbered as shown in table 1. Blood samples 1 and 7, and 21 and 27 were obtained prior to treatment at 50 kg and 100 kg, respectively.

TABLE 1. NUMBERING SCHEME FOR SERUM SAMPLES COLLECTED IN THE ENVIRONMENTAL CHAMBER

Stressful Environment

Sample Number		
50 kg	100 kg	Chamber Environment
1	21	Before treatment
2	22	After 90 min. of 35 C 65% R.H.
3	23	After 180 min. of 35 C 65% R.H.
4	24	After 120 min. of 22 C 65% R.H.
5	25	After 240 min. of 22 C 65% R.H.
6	26	After 360 min. of 22 C 65% R.H.

Control Environment

Sample Number		
50 kg	100 kg	Chamber Environment
7	27	Before treatment
8	28	After 90 min. of 22 C 65% R.H.
9	29	After 180 min. of 22 C 65% R.H.
10	30	After 300 min. of 22 C 65% R.H.
11	31	After 420 min. of 22 C 65% R.H.
12	32	After 540 min. of 22 C 65% R.H.

Blood samples 2 and 22 (stressful environment) were taken 90 min. after the chamber had reached 35 C and 65% R.H. (usually about 30 min.). Pigs remained in the 35 C and 65% R.H. environment for 180 min., or until one of the pair had a rectal temperature of 106 C or exhibited extreme

anxiety. Control and stressful environmental runs were made on all healthy pigs at 50 kg and again at 100 kg.

ACTH Injection

Six to 7 days after being exposed to the environmental chamber at 50 kg live weight all healthy pigs (10) were given a 40 IU injection (IM) of ACTH in each ham for experiment 2. Serum samples were numbered as shown in table 2.

TABLE 2. NUMBERING SCHEME FOR SERUM SAMPLES FOLLOWING ACTH INJECTION

Sample Number	Treatment
13	Before treatment
14	1 hr. after ACTH
15	2 hr. after ACTH
16	4 hr. after ACTH

Collection of Adrenals

Twelve days after the completion of experiment 1 at 100 kg live body weight the pigs were delivered to the meat laboratory at 1500 hr. on the day before slaughter. Right and left adrenal glands were removed immediately postexsanguination and placed in ice water until trimmed. Extraneous adhering tissues were removed, and the trimmed adrenals were weighed. Thin cross sectional slices (approximately 1 mm thick) were made from the central portion of each adrenal gland. A 1 cm section of

the right adrenal gland (central portion) and the remaining posterior portion of the left gland were placed in 10% formalin and later sectioned and stained with H & E (appendix I.C.2).

Adrenal Incubation

For the in vitro incubation studies in experiment 3, 150 to 300 mg of sliced adrenal tissue were weighed, and then placed in each of three flasks containing 5 ml of Krebs-Ringer bicarbonate buffer (appendix I.C.1). Flask A was frozen immediately, Flask B was incubated for 2 hr. at 37 C and frozen. Flask C was incubated for 2 hr. with 1 IU ACTH (Armour Baldwin) at 37 C and frozen until assayed for corticoids.

Insulin Assay

1) Three hundred and fifty and 250 µl of serum at room temperature were added, with a Hamilton microliter syringe (Hamilton Co.), to disposable glass culture tubes (12X75 mm) containing enough buffer B (0.05 M phosphate buffered saline - 1% bovine serum albumin, pH 7.4; appendix I.A.2) to make the total volume 500 microliters. Standard porcine insulin (Eli Lily; appendix I.A.3) prepared in buffer B at 16 different concentrations ranging from 1.43 to 239 µU was also pipetted as described above.

2) On day zero, 200 µl of GPAPI serum (Miles Labs) diluted 1:100,000 (appendix I.A.5) were added to each tube, then gently agitated and stored at 4 C for 24 hours.

3) On day one 13,000 cpm of ^{125}I-insulin (IM-38, specific activity-50 µCi/µg, Amersham Searle; appendix I.A.6) were added to each tube, the tubes were gently agitated, and then stored at 4 C for 24 hours.

4) On day two, 200 µl of SAGPGG (appendix I.A.7) were added, the tubes were agitated and then incubated for 96 hr. at 4 centrigrade.

5) Following the 96 hr. incubation, 3 ml of PBS (appendix I.A.8) were added to each tube and all tubes were then centrifuged at 2500 x g for 30 min. in a refrigerated centrifuge (Sorvall, RC-3).

6) The supernatant was decanted and the tubes were wiped dry after they had dried on absorbant paper for 30 minutes. After drying the tubes were counted in a Nuclear-Chicago Model 4230 autogamma scintillation counter for 10 min. or 10,000 counts.

7) The standard curve was calculated by multiple regression analysis on a CDC 3600 computer and fit linear, quadratic and cubic components of the regression equation. Serum insulin concentrations were calculated with the regression equation using an Olivetti computer (Programma 101, Olivetti Underwood, New York).

Glucocorticoids

This procedure was modified from that described by Smith, Convey and Edgerton (1972). Corticosterone was determined only on the serum samples in experiment 2. Cortisol was determined on all serum samples.

Extraction of Corticoids from Serum

1) To account for procedural losses, 2000 cpm of ^{3}H-cortisol and of ^{3}H-corticosterone (purified by column chromatography as described below; New England Nuclear) were placed in 12X75 mm disposable culture tubes. Unknown serum (0.100 ml or 0.200 ml) samples were added to the tube and allowed to equilibrate with the two tracer steroids for 20 minutes.

2) To wash out progestogens, the serum was vortexed vigorously for 1 min. with 2.0 ml trimethylpentane (nanograde). Then the serum was held at -20 C for 1 hr. and with precaution taken to avoid thawing the serum, the trimethylpentane layer was decanted and discarded.

3) To extract the corticoids, the washed serum was vortexed for 1 min. with 2.0 ml methylene chloride (reagent grade). The methylene chloride (lower) phase was aspirated with a disposable pipette, placed in a second 12X75 mm disposable culture tube, and the methylene chloride evaporated. The sides of these tubes were rinsed three times with the solvent (appendix I.B.1) used for column chromatography and dried each time.

Chromatographic Isolation of Corticoids

1) Two hundred and fifty µl of chromatography solvent were added to each 12X75 mm tube and layered on top of a column of LH-20 Sephadex (Sigma; appendix I.B.2). The culture tube was rinsed with another 250 µl solvent and the rinse was also transferred to the top of the column.

