Porcine Adrenocortical Lipids in Relation to Striated Muscle Characteristics.* (30673)

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(Introduced by W. G. Hoekstra)
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Rate and extent of post-mortem glycolysis in porcine muscle may markedly affect ultimate muscle appearance(1). Pale, soft, exudative muscle often results when post-mortem pH decline is rapid and values approaching pH 5.5 are reached while the muscle temperature is still near body temperature(1-3). Ludvigsen(4) suggested that porcine animals having a rapid rate of post-mortem glycolysis are often deficient in adrenal steroids, and Judge *et al*(5) have recently obtained evidence that such animals excrete lower than normal amounts of adrenal steroids in the urine.

In view of this information we designed an experiment with the following objectives: (a) to examine the porcine adrenal gland with a histochemical method (Sudan black B) in order to ascertain the amount and distribution of lipid material which might be indicative of functional status of the gland, and (b) to determine the association of the type and quantity of sudanophilic material in the adrenal cortex with post-mortem muscle glycolysis and associated properties in 2 porcine breeds known to differ in rate of muscle anaerobic glycolysis(6).

Methods. Thirty-two Poland China (fast glycolysis) and 15 Chester White (normal glycolysis) castrated male porcine animals, approximately 6 months of age, were used in this experiment. All animals were maintained at ambient temperatures of 21-24°C. Immediately after the animals were exsanguinated, the left adrenal gland was removed and portions of it were placed in 10% formol-calcium for subsequent histochemical test. Ten-micron sections were cut in a cryostat and stained with Sudan black B. Quantities of sudanophilic fine granules distributed throughout the cortex and quantities of sudanophilic large masses in the zona reticulares were each scored on a scale of 0 to 5 with 5 representing the greatest quantity. Other portions of the glands were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

At 45 minutes and 24 hours post-mortem, samples of the *longissimus dorsi* muscle were excised for pH determination. Post-rigor sarcomere length(7) was measured in the *longissimus dorsi* of randomly selected animals at 24 hours post-mortem.

Results and discussion. Examination of the stained adrenal glands revealed that the sudanophilic material appeared as two general types: (a) fine granules and (b) large masses. The cortices of both porcine breeds had varying amounts of fine sudanophilic granules throughout the zona fasciculata and zona reticularis. However, of greater interest was the frequent appearance of large masses of sudanophilic material localized in the zona reticularis (Fig. 1) of adrenal glands from the Poland China animals. These large masses of lipid were seen very infrequently in the glands from Chester White pigs. 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. The staining reaction was so heavy that numerous cells appeared as completely black, opaque bodies. This phenomenon is probably not due to a pigment accumulation in the zona reticularis since examination of several sections with hematoxylin and eosin revealed no apparent accumulation of granules or pigment.

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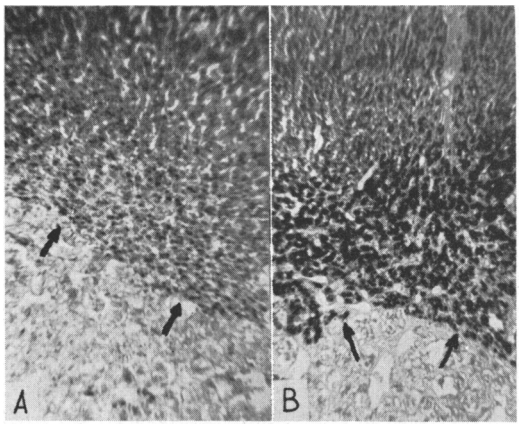

FIG. 1. Sudanophilic material in zona reticularis. Cortico-medullary junctions are shown by arrows. A. Uniform distribution of lipid fine granules. B. Invasion by lipid masses.

Poland China animals had significantly greater amounts ($P<.01$) of sudanophilic masses in the zona reticularis (Table I) than did the Chester White animals. The Chester White animals showed significantly more fine granules ($P<.05$) than did the Poland Chinas.

The importance of the heavy sudanophilic masses in the zona reticularis of the Poland China animals is not known. However, the masses may be indicative of a degenerative process since there is a predisposition in the Poland China animals to be particularly susceptible to stress conditions and also to have extremely rapid rates of post-mortem anaerobic glycolysis in certain muscles. Further support is added to the hypothesis of cortical degeneration by the work of Judge *et al*(5) which indicated that porcine animals with rapid post-mortem muscle glycolysis tended to excrete low levels of urinary steroids. Meyers and Charipper(8) reported that the adrenal cortex of the golden hamster, which has a lipid distribution similar to that found in the adrenal cortex of ungulate animals, undergoes age-associated degeneration and lipid invasion of the zona reticularis. In the present study age of the animals was relatively constant, but the quantity of zona reticularis lipid was correlated with body weight in the Poland China animals ($r = 0.44$, $P<.05$).

