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INTENSITY OF AUTOLYTIC PROCESSES IN EXSANGUINATED AND UNEXSANGUINATED MUSCLES

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With the death of an animal the correlation between the anabolic and catabolic phases of metabolism is disturbed, the intake of new external matter ceases, decomposition products are detained, and the coordination of enzyme action, existing during life, vanishes. Splitting of tissue components sets in, and a number of processes arise, coming under the general heading of autolysis (1).

Salkowski (2), who investigated the autolytic process in exsanguinated minced organs infused in ten times the quantity of chloroform water, found that leucin and tyrosin appear in the solution after a relatively short time. Leucin and tyrosin are not detected in an extract from a previously boiled organ, nor are these amino-acids to be found in the free state in fresh organs. Hence, this investigator concluded that the formation of leucin and tyrosin occurs under the influence of the enzymes of the organ itself.

Schwiening (3) obtained the same results on using extracts of organs freed from cells by filtration.

At present particular attention is paid to protease when studying autolytic processes. Some authors even identify autolytic processes with the action of proteolytic ferments. During autolytic processes, however, there occurs splitting not only of proteids, but of glucides and lipids as well. Hence, autolysis is the splitting under the influence of the organism's own enzyme system of all component parts of the organs and tissues.

Except for Prof. Sadikov (4) and Prof. Smorodintsev (5), very few authors have made a profound study of the post-mortem changes in muscle tissue; uncoordinated investigations of certain aspects of the state of the muscle tissue after death fail to give a distinct notion of the long chain of the processes of transformation of matter occurring in this tissue.

There are also no data on the effect of blood on the intensity of autolysis in muscle tissue, which is why we have decided to study this question.

EXPERIMENTAL PROCEDURE

Muscles were taken for investigation from carcasses of Simental cattle aged 7—8 years of medium fatness. Exsanguinated muscles were obtained from animals killed at the slaughter-house and drained of blood in the usual way. To attain complete bloodlessness physiological solution was passed through the blood vessels. To obtain unexsanguinated muscles the abdominal cavity was cut open after the animal had been stunned, and the aorta and posterior vena cava were ligated.

To ensure equal conditions in all investigations the same muscles were always taken, namely: m. semimembranosus, m. semitendinosus and m. biceps femoris.

The muscles taken for analysis were freed of connective and fatty tissue, minced in a meat-grinder, and the minced meat was thoroughly mixed. An extract was then prepared by adding 500 ml of distilled water to 50 g of minced muscle, and the mixture was shaken for one hour on a shuttle apparatus at a speed of 40 r. p. m.

The resulting extract was filtered through a triple layer of gauze and then analysed. The procedure for obtaining the extract was the same in all experiments.

The following determinations were made:

1. The hydrogen exponent (determination made electrometrically by means of a potentiometer).
2. Total quantity of nitrogen (determination by Kjeldahl's method).
3. Residual nitrogen (determination by the micro-Kjeldahl method after precipitating the proteins in an extract of trichloroacetic acid).
4. The free amino-acid content (determination by distributive paper chromatography).
5. Lactic acid (determination by the Fürt-Embden method).
6. Reducing substances (determination by the Hagedorn-Jensen method).
7. Total and inorganic phosphorus (determination made colorimetrically by the Fiske-Subborow method).

In addition, the total nitrogen was determined in the muscles. The quantity of total nitrogen in the muscles was 3.2 p. c. on the average. No marked fluctuations of total nitrogen were observed. The muscles were kept at a temperature of 15—16°C.

As shown by our investigations (6) the intensity of decomposition of substances is considerably greater in non-sterile muscles than in sterile ones.

To avoid contamination with microflora the muscles were treated with toluol, which is a reliable means for inhibiting the development of microorganisms.

RESULTS

14 series of experiments were carried out — 7 for studying autolytic processes in exsanguinated muscles, and 7 for unexsanguinated muscles. The average results are given below. (*Tables 1, 2, 3, 4, 5, 6, 7, 8*).

Table 1.

CONCENTRATION OF HYDROGEN IONS

	Experimental time				
	1 h.	24 h.	48 h.	72 h.	96 h.
Exsanguinated muscles . . .	6.60	6.14	6.10	6.05	6.08
Unexsanguinated muscles . .	6.50	6.12	6.10	6.00	6.00

Table 2.