2) Eight ml of solvent were placed in the reservoir on top of the column and six 1 ml fractions were collected into 12X75 mm disposable culture tubes.

3) Aliquants (250 µl) were taken from fraction 2 (corticosterone) and from fractions 4, 5 and 6 (cortisol) to measure radioactivity. The fraction with the greatest radioactivity was then assayed for cortisol. The same determination of radioactivity was used to correct for procedural losses. Recovery ranged from 15 to 50%.

Competitive Protein Binding Assay

1) Standard cortisol and standard corticosterone (Sigma) were pipetted from stock solutions of 10 ng/ml in ethanol for each assay, and at least two sets of standards (0, 0.1, 0.25, 0.5, 1.0, 1.5, 2.5, 5.0 and 10.0 ng) were included in each assay.

2) From the column fraction of cortisol and corticosterone with the greatest radioactivity, aliquants of 100 and 200 µl for cortisol and 200 and 400 µl for corticosterone were transferred to 12X75 mm disposable culture tubes and the solvent was evaporated.

3) One ml of 1.25% dog plasma containing 20,000 cpm/ml of ^{3}H-cortisol (appendix I.B.3) was added to each standard or unknown tube, vortexed and stored at 4 C for 12 to 13 hours.

4) To separate bound and free cortisol or corticosterone, 0.5 ml of 0.05% dextran 150 (Pharmacia) and 0.50% carbon decolorizing neutral norit (Fisher Scientific Co.; appendix I.B.4) were added, while stirring, to each tube at 5 C. Each tube was vortexed and centrifuged at 2500 x g for 10 minutes. The total time period from addition of dextran coated charcoal to the first tube until addition to the last tube did not exceed 10 min., and the tubes were centrifuged immediately thereafter. After centrifugation, 0.500 ml of supernatant with 10.0 ml PCS scintillation fluid (Amersham Searle) were added to a glass scintillation vial and radioactivity determined.

5) Serum concentrations were determined by interpolation with the standard curve.

Extraction of Corticoids from Incubated Adrenal Tissue

1) To account for procedural losses, 2000 cpm of ^{3}H-cortisol, ^{3}H-corticosterone and of ^{3}H-progesterone (purified by column chromatography as described previously) were added to 7 ml pyrex glass homogenizer flasks. The contents of the incubation flask were transferred to the homogenizer flask, the adrenal slices were ground thoroughly and the suspension was poured into a 30 ml extraction vial. Extraction vials, homogenizer flasks and conical centrifuge tubes were previously coated with 5% chlorotrimethylsilone (Eastman Kodak Co.) in toluene. The incubation flask was rinsed with 5 ml reagent grade methylene chloride and poured into the extraction vial.

2) An additional 9 ml methylene chloride were added to the extraction vial and the vials were slowly inverted for 4 minutes. The clear lower layer of methylene chloride was aspirated into a 40 ml conical centrifuge tube and dried. The sides of the conical centrifuge tubes were washed three times with chromatography solvent (2) (appendix I.B.5) and dried after each washing.

3) After drying, two hundred and fifty µl of solvent (2) were added to each conical centrifuge tube and the solvent was transferred to a 1X40 cm column of Sephadex LH-20 (appendix I.B.6). The centrifuge tube was rinsed with an additional 250 µl of solvent (2) which was added to the top of the same column. Twenty eight-2.5 ml fractions were collected. The fractions with the greatest radioactivity were assayed as described previously. Recovery ranged from 10 to 20% for both cortisol and corticosterone.

Serum Zinc

Serum was diluted 1:8 in duplicate with distilled water using acid washed glassware and then vortexed. The absorption of diluted serum and standards (0.0, 0.25, 0.5, 1.0 ppm) in an acetylene flame was converted to integration units by an IL 453 atomic absorption emission spectrophotometer (Instrumental Laboratory Inc.). Linear and quadratic equations using the standards were employed to calculate serum zinc concentrations.

Statistical Analysis

One way analysis of variance was determined on the data in the three experiments (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Selection and Behavioral Observations

In addition to using the PSS characteristics described by Topel (1969), additional visual observations were made when pigs were selected at 35 kg and determined to be either SS or SR. A number of pigs were removed from their close confinement pens and moved to a large exercise area to observe their behavior. Those pigs that remained active after 5 min. of exercise were classified as SR while those that assumed a recumbent position immediately or shortly after limited exercise were classified as SS. When forced to move in the exercise area, SS pigs often developed the early signs of PSS (Topel, 1969) while SR pigs remained active. A total of eight SS and 8 SR pigs were selected for these experiments.

From an anatomical viewpoint, there appeared to be differences in the amount and shape of the muscle mass between the two types (SS and SR) of pigs. The SS pigs appeared to have a "lower set" stature as compared to the much taller stature of SR pigs at the same weight. In addition, the SS pigs had more muscle mass and much thicker, more bulging muscles along with less fat than the thinner, narrower, less bulging muscles of SR pigs. The SR pigs also appeared to have less total muscle mass and more total body fat than the SS pigs.

During the three month experimental period the pigs were exposed to frequent handling and potentially stressful conditions, and it was apparent that the behavior of the SS pigs became progressively more like the SR pigs. With time the SS pigs exhibited less anxiety during transport to and from the environmental chamber, while housed in the

chamber overnight, and while exposed to the control environment temperature for 9 hours. However the SS pigs remained reluctant to move when loading and unloading for transport to and from the environmental chamber. When placed in a free exercise area, SS pigs often laid down after only limited movement while SR pigs were more likely to remain active, even after extended periods of free exercise.

Reaction to Environmental Chamber

The pigs responded differently to the environmental chamber at 50 kg compared to that at 100 kilograms. In most cases the pigs at 50 kg moved about before the chamber was enclosed before the control or heat treatment. In the control temperature environment the pigs usually laid down and seldom were visibly disturbed while blood samples were taken. During the heat stress period, the pigs were seldom visibly affected until 60 min. in the chamber at either 50 kg or 100 kg bodyweight. After 60 to 90 min. of heat stress the early signs of PSS (Topel, 1969) developed among SS pigs at 50 kilograms. Open mouth breathing usually began at this point and 30 to 90 min. later rectal temperature reached 106 Centigrade. When rectal temperature of the SS pigs reached 106 C heat treatment was terminated. In some cases heat stress was also terminated because the SS pig exhibited extreme anxiety even though rectal temperature had not reached 106 Centigrade. However, in all cases at 50 kg the SS pig had higher rectal temperature or showed greater anxiety than SR pigs. At the termination of heat stress, the SR pigs had 1 to 2 C lower rectal temperature than SS pigs and they showed less anxiety, were less likely to have developed open mouth breathing and moved about the chamber more freely.

