

Factors Affecting the Water Retention of Beef. V. Variation of the Zinc-Containing Enzymes

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SUMMARY

A comparative study of the lactic dehydrogenase, glutamic dehydrogenase, carbonic anhydrase, zinc, water-soluble nitrogen, moisture, and protein content of six different muscles from three bovine animals was conducted in an investigation of factors contributing to differences in the ultimate pH values of the muscles. The results indicate that variation was considerable in content of zinc, lactic dehydrogenase, glutamic dehydrogenase, and carbonic anhydrase. A highly significant direct correlation was found between pH and zinc content, and a highly significant inverse correlation between lactic dehydrogenase and pH. Lactic dehydrogenase significantly correlated with soluble nitrogen content. No relation was found between glutamic dehydrogenase, carbonic anhydrase, and pH.

Previous studies have indicated marked differences in the water retention, pH value, and zinc content of different muscles of bovine animals (Swift and Berman, 1959; Swift *et al.*, 1960). A highly significant positive correlation was found between water retention, zinc content, and pH. The parallel relationship of zinc and pH, which is in contrast to that between pH and the divalent cations calcium and magnesium, suggested that zinc may participate as a component of enzymes, the action of which may in part determine pH differentials. Carbonic anhydrase, glutamic dehydrogenase, and lactic dehydrogenase, three enzymes present in muscle tissue, contain zinc as an integral part of the protein molecule (Vallee, 1956; Vallee and Wacker, 1956). Removal of the metal results in irreversible inactivation of the enzyme.

Carbonic anhydrase catalyzes the reaction $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$. Zinc is present to the extent of 0.33% (Keilin and Mann, 1940). Studies (Ashby and Chan, 1943) of carbonic anhydrase in human autopsy tissue suggest that carbonic anhydrase plays a role in intermediary metabolism of carbohydrate as an adjunct in decarboxilating reactions. Glutamic dehydrogenase contains 4-5 zinc

atoms per molecule (Vallee *et al.* 1955). The enzyme catalyzes the reaction α -ketoglutarate + DPNH + $\text{NH}_4^+ \rightarrow \text{DPN}^+ + \text{H}_2\text{O} + \text{L-glutamate}$, a reaction that is an important connecting link between carbohydrates and proteins. Lactic dehydrogenase catalyzes the reaction $\text{pyruvic acid} + \text{DPNH} \rightleftharpoons \text{DPN}^+ + \text{lactic acid}$, the end product of muscle glycolysis.

The studies of Andrews *et al.* (1952) and Grant (1955) have indicated the post-mortem stability of the glycolytic and of the citric acid cycle enzymes. Post-mortem inactivity was shown to be due to substrate dearth. Therefore, determination of the rate of substrate turnover of the three zinc-containing enzymes would be a measure of their activity during post-mortem metabolism, the period during which the ultimate pH is attained.

The present investigation followed the general procedure of previous papers of the series. Its purpose was to compare the enzyme and zinc content of six muscles from three animals; it involved determination of ultimate pH values, lactic dehydrogenase (LDH), carbonic anhydrase (CA), glutamic dehydrogenase (GDH), zinc, soluble nitrogen, water, and protein content. Ex-

periments were also conducted to determine the stability and optimum conditions for measurement of the three enzymes.

EXPERIMENTAL

Preparation of samples. Carcasses of three animals were obtained immediately after slaughter. Six muscles designated in Table 1 were prepared as previously described (Berman, 1960). For the enzyme determinations, 10-g portions of the meat were weighed into beakers, covered with parafilm, and stored at -30°C until used. The remaining meat was stored at 2°C for determination of pH, zinc, water-soluble nitrogen, moisture, and protein. At the time of storage, samples were about 5 hr post-mortem.

Table 1. Muscles selected and locations.

