HISTOLOGY OF THE ADRENAL GLAND AS RELATED TO MUSCLE PROPERTIES

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In the studies conducted on porcine adrenal histology at the University of Wisconsin, we have attempted to relate the staining characteristics of the cells of the cortex to adrenal hormone production, breed of animal, and postmortem muscle properties (3,7). This research was begun to help clarify the role of adrenal gland function as it may influence meat quality. The findings of Ludvigsen (10), Adis et al. (1), and workers at the Wisconsin Station (5, 6, 8) have indicated that the secretions and size of the adrenal gland are related to ultimate color, structure, tenderness, and other properties of muscle.

A review of the general and comparative features of mammalian adrenal morphology may provide a framework within which the results of the work may be evaluated. The adrenal cortex and adrenal medulla differ in embryonic origin, morphology, and type of secretion. The chromaffin cells of the medulla produce the quick-acting catecholamines (epinephrine and norepinephrine) which are elaborated during the early responses to stress. The cortex produces many steroid hormones that are important in several physiological and biochemical processes.

The adrenal cortex of better-known laboratory animals is divided into three distinct zones (Figure 1). The zona glomerulosa, the most peripheral functional zone, has been shown to produce the principal quantities of the mineralocorticoids, those hormones which influence salt and water balance in the body. The next zone, the zona fasciculata is usually broad and often has heavy lipid deposits. These stored lipids, believed to be precursors of steroid hormones, are clear in paraffin preparations and stain darkly with lipid stains such as Sudan black B. After ACTH stimulation or chronic stress, these "clear cells" are converted to "compact cells" similar to those of the zona reticularis, the innermost zone of the cortex. The cells of the latter zone are usually lipid-poor and enzyme-rich. Both types of cells have been shown to produce various species of the class of hormones that influences carbohydrate metabolism (glucocorticoids) and the compact cells have been reported to produce adrenal androgens and estrogens (14).

The adrenals of several animal species deviate distinctly from the above histological pattern. The ungulates have little stainable lipid and do not have clear cells in the zona fasciculata. In ruminants the three classical zones are not present. In pigs (Figure 2) the boundary between the zona fasciculata and the zona reticularis is indistinct and the cells of both zones are of the compact type.
In the adrenal studies to be reviewed, we have used a histochemical method to estimate the functional status of the gland and to compare histological differences in animals of breeds known to differ in post-mortem muscle properties. Since the quality attributes of Poland China and Chester White muscle differ so markedly(12), the initial work consisted of adrenal comparisons in these breeds.

The adrenal glands were secured immediately after death and portions were placed in 10% formol-calcium. These tissues were then sectioned with a cryostat (10 u thick) and stained with Sudan black B. This permitted observation of the quantity, size and location of lipid (sudanophilic) particles in the glands.

Two types of lipid depositions were observed in these adrenal sections. Lipid fine granules were seen and were distributed rather uniformly throughout the zona fasciculata and zona reticularis (Figure 3). Variable quantities of these granules were observed. It is probable that these lipids are precursors of the adrenal corticoids.

Other lipid inclusions were seen which always appeared in the inner layers of the cortex, the zona reticularis (Figure 4). In many glands these large masses of lipids completely obliterated all the other structures of the cells. When present they usually extended over the entire reticulomedullary border.

Quantities of the two types of cortical lipids were scored subjectively on a 0-5 scale at a magnification of 15X. Statistical analysis of these ratings revealed no marked breed difference in the quantity of lipid fine granules but the zona reticularis masses were much more prominent in the adrenals of 32 Poland China pigs than in the glands of 15 Chester White pigs (P< .01).

The function of these lipid-laden cells is not known. In the hamster, similar cells have been observed in the adrenals of old animals and have been considered to be signs of age-degeneration(11). Some investigators believe that androgens are produced by the cells of the zona reticularis. However this area of the adrenal cortex is lipid-poor in many species(14).