At 100 kg SS and SR pigs appeared to react similarly and less variably to the heat stress conditions than at 50 kilograms. During stress conditions at 100 kg, the SS as well as some of the SR pigs developed the characteristic signs of PSS. In general all pigs were better able to withstand the stress at 100 kg than at 50 kilograms. At 100 kg more of the pigs tolerated the entire 180 min. of heat treatment and showed less anxiety than those that withstood 180 min. at 50 kg bodyweight. Nevertheless, SS pigs continued to exhibit more PSS characteristics at 100 kg than SR pigs.

The apparent similarity in the observed response to heat stress of SS and SR pigs at 100 kg compared to that at 50 kg suggests that either SS pigs became more stress resistant or SR pigs became more stress susceptible with increased age and bodyweight, and repeated handling and exposure to the chamber.

Experiment 1 Environmental Chamber Studies

Insulin

Serum insulin of all pigs during heat stress (times 1 to 6, 21 to 26) and control environment (times 7 to 12, 27 to 32) are presented in appendix II.A. The mean control environment levels agree with those reported by Hertelendy *et al.* (1970), and with values reported by Swaitek *et al.* (1968) for young pigs.

TABLE 3. PERCENTAGE CHANGE IN SERUM INSULIN DURING HEAT STRESS COMPARED TO CONTROL CHAMBER ENVIRONMENT[a]

Time of sampling	SS 50 kg	SR 50 kg	SS 100 kg	SR 100 kg
Pre stress	54.3	25.3	-66.7	22.3
1 1/2 hr. of stress	-58.2	-35.9	-40.9	-41.5
Endpoint of stress	-37.2	-31.8	-50.4	-41.0
2 hr. post stress	69.5[b]	-19.1	-17.9	-32.8
4 hr. post stress	62.5	21.1	-16.7	-40.8
6 hr. post stress	-10.2	12.4	8.8[b]	-2.3

[a]Endpoint of stress sample was when heat stress was terminated because of high rectal temperature or anxiety conditions, or after 180 min. of heat stress.

[b]S.D. 3 times the mean percentage.

Serum insulin at 1 1/2 hr. of heat stress and at the endpoint of stress was lower than the corresponding levels taken during the control environment in both SS and SR pigs (thus the negative percentages shown in Table 3). However, none of these percentages was statistically significant ($P>.05$). Two and 4 hr. after the termination of heat stress the SS pigs at 50 kg had higher serum insulin than during the control environment while serum insulin of SR pigs remained nearer control levels (smaller percentage changes, Table 3). At 100 kg both SS and SR pigs had serum insulin levels similar to or lower than control samples at 2, 4 and 6 hr. post stress.

Zinc

At 50 kg bodyweight serum zinc was similar during and after heat stress compared to the control chamber environment (Table 4). At 100 kg SS pigs showed a significantly ($P<.05$) greater change in serum zinc at 1 1/2 hr. of heat stress than SR pigs. At 2 and 4 hr. post stress serum zinc of SS pigs was elevated and was higher than control values compared to SR pigs (Table 4).

TABLE 4. PERCENTAGE CHANGE IN SERUM ZINC DURING HEAT STRESS COMPARED TO CONTROL CHAMBER ENVIRONMENT

Time of sampling	SS 50 kg	SR 50 kg	SS 100 kg	SR 100 kg
Pre stress	-12.5[a]	15.4[a]	75.4	12.0
1 1/2 hr. of stress	-0.48	92.8[a]	86.5*	13.6*
Endpoint of stress	-3.24	-0.92	44.6	22.2
2 hr. post stress	19.2	-4.86	35.6	21.3
4 hr. post stress	-3.60	-5.47	45.8	21.8
6 hr. post stress	0.0	-4.02	60.3	84.8[b]

[a]S.D. larger than mean percentage.

[b]S.D. twice the mean percentage.

*$P<.05$

Cortisol

The SS and SR pigs showed greater changes in serum cortisol during heat stress at 50 kg than at 100 kg (Table 5). These results are consistent with those of Aberle et al. (1973) who showed only a slight increase in serum corticoids in replicate 2 during heat stress compared

to a large serum corticoid increase during heat stress in replicate one.

In the present study, SR pigs at 50 kg had a significantly ($P<.10$) larger increase than SS pigs in serum cortisol at the end of the heat stress period compared to the control chamber environment. Although not significant, SR pigs at 50 kg had more than twice the serum cortisol at 2, 4 and 6 hr. after stress compared to the same periods of control conditions, while SS pigs had decreased serum cortisol at the same time periods after heat stress (Table 5).

TABLE 5. PERCENTAGE CHANGE IN SERUM CORTISOL DURING HEAT STRESS COMPARED TO CONTROL CHAMBER ENVIRONMENT

Time of sampling	SS 50 kg	SR 50 kg	SS 100 kg	SR 100 kg
Pre stress	102.8[a]	128.4[a]	-21.9	-4.4
1 1/2 hr. of stress	46.2	68.6	-18.6	-53.2
Endpoint of stress	38.1*	213.8*	53.0	69.6
2 hr. post stress	-19.0	217.4	22.9	28.4[b]
4 hr. post stress	5.0	159.8	-76.7	149.3[c]
6 hr. post stress	74.3	213.4	-57.6	-15.1

[a]S.D. larger than mean.

[b]S.D. of 3 times mean.

[c]S.D. of 2 times mean.

*$P<.10$

At 1 1/2 hr. of heat stress in both SS and SR pigs serum cortisol increased during heat stress at 50 kg but serum cortisol decreased at 100 kg and the percentage changes were greater in SR pigs at both weights. At the endpoint of heat stress SS and SR pigs showed higher serum cortisol (higher percentages) compared to control conditions, but the differences were greater at 50 kg than at 100 kg bodyweight. Aberle et al. (1973) found a greater increase in serum corticoids of SR pigs than SS pigs during heat stress in replicate 1 but consistent with the present study at 100 kg, they found a smaller increase in plasma corticoids in both SS and SR pigs in replicate two. At 2, 4 and 6 hr. after heat stress SS pigs at 100 kg had nearly the same or lower serum cortisol compared to the same time periods after the control environment, while SR pigs had nearly the same or elevated serum cortisol after stress conditions (Table 5).