No.	Muscle	Location	
	Name	Forequarter	Hind-quarter
1	Longissimus dorsi	Rib, chuck	
3	Semimembranosus		Round
4	Serratus ventralis (thoracic part)	Rib, chuck	
5	Rectus abdominis	Plate	Flank
6	Semitendinosus		Round
8	Trapezius	Rib, chuck	

Determining CA, LDH, and GDH. Homogenates for the determination of CA, LDH, and GDH were prepared by blending 10 g of frozen meat with 100 ml of ice-cold water for 1 min in a Serval Omnimixer immersed in an ice bath. (Mention of trade names in this paper is for identification and implies no endorsement of the products.)

CA activity was measured manometrically at 15°C by the method of Krebs and Roughton (1948). Twenty-five ml of the slurry were diluted to 100 ml with cold 0.05% Difco bacto-peptone solution. Scott and Mendive (1941) showed that stability and activity are maximum when CA solution is diluted with 0.05% peptone solution. To the main compartment of a Warburg reaction flask were added a few glass beads, 0.5 ml of the diluted slurry (0.5 ml of 0.05% peptone was used for the blank or uncatalyzed reaction), and 1 ml of a phosphate buffer solution prepared by mixing 300 ml of 0.1M Na_2HPO_4 with 200 ml of 0.1M KH_2PO_4 . To the side arm of the flask was added 1 ml of freshly prepared 0.05N NaHCO_3 . The flask was equilibrated for 5 min at 15°C . At zero time, after

equilibration, the two solutions were mixed and shaken at 160 oscillations per min, and, as suggested by Mitchell *et al.* (1945), pressure readings X_1, X_2, \dots, X_n were taken at equal intervals of time (10 readings at 30-sec intervals) beginning 30 sec after mixing. The readings were paired for 60-sec intervals and differences taken, i.e., $(X_4 - X_2), (X_6 - X_3), \dots, (X_{11} - X_6)$. The velocity constant was obtained by plotting the common logarithm of the differences against time. The slope of the line multiplied by 2.303 gives the velocity constant. The amount of enzyme activity was found by measuring the velocity constants for the uncatalyzed and catalyzed reactions. The activity is expressed by the unit K_D defined by the equation $K_D = K_C - K_0$ where K_C = velocity of the catalyzed reaction; K_0 = velocity of the uncatalyzed reaction. Values for K_D are plotted against the logarithm of the amount of meat used in the determination, and a straight line is drawn (Altschule and Lewis, 1949). By extending this line to zero dilution, an extrapolated value for 1 g of undiluted meat is obtained.

The method of Robins *et al.* (1956) was adapted for determination of GDH activity. Twenty-five ml of the homogenate were diluted to 50 ml with 0.1M sodium barbital buffer, pH 8.0. One-tenth ml of the diluted slurry was transferred to a test tube immersed in an ice bath. One ml of a buffer substrate solution, prepared by mixing 18 ml of 0.05M sodium barbital buffer, pH 8.0, with 135 mg diphosphopyridine nucleotide (DPN) and 1.2 ml of neutralized 4.5M sodium glutamate, was added. For the blank determination, 1.2 ml of 0.05M sodium barbital, pH 8.0, were substituted for the 4.5M sodium glutamate. The contents of the tubes were mixed and the tubes capped with parafilm. The tubes were then placed in a water bath at 32°C for exactly 60 min. The tubes were returned to the ice bath, and 1 ml of 0.5mM 3-quinolyhydrazide (QH) (in 0.18N HCl) was added. The contents of the tubes were mixed by tapping and were then allowed to stand 1 hr at room temperature ($20-25^{\circ}\text{C}$). The samples were then diluted with 4 ml of 0.01N HCl. The absorption of the samples at $350\text{ m}\mu$ was determined 10 min after dilution. Calculations were made according to the following equation:

$$\frac{\text{O.D. with substrate} - \text{O.D. without substrate}}{\text{weight of meat in aliquot}} \times \frac{\text{final volume}}{E_t} \times 1000 = \text{moles of substrate oxidized}$$

per kilo per hour,

in which E_t = molar extinction coefficient at $305\text{ m}\mu$ of QH - α -ketoglutaric acid, plus that of reduced diphosphopyridine nucleotide (DPNH) in the presence of QH. The E_{305} for the QH -

Table 2. Effect of blending time on enzyme activity.