Further studies were undertaken to clarify the functional significance of these histological differences and to determine the extent to which they are related to post-mortem muscle properties. Collections of 24 hr. urine were made from 10 Poland China and 5 Chester White barrows. Chemical assays of 17-ketosteroid and 17-OH-corticosteroid metabolites were conducted(13). The quantities of the urinary hormones were compared to the adrenal scores for Sudanophilic material. The correlations revealed that 17-OH-corticosteroid levels were positively related to the scores for lipid fine granules (0.51, P< .05) but the 17-ketosteroid levels were unrelated to the histological observations. The lipid fine granules appeared to be more indicative of hormone production by the gland than were the lipids of the zona reticularis.

Data on post-mortem muscle properties were collected and compared with the sudanophilic materials of the adrenal cortices. The rate of
Glycolysis in longissimus dorsi muscle was related to the quantity of the zona reticularis masses since a low but significant negative correlation was found for 45 min pH versus zona reticularis score in the combined breeds ($-0.32, P < 0.05$). The scores for fine granules of the cortex appeared to be positively related to the extent of glycolysis (24 hr pH) in Poland China (0.40, $P < 0.05$) and Chester White (0.56, $P < 0.05$) pigs. It is possible that these correlations reflect the influence of adrenal corticoids on carbohydrate metabolism, or they may have resulted from the more direct action of these hormones on the circulatory system. Ludvigsen (10) has postulated that the adrenal hormones prevent accumulation of lactic acid in muscle by supporting circulatory function in the ante-mortem period.

Bendall and Lawrie (2) have shown that pre-treatment with cortisone prevents the glycogen-mobilizing effects of epinephrine in rabbits. The loss of muscle extensibility caused by epinephrine was also prevented by cortisone. Their data suggested that the adrenal corticoids influence the contractile state of the muscle; the present study was designed to include the evaluation of the ultimate degree of muscle contraction. For this work, samples of longissimus dorsi were excised 24 hr post-mortem and suspensions of myofibrils were prepared for determinations of sarcomere length (9). Correlations for mean sarcomere length versus adrenal lipid content were then calculated.

Poland China pigs with extensive lipid masses in the zona reticularis had comparatively short sarcomeres ($-0.58, P < 0.05$). The scores for lipid fine granules were unrelated to the sarcomere length of muscle in both breeds. Since the lipid fine granules were more closely related to adrenal hormone production than were the zona reticularis masses, the contracted sarcomeres may reflect some hormone deficiency. The possibility of cortical degeneration is given some support by the observation of a positive correlation between the zona reticularis masses and live weight in Poland China pigs (0.44, $P < 0.05$).

The data reviewed here indicate that pigs with muscle that undergoes rapid post-mortem pH decline, develops low ultimate pH, and becomes severely contracted may have adrenal cortices with little granular lipid material but with heavy invasions of lipid in the zona reticularis. These differences in adrenal glands and their relation to muscle properties implicate the hormones of the cortex in the etiology of pale, soft, exudative porcine muscle. Further studies have been conducted and are being planned in this important area. The results also emphasize the importance of the endocrine control of muscle physiology and meat characteristics. As better techniques are discovered to study hormone actions, new approaches to existing problems may become available. A better understanding of the endocrine mechanisms by which animals maintain their metabolic balance might result in improved productive efficiency, handling practices, and ultimate meat quality.
REFERENCES


Figure 1.

Section of adrenal gland of the dog.

Figure 2.

Section of adrenal gland of the pig. Hematoxylin and eosin. The cells of the zona glomerulosa are evident but the zones of the fasciculata and reticularis are indistinct.
Figure 3.

Normal zona reticularis of pig adrenal cortex. Sudan black B.
Some lipid fine granules are visible.

Figure 4.

Lipid masses in the zona reticularis of pig adrenal cortex.
Sudan black B.
R. G. CASSENS: Thank you Dr. Judge for sharing some of your research results with us. We now have time to entertain some questions.

R. A. FIELD: I would like to ask Mr. Everett if the hide from old animals has more collagen than the hide from young animals as shown by histological techniques? Also, do you have any information concerning reticulin?