Thus, SS pigs appeared to show more anxiety during heat stress at 50 kg than at 100 kg and they had significantly ($P<.10$) less serum cortisol at the endpoint of heat stress at 50 kg than SR pigs. The lower serum cortisol during stress and the higher serum cortisol at 2, 4 and 6 hr. after heat stress are consistent with the data of Marple and Cassens (1973) who reported the metabolic clearance rate of cortisol was 5 times greater in SS compared to SR pigs.

Experiment 2 ACTH Injection

The preinjection blood sample was taken after the catheter had been flushed with 3.5% sodium citrate. The flushing procedure sometimes had a very traumatic effect on the pig. Some pigs became

visibly disturbed by the handling necessary to flush the catheter and draw the blood sample, and especially during the physical restraint necessary for the ACTH injection. Marple, Judge and Aberle (1972) found confinement in a psychrometric chamber to appear to be stressful to SS and SR pigs but found no difference in plasma adrenocorticoids between the groups of pigs while Judge et al. (1968b) found lower urinary corticoid metabolites in SS than in SR pigs confined in metabolism cages.

Zinc and Insulin

When compared to preinjection serum levels, serum zinc did not change at 1, 2 and 4 hr. after an 80 IU intramuscular injection of ACTH (Table 6). These results are not consistent with those of Flynn et al. (1972) who observed that serum zinc paralleled ACTH in rats subjected to control and stress conditions.

TABLE 6. PERCENTAGE CHANGE IN SERUM ZINC AND INSULIN FROM PREINJECTION LEVELS AFTER AN I.M. INJECTION OF ACTH

	Zinc		Insulin	
Time postinjection	SS	SR	SS	SR
0 hr.				
1 hr.	0.05	0.01	18.4	31.2
2 hr.	0.03	0.04	16.7	23.9
4 hr.	0.12	0.00	85.6	66.3

Serum insulin increased slightly at 1 and 2 hr. after ACTH injection in both SS and SR (Table 6). Serum insulin showed further increases (86 and 66% above 0 hr. for SS and SR pigs respectively) at 4 hr. after the ACTH injection (Table 6).

Cortisol and Corticosterone

Serum corticosterone changed very little (<20%) at 1, 2 and 4 hr. after ACTH injection compared to preinjection serum levels in both SS and SR pigs. Serum cortisol had decreased slightly (-12%) 1 hr. after ACTH injection in SR pigs and rose approximately 50% above 0 hr. levels in SS pigs. Serum cortisol decreased slightly from the 1 hr. serum level at 2 and 4 hr. postinjection in SS pigs, while SR pigs had increased serum cortisol 2 and 4 hr. after the ACTH injection from that at 1 hour. At 4 hr. postinjection SR pigs had nearly 50% more serum cortisol than preinjection serum levels as shown in Table 7, but cortisol at any time sampled was not significantly (P>.05) different from that of SS pigs.

TABLE 7. PERCENTAGE CHANGE IN SERUM CORTISOL AND CORTICOSTERONE FROM PREINJECTION LEVELS AFTER AN I.M. INJECTION OF ACTH

	Corticosterone		Cortisol	
Time postinjection	SS	SR	SS	SR
0 hr.				
1 hr.	17.0	14.2	48.9	-12.4
2 hr.	18.8	14.2	30.4	40.7
4 hr.	9.3	15.5	32.7	46.5

The differences in response of SS and SR pigs to ACTH injection do not agree with the results of Reichel and Braun (1962) who found a 3-fold increase in corticoids 5 hr. after an I.M. injection of 0.5 IU of ACTH. Lister, Lucke and Perry (1971) and Sebranek et al. (1973) reported a 3-fold rise in plasma cortisol 60 to 90 min. after an I.M. injection of ACTH which declined to double preinjection cortisol 2 hr. after injection in both SS and SR pigs. Sebranek et al. (1973) observed a 4-fold rise in plasma corticosterone 2 hr. after an I.M. injection of ACTH followed by a drop to double preinjection plasma corticosterone levels 3 hr. after injection.

Thus at the time periods sampled, SS pigs apparently showed the greatest response (increased serum cortisol) at 1 hr. after injection and then serum cortisol decreased from the 1 hr. level 2 and 4 hr. after injection (Table 7). In contrast to the SS pigs, the SR pigs showed little response at 1 hr. after injection but serum cortisol increased at 2 and 4 hr. after ACTH injection. The different serum cortisol responses in SS and SR pigs is in direct contradiction to the report by Lister, Lucke and Perry (1971) who described mesomorphic (Pietrain) and controls (Large White) as having similar response to an I.M. injection of ACTH. The SR pigs in the present study responded more like those of Reichel and Braun (1962), who reported a 3-fold increase in corticoids 5 hr. after a 0.5 IU injection of ACTH.

The more rapid increase in serum cortisol following an ACTH injection in SS pigs followed by a decrease in serum cortisol 2 and 4 hr. after ACTH injection is in direct agreement with the findings of Marple and Cassens (1973) who observed that SS pigs have a 3-fold

higher turnover of cortisol. In the present study the SR pigs also had 50% more cortisol at 4 hr. after ACTH injection than at 0 hour. The increase (50%) in serum cortisol in SS directly refutes the reports of Marple et al. (1972) and Judge et al. (1968b) that SS pigs have deficiencies in glucocorticoid production.

Experiment 3 In Vitro Incubation

The cortisol and corticosterone content of adrenal glands from SS and SR pigs responded similarly in vitro and also contained similar levels of these corticoids immediately post exsanguination (Table 8). Adrenal tissue from SS and SR pigs incubated for 2 hr. without ACTH showed similar increases in cortisol and corresponding similar decreases in corticosterone from that of adrenal slices frozen immediately post exsanguination. Corticosterone increased slightly and cortisol increased several fold in adrenal tissue from SS and SR pigs when ACTH was added to the incubation medium compared to that without ACTH (Table 8).

TABLE 8. ADRENAL CONTENT OF CORTISOL AND CORTICOSTERONE AND INCUBATION WITH AND WITHOUT ACTH[a]

	Corticosterone		Cortisol	
Incubation treatment	SS	SR	SS	SR
Preincubation	63.6	42.3	97.3	92.3
2 hr. incubation	27.3	32.1	192.8	197.4
2 hr. incubation with ACTH	45.6	49.6	519.2	660.7

[a]Total cortisol and corticosterone expressed as ng/mg adrenal tissue

Although the quantity of ACTH used in the incubation and the time of incubation was similar to that used by Dvorak (1972) he reported mean production of total 17-OHCS of 86 ng/mg of adrenal tissue. The values shown in Table 8 are considerably higher, even without ACTH in the incubation medium than those reported by Dvorak (1972). However, Dvorak (1972) discarded the preincubation (1/2 hr.) medium before his 2 hr. incubation and may have also discarded much of the cortisol production. In both SS and SR pigs mean corticosterone synthesis decreased while cortisol synthesis more than doubled without ACTH. These results may be accounted for by a shift in corticoid precursors required for corticosterone synthesis to those for cortisol synthesis. Also, these results may possibly be due to decomposition of corticosterone in the incubation medium.