Enzyme	30 sec	60 sec	120 sec	480 sec
Lactic dehydrogenase ^a	3.65	3.62	3.56	3.62
Glutamic dehydrogenase ^b	411	416	411	406
Carbonic anhydrase ^c	0.753	0.784	0.773	0.829

^a Moles substrate oxidized per kg wet weight per 3 min.

^b Micromoles substrate oxidized per kg wet weight per hr.

^c Log of the pressure change (K_D) per min per g wet weight.

α -ketoglutaric acid product is 21,700, and the DPNH in the presence of QH has an $E_{305} = 5960$ at 10 min; therefore, $E_t = 21,700 + 5960 = 27,660$.

The method of Meister (1950) was adapted for determination of LDH activity. The decrease in absorbance, measured at 340 $m\mu$, due to the oxidation of DPNH to DPN^+ , was determined as a direct measure of the reduction of pyruvate to lactate (Colowick and Kaplan, 1955). Twenty-five ml of the homogenate were diluted two-hundred-fold with 0.1M PO_4 buffer, pH 7.6. Three ml of a buffer substrate solution, containing one micromole DPNH, 10 micromoles sodium pyruvate, 165 micromoles nicotinamide, and 100 micromoles of PO_4 buffer, pH 7.6, were placed in a cuvette. The reaction was started by addition of 0.1 ml of the diluted homogenate. Readings were taken for 3 min at 30-sec intervals, beginning 30 sec after introduction of the homogenate. The change in absorbance during this 3-min period, converted to micromoles of pyruvate oxidized per kg wet weight of meat, represented the enzyme activity of the sample.

Determining pH, moisture, protein, water-soluble nitrogen, and zinc. pH, moisture, protein, and water-soluble nitrogen were determined as described in earlier papers (Swift and Berman, 1959; Swift *et al.*, 1960). Zinc as the zinc dithizonate in carbon tetrachloride (Sandell, 1950).

RESULTS AND DISCUSSION

In determining the enzyme activity, an important consideration was determination of optimum extraction and assay conditions. Therefore, a series of experiments were conducted to determine these parameters. Results are in Fig. 1 and Tables 2 and 3. The data show that, under the conditions chosen, all three enzyme activities are linear with respect to quantity of tissue assayed, extraction is complete in 30 sec, blending does not inactivate or denature the enzyme, and quantitative extraction of the enzymes from the tissue was achieved.

Optimum conditions for the assay of

Table 3. Effect of meat-to-water ratio during blending on the extraction of lactic dehydrogenase (LDH), glutamic dehydrogenase (GDH), and carbonic anhydrase (CA).

Ratio of meat to water (g)	LDH ^a	GDH ^b	CA ^c
5:100	3.25	0.947	0.793
10:100	3.21	0.942	0.719
15:100	3.21	0.970	0.847
20:100	3.36	0.915	0.756

^a Moles substrate oxidized per kg wet weight per 3 min.

^b Micromoles substrate oxidized per kg wet weight per hr.

^c Log of the pressure change (K_D) per min per g wet weight.

GDH and LDH were at pH 8.0 in a 0.05N sodium barbital buffer containing 9.5 micromoles of DPN per ml and 300 micromoles of sodium glutamate per ml, and at pH 7.6 in a solution containing 0.333 micromoles DPNH, 313 micromoles sodium pyruvate, and 33 micromoles phosphate, respectively.

The standard error of measurement for 12 replicates was $\pm 2.3\%$ for LDH, $\pm 5.7\%$ for GDH, and $\pm 14.0\%$ for CA.