A. L. EVERETT: I do not think the percent collagen changes much with age, however, there are some qualitative changes. I have no knowledge of the percent composition reticulin in hide.

J. J. GUENTHER: Does the number of muscle fibers change during the period from birth of an animal to maturity?

W. NORMAN: That is a difficult question to answer. Some people believe that there is an increase in the number of muscle fibers during growth. There are changes in number as the striated muscle fibers develop during embryonic growth, but during latter stages I believe there is probably no change as far as increase in number of muscle fibers. There may of course be a change in muscle fiber size.

R. G. CASSENS: We must close the discussion at this time, but I am sure if you have any further questions of these three men, they will be happy to discuss them with you later. I would again like to thank each of the three speakers for commendable presentations and I will now turn the meeting back to Chairman Palmer.

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A. Z. PALMER: Thank you very much, Bob. You are to be commended, you and your committee, for a fine program and I am sure that everybody appreciates it. I would like to make one short announcement. Jim Carpenter and I have discussed with some of you individually a problem that has been brought to our attention by some of our state meat inspection people in Florida concerning incidence of petechial hemorrhages in some of our slaughter animals in Florida and we would like to very informally discuss this with some of those who might be interested tonight after the banquet, meeting in here. We brought up a few slides that were taken by one of the men in Florida so you can see what their concern is. We would like to get your views about the incidence of this in your respective areas. I'll turn this back to Ellis Pierce at this time.

ELLIS PIERCE: We are rolling right along, almost on time and we are now returning to the Committees on Education and Don Kropf, I think, has his next committee all set to go so Don I'll turn the microphone over to you.

DONALD KROPF: Thank you, very much. Yesterday you heard a stimulating talk on Student Recruitment by Dr. Duane Acker. I think the Intercollegiate and Youth Activities Committee are very importantly concerned in this area. First of all in youth activity because here we have an opportunity to recruit potential college material from our youngsters out in
the state and intercollegiate meat judging team activities because I am sure we will all agree that they have served as a principal recruitment for potential graduate students. I think without any further comment I will turn this over to Dr. Bob Merkel, Chairman of the Intercollegiate and Youth Activities Committee.

DR. R. A. MERKEL: Thank you, Don. Members of the Reciprocal Meat Conference and Guests, Don just explained to you the purpose of the Intercollegiate and Youth Activities Committee. Before we get into our program, however, I would like to recognize the members of this Committee. They are Dr. V. K. Johnson, Dr. George Skelley, Mr. Gary Smith of Washington State, who is not here, Dave Stroud from the National Livestock and Meat Board and Ned Tyler of the U.S.D.A.; Ned also is not here. In previous reports of this particular Committee the usual report was the reading of the minutes of the Annual Coaches Meeting and the approval of these minutes. However, with the reorganization we added youth activities in addition to intercollegiate activities and this year the Committee felt that it would be highly desirable to include a report of some phase of youth work that is being done in the area of meats. In surveying the Committee, they were quite unanimous in selecting the State of Kansas as the State that should make this report since they have been one of the pioneers in the area of youth activities, both in the F.F.A and 4-H programs. The individual that was suggested by all of them is certainly no stranger to this group, she is a graduate of Kansas State University in Home Economics. She was a member of the Meat Judging Team here, one of Davy Mackintosh's Home Ec Meat Teams, and her husband also a member of the Kansas State Meat Team. In addition I had the good fortune of having their son Bob, as a member of one of my meat teams. Bob Lewis incidentally is a member of this group and is working on his doctoral program at Wisconsin. Mrs. Lewis has been very active with 4-H work since she left Kansas State and has been working with a meat judging team and the meat utilization program in Pawnee County. She has won the state meat contest and competed in national competition and I think she brings a great deal of training and experience to this program. She certainly has one of the more successful programs in the state and with this background it is a great deal of pleasure for me to introduce to you Mrs. Frances Lewis of the Alfalfa Lawn Farms of Pawnee County here in Kansas and she is going to speak to you on the subject entitled "The Pawnee County 4-H Meat Utilization Program." Mrs. Lewis.