These data suggest that there were no differences in the adrenal content of cortisol and corticosterone between the groups of pigs and that there were no differences in the ability of adrenal tissue from SS and SR pigs to synthesize cortisol or respond to ACTH. The similar response *in vitro* of adrenal tissue from SS and SR pigs is in direct contradiction to the reports of Marple *et al.* (1972) and Judge *et al.* (1968b) that SS pigs have deficiencies in glucocorticoid production.

Adrenal Morphology

No apparent differences were found in corticol morphology in adrenal glands of SS and SR pigs. Ludvigsen (1968) reported that the adrenal cortex of Poland China pigs was thicker than that of Chester White pigs but he made no attempt to categorize them as SS or SR. The zona

fasciculata and zona reticularis appeared to be of similar width, with only slight variation, in adrenals of SS and SR pigs. Cell integrity also appeared to be similar between the two groups. These data do not support the conclusion of Cassens et al. (1968) who reported that SS pigs had a degenerative adrenal cortex compared to SR pigs.

SUMMARY

Three separate experiments using pigs determined to be either SS or SR were conducted for this study. In Experiment 1, changes in serum cortisol, zinc and insulin during and after heat stress were compared to serum levels at ambient conditions in both SS and SR pigs at 50 and at 100 kg bodyweight. In Experiment 2, serum cortisol, corticosterone, zinc and insulin were measured after an I.M. injection of ACTH in SS and SR pigs. The cortisol and corticosterone content of adrenal glands from SS and SR pigs was measured, as well as the *in vitro* synthesis of both corticoids with and without ACTH in the incubation medium in Experiment 3. Serum insulin was quantified by radioimmunoassay and serum zinc by atomic absorption spectrophotometry, and serum and *in vitro* corticoids were isolated on Sephadex LH-20 columns and quantified by protein binding assay.

During heat stress, serum insulin was lower than levels at ambient conditions in both SS and SR pigs at 50 and 100 kg bodyweight. At 50 kg serum zinc was similar during and after heat stress compared to the ambient chamber environment in SS and SR pigs. However, at 100 kg SS pigs had a significantly greater ($P<.05$) increase in serum zinc at 1 1/2 hr. of heat stress (compared to ambient conditions) than SR pigs. The SR pigs had significantly ($P<.10$) more serum cortisol at the endpoint of heat stress compared to control conditions than SS pigs at 50 kg. SR pigs also had greater serum cortisol after heat stress than SS pigs at 50 kg. At 100 kg SR pigs had nearly the same or elevated serum cortisol after heat stress while SS pigs showed a corresponding decrease in serum cortisol after stress.

In Experiment 2, SS pigs responded to an I.M. injection of ACTH with increased serum cortisol 1 hr. after injection which decreased slightly at 2 and 4 hr. after injection compared to the 1 hr. postinjection level. The SR pigs had little change in serum cortisol 1 hr. after injection, but serum cortisol had increased at 2 and 4 hr. after injection. The increase in cortisol at 1 hr. after injection in SS pigs and after 4 hr. in SR pigs was nearly identical. Serum zinc did not change after the ACTH injection and serum insulin increased only at 4 hr. in both SS and SR pigs.

The content of cortisol and corticosterone of adrenal tissue from SS and SR pigs was not different in the in vitro study in Experiment 3. Likewise, the quantity of cortisol and corticosterone synthesized in vitro by adrenal tissue from SS and SR pigs during the 2 hr. incubation without ACTH was similar. The increase in cortisol synthesized in response to ACTH addition to the incubation medium was not different between adrenal tissue of SS and SR pigs.

No differences in morphology of the adrenal cortices between SS and SR pigs were apparent.

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APPENDIX I.

A. Reagents for radioimmunoassay

1. Buffer A
   $NaH_2PO_4 \cdot 2H_2O$---------- 6.2 g
   Merthiolate---------- 0.25 g
   BSA (Fraction V, sterile, 35% solution
   serological, NBC, Cleveland)---------- 14.6 ml
   Add 950 ml distilled H_2O.
   Adjust pH to 7.4.
   Dilute to 1 l, store at 4 C.

2. Buffer B
   NaCl---------- 9.0 g
   Dissolve in 1 l Buffer A.
   Store at 4 C.

3. Insulin standards
   Weigh 200-500 µg insulin (wt PJ-5682) on a Cahn
   Electrobalance.
   Dissolve in 0.85% saline (pH 5.0) to 1 mg/ml.
   Make stock insulin solution to 500 ng/ml in Buffer B.
   Further dilute to working standards of:
   0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.5,
   2.0, 3.0, 4.0, 5.0, 10.0 ng/100 µl.
   Multiply each standard concentration times 23.9 µU/ng
   and express serum in µU/ml.
   Dispense each standard in culture tubes at a quantity
   suitable for one assay (2 ml).
   Freeze and store at -20 C.

4. 0.05 disodium ethylenediamine tetraacetate
   (EDTA) - PBS, pH 7.0.
   Disodium EDTA---------- 18.612 g
   Add 950 ml PBS.
   Adjust pH to 7.0.
   Dilute to 1 l and store at 4 C.

5. Guinea pig anti-porcine insulin serum (GPAPI)
   Dilute antisera to 1:400 in 0.05 M PBS-EDTA.
   Dispense in small quantities, store at -20 C.
   On day of use, dilute the 1:400 antisera to 1:100,000 in
   1:400 NGPS (normal guinea pig serum diluted 1:400 in
   0.05 M PBS-EDTA, pH 7.0).

6. Dilute ^{125}I insulin in buffer A to an activity of
   13,000 cpm/100 µl
   Store at 4 C.

7. Sheep anti-guinea pig gamma globulin (SAGPGG)
   Dilute antisera in 0.05 M PBS-EDTA, pH 7.0, to concentration which will give maximum binding of ^{125}I insulin by GPAPI at 1:100,000 dilution.
   Store at 4 C.