Table 4 shows the average moisture, protein, and soluble nitrogen contents of six

Table 4. Average moisture, protein, and soluble nitrogen content of six muscles from each of three animals.

No.	Muscle Name	% wet weight of meat		
		Moisture	Protein (N $\times 6.25$)	Soluble nitrogen (mg/g tissue)
1	Longissimus dorsi	74.46	21.75	7.80
2	Semimembranosus	75.11	21.80	8.37
4	Serratus ventralis	74.56	18.89	5.66
5	Rectus abdominis	76.07	20.00	6.45
6	Semitendinosus	75.67	21.41	7.52
8	Trapezius	76.27	19.99	6.16

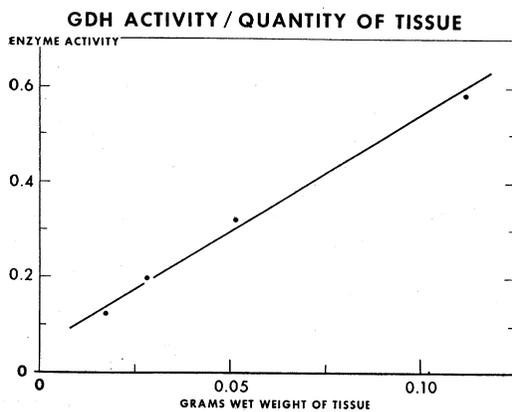


Fig. 1a. Linearity of glutamic dehydrogenase activity with respect to quantity of tissue.

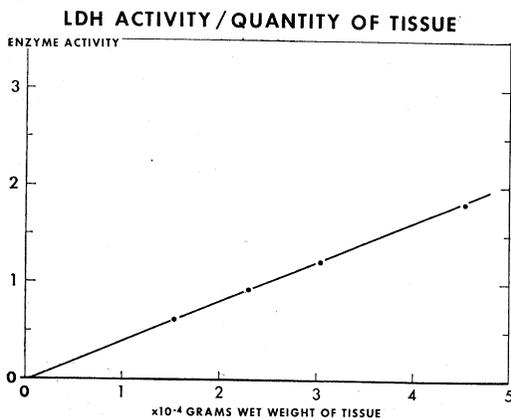


Fig. 1b. Linearity of lactic dehydrogenase activity with respect to quantity of tissue.

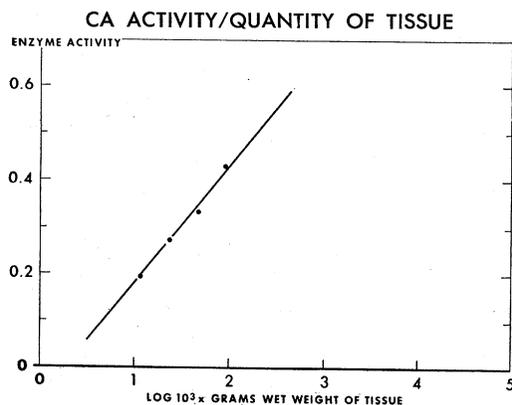


Fig. 1c. Linearity of carbonic anhydrase activity with respect to quantity of tissue.

muscles from three animals. As indicated in the data, muscles 4, 5, and 8 differ from the others in protein and soluble nitrogen content in accordance with the tendency of muscle properties to differ in patterns (Swift and Berman, 1959; Swift *et al.*, 1960).

The curve in Fig. 2 expresses the relation of LDH activity and pH. An interesting feature is that at pH 5.6 the enzyme exerts half of its optimum activity. It is significant, therefore, that even when the ultimate pH has been attained in post-rigor meat, LDH activity is capable of converting any pyruvate formed to lactic acid.