Table II presents the correlation coefficients between adrenocortical lipid scores and post-mortem muscle parameters. The zona reticularis lipid masses in the combined breeds were possibly related to a more rapid rate of pH decline in muscle as measured by 45-minute post-mortem pH values. The cortical fine granules were related to a higher ultimate pH (24 hours) in all cases. Correlations in Table II also reveal that Poland China animals with heavy lipid depositions in the zona reticularis had more highly contracted muscle post-rigor as indicated by their shorter sarcomeres.

The data indicate that porcine animals with muscle that undergoes a rapid post-mortem pH decline, develops low ultimate pH and becomes rather highly contracted, may have adrenal cortices with small amounts

TABLE I. Adrenocortical Lipid Scores* in 2 Porcine Breeds.

Adrenocortical lipids	Poland China (32)†	Chester White (15)†
Fine granules	1.2 ± .2‡	2.1 ± .4
Zona reticularis masses	1.4 ± .3§	.1 ± .1

* Histological rating 0-5 for quantity of sudanophilic material; mean ± standard error of mean.
† No. of animals.
‡ $P<.05$ significant mean difference.
§ $P<.01$ significant mean difference.

TABLE II. Relation of Adrenocortical Lipids to Post-Mortem Muscle Characteristics in Two Porcine Breeds.*

Muscle parameter	Adrenocortical lipids† Fine granules	Zona reticularis masses	Breed§
pH,‡ 45 min post-mortem	0.05	−.06	PC (32)‖
	−.39	−.22	CW (15)
	0.15	−.32¶	All (47)
pH,‡ 24 hr post-mortem	0.40¶	−.05	PC (32)
	0.56¶	0.41	CW (15)
	0.51**	−.09	All (47)
Sarcomere length,‡ 24 hr post-mortem (μ)	0.18	−.58¶	PC (13)
	−.21	−.06	CW (8)
	0.04	−.46¶	All (21)

* Correlation coefficients.
† Histological rating 0-5 for quantity of sudanophilic material.
‡ *Longissimus dorsi.*
§ PC = Poland China, CW = Chester White.
‖ Number of animals.
¶ $P<.05$.
** $P<.01$.

of fine granular lipid material and heavy invasions of large lipid masses in the zona reticularis.

Summary. Large lipid masses were found in zona reticularis of certain porcine animals and it was suggested that they might represent a degenerative change. Correlation coefficients indicated that the zona reticularis lipid masses may be associated with a rapid post-mortem pH decline in muscle and a short post-mortem sarcomere length.

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Lipogenesis and Cholesterol Turnover in the Chick as Influenced by Dietary Lithocholic Acid. (30674)

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Dietary lithocholic acid markedly elevates plasma lipids in the chicken(1). The ingestion of this bile acid also results in an increased liver size and a proliferation of bile ductules(1,2). The mechanism(s) by which plasma lipids are elevated as a consequence of lithocholic acid ingestion remains obscure. Elevated plasma lipid levels might result as a consequence of increased synthetic rates and/or a decreased rate of turnover.

The data presented here were obtained in an effort to shed light on the mechanism(s) responsible for the lipemia observed in lithocholic acid-fed chicks.

Materials and methods. Male White Leghorn Hy-line chicks were fed a stock ration prior to being fed the experimental diets. Chicks used in these experiments were fed the lithocholic acid supplemented diet for at least 3 weeks, a period previously shown to be sufficient for the induction of the reported hyperlipemia and ductular hyperplasia(1,2). The diets were identical to those used in previous studies(1) and were fed on an *ad libitum* basis as was water.

The chicks used for *in vitro* studies were sacrificed by cervical fracture and the liver was quickly excised, weighed and slices prepared from the left lateral lobe with a Stadie-Riggs hand microtome. The slices were weighed on a rapid weighing torsion balance and approximately 100 mg of slices were incubated in 25 ml Erlenmeyer flasks having 1.5 × 3 cm glass center wells. The main compartment contained 3.0 ml of Krebs-Ringer bicarbonate buffer pH 7.4. The center wells contained a 2 cm × 2 cm piece of Whatman #1 filter paper and the flasks were capped with rubber plasma stoppers. Incubations were carried out in an atmosphere of 95% O_2-5% CO_2 at 38°C in a metabolic shaker (90 strokes/min). At the completion of the 3-hour incubation period, 0.1 ml of 25% KOH was introduced through the rubber cap with a syringe onto the filter paper and 0.5 ml of 0.2 N H_2SO_4 into the main compartment to insure complete liberation of CO_2 from the medium. Following the incubation, $^{14}CO_2$, fatty acid-^{14}C and cholesterol-^{14}C were determined as previously outlined (3).

Liver used for enzyme assays was homogenized with cold 0.15 M KCl, the homogenate was centrifuged at 1000 × *g* for 15 minutes and the supernatant was used for assay. Glucose-6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconate dehydrogenase (6-PGD) were measured separately by the meth-