8. 0.01 M phosphate buffered saline (PBS), pH 7.0

NaCl	43 g
Monobasic phosphate (69.05 g $NaH_2PO_4 \cdot H_2O$ in 1 l)	120 ml
Dibasic phosphate (70.98 g Na_2HPO_4 in 1 l)	240 ml
Merthiolate	1.75 g

   Dissolve in water and dilute to 17.5 l with distilled water.
   Adjust pH to 7.0 and store at 4 C.

B. Reagents for protein binding assay and separation

1. Mini column chromatography solvent

Redistilled chloroform	99 ml
Redistilled methanol	1 ml
Distilled water (saturated)	<0.5 ml

2. Sephadex LH-20 columns (mini columns)
   Sephadex LH-20 was equilibrated at least 12 hr. with solvent.
   The columns were 0.5X12 cm pipettes fitted with 12 ml top reservoirs.
   The bottom of the column was plugged with cotton, and the cotton was washed with solvent.
   The Sephadex was poured to a height of 10 cm and a plug of cotton was fitted on top of the column.
   The packed column was washed with 10 ml solvent and then grouped with four others with similar flow rates.

3. 1.25% dog plasma with 20,000 cpm/ml
   Dog plasma (Colorado Serum Co.) was diluted to 2.5% in 1 l distilled water.
   Dog plasma was vortexed with 60 g Florisil (80 mesh; Matheson Coleman and Bell) for 3 hr.
   The suspension was centrifuged at 2500 x g for 15 min. and the supernatant volume was doubled with water to give 1.25% plasma.
   Repurified (by column chromatography, as above) 1,2-^{3}H-cortisol in ethanol was added to the 1.25% plasma to 20,000 cpm/ml.
   This solution was used for both cortisol and corticosterone assay for up to 1 month when stored at 4 C.

4. Dextran coated charcoal
   Wash neutral norit with water, then wash with methanol and oven dry.
   Washed neutral norit---------- 0.50 g
   Dextran 150---------- 0.050 g
   Glass distilled water---------- 100 ml
   Store at 4 C for at least 12 hr. before use.

5. Large column chromatography solvent (2)
   Redistilled chloroform---------- 98 ml
   Redistilled methanol---------- 2 ml

6. Sephadex LH-20 columns (large columns)
   Sephadex LH-20 was equilibrated at least 12 hr. with solvent (2)
   The columns were 1X40 cm pyrex glass fitted with a 250 ml top reservoir.
   The bottom of the column was plugged with cotton, the cotton was washed with solvent and air bubbles were removed.
   The Sephadex was poured to a height of 40 cm and a cotton plug was fitted on top of the column.

C. Other reagents

1. Krebs-Ringer Bicarbonate Buffer
   Stock solutions
   1) KCl---------- 5.75%
   2) $CaCl_2$---------- 6.10
   3) KH_2PO_4---------- 10.55
   4) $MgSO_4 \cdot 7H_2O$---------- 19.10
   5) NaCl---------- 0.90
   To make a working Krebs-Ringer salt solution, mix 8 ml of (1), 6 ml of (2), 2 ml of (3), 2 ml of (4) and add water to 90 ml. This working solution may be stored at 4 C for up to 1 week.
   Weigh 1.30 g $NaHCO_3$ in a 100 ml cylinder, add water to 100 ml and gas with CO_2 for at least 30 min. Prepare immediately before use each day.
   Weigh 130 mg glucose into a 250 ml erlenmeyer flask, add 100 ml saline, add 9 ml Krebs-Ringer salt solution from above, add 21 ml $NaHCO_3$ and gas with O_2/CO_2 (95.5) for at least 15 min.

2. Hematoxylin and eosin stain
   Fixative:
   10% formalin solution
   37.40% formaldehyde---------- 100 ml
   Distilled water---------- 900 ml

Autotechnicon - dehydrating, clearing and infiltration

1) 70% ethyl alcohol---------- 1 hr.
2) 70% ethyl alcohol---------- 1 hr.
3) 80% ethyl alcohol---------- 1 hr.
4) 95% ethyl alcohol---------- 1 hr.
5) 95% ethyl alcohol---------- 1 hr.
6) Absolute ethyl alcohol---------- 1 hr.
7) Absolute ethyl alcohol---------- 1 hr.
8) Xylene---------- 1 hr.
9) Xylene---------- 1 hr.
10) Paraffin (paraplast)---------- 1 hr.
11) Paraffin (papaplast)---------- 2 hr.
12) Embed in paraffin (papaplast) and cool rapidly.

Dehydration - clearing and infiltration (without Autotechnicon)

1) Fixative - 10% formalin
2) Wash tap water---------- overnight
3) 50% ethyl alcohol---------- 4 hr.
4) 70% ethyl alcohol---------- 4 hr.
5) 80% ethyl alcohol---------- 2 hr.
6) 95% ethyl alcohol (3 changes)---------- 1 hr. each
7) Absolute ethyl alcohol (2 changes)---------- 1 hr. each
8) Absolute alcohol and terpinol (1:1, v/v)---- overnight at 2°C
9) Paraffin (4 changes)---------- 1 hr. each at 60°C
10) Embed as described above.

Stock solutions and staining procedures

Solutions

Hematoxylin and eosin

Eosin (stock solution)

Eosin---------- 1 g
95% ethyl alcohol---------- 100 ml
Dilute 1:1 with distilled water before use.

Harris hematoxylin (stock solution)

Hematoxylin---------- 2.5 g
Aluminum ammonium sulfate---------- 50.0 g
Mercuric oxide---------- 1.25 g
Absolute alcohol---------- 25.0 ml
Distilled water ---------- 500.0 ml
Dilute 1:1 with distilled water and filter before use.

Staining

1) Xylene---------- 3 min.
2) Xylene---------- 3 min.
3) Absolute alcohol---------- 2 min.
4) Absolute alcohol---------- 2 min.
5) 95% ethyl alcohol---------- 2 min.
6) 80% ethyl alcohol---------- 2 min.
7) 70% ethyl alcohol---------- 2 min.
8) 50% ethyl alcohol---------- 2 min.

9) Distilled water---------- rinse until clear
10) Harris hematoxylin---------- 5 min.
11) Tap water---------- rinse until clear
12) Acid alcohol (0.25% HCl)---------- 5 dips
13) Ammonia in tap water---------- dip until blue
14) Distilled water---------- rinse
15) Eosin---------- 1 min.
16) 95% ethyl alcohol---------- wash (2-5 dips)
17) Absolute alcohol---------- wash (5-10 dips)
18) Xylene---------- 2 min.
19) Xylene---------- 5-10 min.
20) Remove excess moisture from freshly stained slide and mount in permamount and coverslip.