Table 5 shows the results of determination of zinc content and enzyme activity. The content of zinc and enzyme activity of individual muscles ranged widely. Muscles 4, 5, and 8 differ from the others in LDH activity. The specific activities (enzyme activity/soluble nitrogen) indicate that the enzyme is a smaller percentage of the total water-soluble fraction in muscles 4, 5, and 8 than in muscles 1, 3, and 6. Similar results were reported by Kronman and Winterbottom (1960) relative to the enzyme aldolase.

The data in Table 6 show the results of statistical analyses of the relations between pH, soluble nitrogen, and components of the muscles. The results indicate that pH was directly related to zinc content and inversely related to LDH activity. The latter supports the view that variations in the amount of lactic acid (Bate-Smith and Bendall,

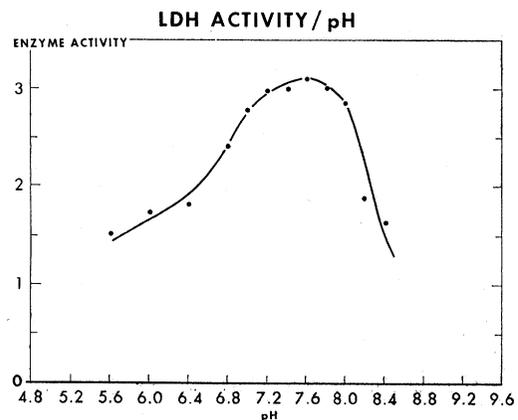


Fig. 2. Enzyme activity vs pH of reaction solution.

Table 5. Average zinc content and activity of lactic dehydrogenase (LDH), glutamic dehydrogenase (GDH), and carbonic anhydrase (CA) in six muscles from three animals.

Muscle		Zn ^a	LDH ^b	GDH ^c	CA ^d	pH
No.	Name					
1	Longissimus dorsi	4.60	3.53	660.6	0.590	5.54
3	Semimembranosus	3.79	3.73	729.9	0.632	5.51
4	Serratus ventralis	6.49	1.08	960.1	0.626	5.78
5	Rectus abdominis	5.58	2.58	674.5	0.660	5.68
6	Semitendinosus	3.60	3.74	813.1	0.468	5.51
8	Trapezius	5.03	2.14	836.2	0.473	5.65

^a Milligrams per 100 g wet weight of tissue.

^b Moles substrate oxidized per kg wet weight per 3 min.

^c Micromoles substrate oxidized per kg wet weight per hr.

^d Log of the pressure change (K_D) per min per g wet weight.

Table 6. Statistical relationships between properties of six muscles.

	Correlation coefficient	Probability ^a
Correlation with pH		
Zinc	+0.966±0.090	.01
Lactic dehydrogenase	-0.972±0.056	.01
Glutamic dehydrogenase	+0.330±0.318	n.s.
Carbonic anhydrase	+0.609±0.270	n.s.
Correlation with soluble N		
Lactic dehydrogenase	+0.931±0.123	.01
Glutamic dehydrogenase	-0.541±0.284	n.s.
Carbonic anhydrase	+0.215±0.160	n.s.

^a Five degrees of freedom.

1956) may be responsible for ultimate pH differences in muscles.

Previous work (Swift *et al.*, 1960) has shown the marked decrease in the glycogen content of muscles attaining low ultimate pH values (Nos. 1, 3, and 6) in contrast with the lesser decrease in those attaining higher ultimate pH values (Nos. 4, 5, and 8). This was explained on the basis that ultimate pH varied with the amount of glycolysis occurring prior to ATP disappearance and that this interval was affected by variations in rates. The present results indicate that the variation in rates was associated with parallel variations in the capacity of the glycolytic enzyme systems in the muscles.

The data in Table 6 show that soluble nitrogen content was related directly to LDH activity. No relation was found be-

tween GDH and CA activity and the pH or soluble nitrogen.

The results show that, of the three zinc-containing enzymes, only LDH plays a significant role in influencing the ultimate pH of meat. The direct, highly significant correlation between pH and zinc content remains to be explained.

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