Staining procedure for eosin (Experiment I)

Follow procedure for hematoxylin and eosin except for steps 9-13.

Solutions and staining procedures of nerve fibers

Fixative - 10% formalin

Solutions

1) Luxol fast blue solution
   Luxol fast blue---------- 0.1 g
   95% ethyl alcohol---------- 100.0 ml
   Add 5 ml of 10% acetic acid to each.
   1000 ml of solution. Solution is stable.
2) Cresyl-echt violet solution
   Cresyl-echt violet acetate---------- 0.1 g
   Distilled water---------- 100.0 ml
   Just before using, add five drops of 10% acetic acid to every 30 ml of solution and filter.
3) Lithium carbonate solution
   Lithium carbonate---------- 0.05 g
   Distilled water---------- 100.0 ml

Staining procedure

1) Deparaffinized through xylene and absolute alcohol and then through several changes (minimum of 3) of 95% alcohol.
2) Stain overnight (16 to 24 hr.) in Luxol fast blue solution at 37°C.
3) Wash in 95% alcohol to remove excess stain.
4) Wash in distilled water.
5) Begin differentiation by quick immersion in 0.05% lithium carbonate.
6) Continue differentiation in 70% alcohol until gray and white matters can be distinguished.
7) Wash in distilled water.

8) Finish differentiation by rinsing briefly in 0.05% lithium carbonate and then put through several changes (minimum of 3) of 70% alcohol until the white matter contrasts (greenish blue) sharply with the gray matter (colorless).
9) Wash thoroughly in distilled water.
10) Stain for 6 min. in warm cresyl violet solution.
11) Differentiate in several changes of 95% alcohol.
12) Dehydrate in absolute alcohol, clear in xylene and mount.

APPENDIX II

A. Heat Stress and Control Temperature Data at 50 kg (Experiment 1)

Pig No.	Time 1			Time 2			Time 3			Time 4			Time 5			Time 6		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1-1	0.743	25.3	103.4	0.514	9.5	310.0	0.476	7.6	242.6									
2-1	0.820	88.0	37.1	0.405	14.0	44.4	0.794	14.3		1.025	28.6	76.6				0.547	26.2	23.5
3-1	1.008	78.2	34.0	0.860	34.6	50.6	0.897	33.8	62.0	0.800	80.6	36.8	0.900		50.5	0.987	41.1	
4-1	0.648	43.3	43.8	0.486		59.8	0.917	19.1	56.6	0.768	47.9		0.832	60.7	24.4	0.732	53.9	14.9
5-1	0.887	25.4	38.3	1.393	38.4	55.3	0.670	27.0	19.3		97.7	42.7	0.786	115.2	44.2	0.752	35.5	52.2
6-1	0.811	32.9	67.3	0.616	15.5	169.5	0.689	15.1		0.788	27.8	68.0	0.665	34.8	41.2	0.557	47.0	21.1
7-1	0.840	39.7	66.8	0.908	23.9	100.7	1.006	27.2	67.6	1.139	35.5	28.6	0.719	32.7	38.8	0.894	32.2	
1-2	0.789	19.7	51.6	0.743	24.0	58.2	0.653	23.0		0.660	20.8	29.6	0.750	64.0		0.713	39.2	
3-2	0.992	71.2	87.2	2.080	11.1	72.0	0.588	48.9	190.5	0.708	43.7	153.3	0.499	37.9	120.4	0.457	54.1	54.4
5-2	0.806	29.6	35.6	1.601	25.6	74.2	0.731	14.4	49.3	0.654	29.5	39.8	0.843		61.9	0.849	15.7	79.0
6-2	0.865	42.5	52.4	0.787	25.8	290.2	0.511	22.1		0.529	27.3	240.8	0.433	36.1	182.0	0.712	20.0	21.6

1 = Zinc; ppm

2 = Insulin; μU/ml

3 = Cortisol; ng/ml

A. Heat Stress and Control Temperature Data at 50 kg (Experiment 1)

Pig No.	Time 7			Time 8			Time 9			Time 10			Time 11			Time 12		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1-1	0.934	32.0	53.7	0.890	32.0	90.9	1.050	37.8	91.7	0.940	37.2	47.3	1.063	50.2	49.0	1.241	43.6	139.4
2-1	0.936	86.9		0.802	66.3		0.828	48.0		0.962			0.960	28.3		0.865	48.1	
3-1	0.920	16.2	9.4	0.744	25.7	61.0	0.756	29.3		0.760	23.1	286.2	0.682	17.6	151.2	0.534	24.7	144.4
4-1	0.893	24.5	43.3	0.542	67.0	68.7	0.911	43.1	42.1	0.946	38.7		1.192	30.8	60.0	0.913	36.0	25.0
5-1	1.117	28.2	13.4	1.037	59.5	39.1	1.124	56.1	58.0	0.987	37.1	31.8	1.177	34.0	56.0	1.019	35.7	
6-1	0.784	33.2		0.481	87.8		0.544	24.1	154.8	0.604	44.1	48.2	0.575	49.2	29.8	0.640	114.8	112.1
7-1	1.042	77.4	91.7	0.752	97.3	128.1	0.771	22.8	56.2	0.659	73.4	79.5	0.735	75.1	21.1	0.806	115.9	95.1
1-2	0.960	46.7	259.9	0.992	18.7	158.4	0.752	30.2	53.8	0.863	23.4	236.0	0.961	30.2	54.7	0.868	25.5	40.8
3-2	0.952	31.1	65.9	0.614	76.2	93.8	0.491	67.6	53.4	0.595	48.7	96.2	0.646	50.6	44.4	0.973	80.0	142.0
5-2	0.568	94.1	10.4	0.746	64.0	25.4	0.651	39.2	18.2	0.746	42.1	7.8	0.575	32.4	17.2	0.609	27.5	6.6
6-2	0.649	21.4	12.5	0.550	35.2	108.0	0.660	25.3	34.9	0.543	36.4	41.0	0.568	47.2	122.8	0.617	27.8	119.0

1 = Zinc; ppm

2 = Insulin; μU/ml

3 = Cortisol; ng/ml

B. Heat Stress and Control Temperature Data at 100 kg (Experiment 1)

Pig No.	Time 21			Time 22			Time 23			Time 24			Time 25			Time 26		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3-1	1.581	24.4	32.4	1.111		47.4	2.000	18.9	67.4	1.569	22.7	42.2	1.200	32.5	26.5	1.072	35.9	26.8
4-1	0.822	38.7	37.0	0.736	28.0		0.842	23.9		1.086	37.3	66.1	0.964		6.4	0.601	29.8	14.4
8-1	1.086	29.6	39.1	1.282	25.4	102.5	0.936	26.4	93.1	0.966	35.3	46.2	0.878	31.9	11.3	0.887	50.0	14.9
1-2	0.674	29.6		0.865	24.8	51.8	0.640	17.4	143.2	0.757	16.5	76.6		17.8	19.4	0.556	21.8	23.2
3-2	1.247	39.8	44.0	1.228	18.2	80.2	1.167	21.9	48.3	1.153	34.0	118.5	0.772	29.2	21.6	0.840	33.2	41.1
4-2	1.339	36.9	53.9	0.963	34.4		1.111	28.2		0.896	37.8		0.887	47.3	46.4	0.862	64.2	91.3
6-2	0.961	27.6	54.0	0.898	18.7	19.5	0.970	17.1		0.746	19.4		0.908	18.9	29.2	0.673	27.4	
7-2	0.864	42.0	90.4	0.772	18.5	87.0	0.870		153.6	0.648	34.6	37.5	0.786	47.8	130.7	0.804	41.2	71.5

1 = Zinc; ppm

2 = Insulin; μU/ml

3 = Cortisol; ng/ml

B. Heat Stress and Control Temperature Data at 100 kg (Experiment 1)

Pig No.	Time 27			Time 28			Time 29			Time 30			Time 31			Time 32		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3-1	0.568	87.8	54.2	0.809	36.9	85.9	1.488		32.9	1.357	26.3	32.1	0.833		87.8	0.533	32.3	57.7
4-1	0.723	99.9	38.4	0.441	48.0	108.3	0.713	70.2	30.9	0.524	48.8	49.2	0.622	46.1	77.9	0.483	44.0	40.5
8-1	0.809			0.502	42.4	95.2	0.516	40.5	92.0	1.152	42.2	44.9	0.634	38.3	35.8	0.571	33.9	33.0
1-2	0.793	29.6	55.6	0.680		139.2	0.578		74.8	0.675	39.4		0.438	56.2	3.4	0.422		114.9
3-2	1.084	27.8	126.0	1.039	36.6	216.8	0.962	43.0		0.902	54.7	61.8	0.886	52.7		0.937	30.6	57.0
4-2	0.901	66.0	117.2	0.713	64.8		0.746	42.0	37.0	0.591	64.7		0.712			0.656	57.4	50.0
6-2	0.968		202.2	0.918		44.4	0.887		240.7	0.714	27.4		0.650	38.0	39.7	0.618	39.5	37.9
7-2	0.771	22.1	32.9	0.861	25.4	125.6	0.722	31.0	103.9	0.586	33.6	57.7	0.584	47.8	126.1	0.174	40.8	110.7

1 = Zinc; ppm

2 = Insulin; μU/ml

3 = Cortisol; ng/ml

C. ACTH Injection Data (Experiment 2)

Pig No.	Time 0				Time 1				Time 2				Time 3			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
2-1	0.530	31.0	33.4	125.2	0.752	96.1	50.4	159.2	0.861	74.7	39.0	19.4				
3-1	0.631	25.2	14.4	31.4	0.630	49.7	24.9	112.8	0.661	63.9	49.4	106.0	0.425	68.0	19.4	100.4
4-1	0.511	145.4	14.6	66.8	0.540	175.4	58.2	95.2	0.640	143.5	50.0	52.2	1.224	210.6	10.0	75.0
6-1	0.680	94.7		24.3	0.693	88.3	30.9	167.6	0.558	81.6	43.4	220.2	0.607	129.5	10.8	121.8
7-1	0.589	63.5	39.5	151.6	0.583	42.5	36.4	109.1	0.371	79.5	38.8	153.6	0.622	263.0	67.2	107.6
1-2	0.373	78.1	14.8	119.4	0.511	49.5	25.2	143.8	0.429	74.4	22.0	152.1	0.440	159.5	34.2	128.5
2-2	0.931	31.2	23.6	130.2	0.752	55.8	15.1	164.6	0.672	50.5	34.6	192.6	0.673	60.3	22.6	162.5
3-2	0.488		9.4	87.5	0.578	250.0	27.6		0.538	268.1	39.0	168.8	0.631	212.4	45.6	186.3
7-2	0.602	86.2	26.8	144.6	0.666	139.9	59.0	40.0	0.672	145.7	39.8	174.7	0.734	202.0	50.5	245.3
8-2	0.733	91.8	39.4	193.6	0.651	166.9	57.9	189.8	0.619	112.2	49.6	190.6	0.657	130.6	38.7	185.4

1 = Zinc; ppm

2 = Insulin; μU/ml

3 = Corticosterone; ng/ml

4 = Cortisol; ng/ml

D. In Vitro Data (Experiment 3)

Left Adrenal

Pig No.	A		B		C	
	1	2	1	2	1	2
2-1	46.5	41.4	14.6	123.8	26.0	357.5
3-1	48.5	173.3	49.7	259.9	45.6	612.4
4-1	21.8	19.3	10.8	200.9	64.4	532.9
8-1	64.3	167.1	28.6	314.9	51.7	835.0
1-2	54.7	83.1	24.8	154.8	17.8	528.4
3-2	107.0	158.2	102.1	409.7	59.9	996.5
4-2	64.0	115.2	27.1	205.6	88.0	592.4
6-2	9.7	71.3	5.0	97.0	31.8	929.1
7-2	77.0	103.6	16.7	171.0	29.0	615.3

Right Adrenal

Pig No.	A		B		C	
	1	2	1	2	1	2
2-1	58.7	63.0	7.6	136.6	49.4	491.9
3-1	143.4	135.4	23.5	183.3	20.1	393.9
4-1	64.4	37.8	32.2	107.9	37.2	283.8
8-1	61.0	140.8	51.3	215.8	69.2	646.0
1-2	43.8	59.4	20.8	84.3	58.0	397.9
3-2	24.4	172.8	15.8	410.0	114.4	1006.0
4-2	13.9	54.8	57.0	109.0	36.4	483.5
6-2	5.8	29.1	22.5	106.6	19.2	459.2
7-2	22.7	75.0	29.0	225.9	41.1	598.6

1 = Corticosterone; ng/mg

2 = Cortisol; ng/mg