TOTAL NITROGEN OF EXTRACT

Time	Milligrams per 100 grams			
	Exsanguinated muscles	in p. c. of 1st hour	Unexsanguinated muscles	in p. c. of 1st hour
1	644.23	100	654.32	100
24	658.72	102.2	693.42	105.9
48	679.60	105.4	710.00	108.6
72	691.30	107.3	770.63	117.7

Table 3.

RESIDUAL NITROGEN

	Milligrams per 100 grams			
	Exsanguinated muscles	in p. c. of 1st hour	Unexsanguinated muscles	in p. c. of 1st hour
1	227.00	100	206.40	100
24	234.32	103.2	233.72	113.2
48	248.14	109.3	244.25	118.3
72	255.22	112.4	250.00	121.1

Analysing the numerical data obtained as a result of investigating the autolytic processes occurring in exsanguinated and unexsanguinated muscles, it can be readily noted that in unexsanguinated muscles the quantity of total and residual nitrogen, as well as that of free amino-acids increases much more rapidly than in exsanguinated muscles.

The increase in the rate of decomposition cannot be attributed, as is usually done, to the activity of microorganisms for which blood is a good nutrient medium, since the muscle samples were taken from freshly killed animals with all the necessary precautions to prevent possible bacterial contamination, and these samples were then treated with toluol.

Table 4.

CONTENTS OF FREE AMINO-ACIDS IN MUSCLES
(IN MILLIGRAMS PER 100 GRAMS)

Amino-acids	Exsanguinated muscles				Unexsanguinated muscles			
	Time of investigation				Time of investigation			
	1 h.	24 h.	48 h.	72 h.	1 h.	24 h.	48 h.	72 h.
Arginine	4.42	6.87	10.31	14.08	4.33	10.05	15.16	22.38
Alanine	36.80	40.03	43.64	49.56	37.29	49.01	52.30	63.20
Aspargic acid	28.65	33.00	38.44	45.01	28.55	42.30	40.16	56.03
Valine	8.14	8.36	9.29	13.17	9.01	16.54	22.34	29.06
Glycine	12.16	13.00	19.11	23.12	12.00	17.22	26.05	32.30
Glutamic acid	14.18	15.06	19.33	22.04	15.16	25.35	39.07	43.77
Histidine	8.06	9.33	12.12	16.19	9.15	11.23	18.16	32.00
Leucine	10.12	13.10	19.51	29.32	9.85	14.00	22.30	38.38
Lysine	5.66	6.39	7.72	10.61	4.44	8.35	12.06	16.39
Methionine	—	—	—	—	—	—	12.34	24.01
Proline	6.39	7.00	9.13	10.01	6.25	12.32	15.06	18.24
Serine	10.00	12.50	12.68	13.07	10.11	14.09	16.36	19.63
Tyrosine	8.34	10.66	12.13	18.19	9.10	16.14	22.16	23.01
Phenylalanin	5.25	6.19	9.13	11.16	6.38	12.16	18.25	22.13
Cystine	6.00	9.13	9.00	6.31	5.95	12.13	9.12	—

The post-mortem chemical changes in the muscle tissue were due to a great extent to the effect of proteolytic enzymes. The proteolytic enzymes may, depending on the nature of the substratum which they split, be divided into proteinases of the pepsin, trypsin and chymotrypsin types.

Proteinase is usually formed in cells as inactive proenzymes passing into active forms under the influence of definite activators. Activation of proenzymes is almost always attended by their proteolysis.

In distinction to digestive enzymes secreted with the secretions of the respective glands of external secretion, proteolytic enzymes are formed in the tissues. These enzymes, embraced by the general term of cathepsins, are found in the tissues in small quantities, which makes it very difficult to study them.

Three cathepsins have been isolated from cattle spleen. Cathepsin I, or A, is similar in its action to pepsin; cathepsin II, or B, is by its specificity similar to trypsin, and cathepsin V, or C, is similar to chymotrypsin.

Muscle tissue contains a small quantity of cathepsins. According to modern ideas muscles contain several cathepsins, one of them being the basic one. But this cathepsin does not correspond to any of the three spleen cathepsins.

Muscle cathepsins have been inadequately studied, but it should be considered that the proteolysis induced by them plays a great part in post-mortem chemical changes in muscle tissue.

A number of other enzymes participate in these changes, among them: V-glutamylcarboxypeptidase, which splits peptides with a

terminal V-glutamylglutamate residue; leucinaminopeptidase (cathepsin III), which splits certain peptides and amides having a free α-amino-group on the terminal residue of leucin or an amino-acid close to it; aminotripeptidase, which acts on tripeptides formed by aliphatic amino-acids; glycyldipeptidase, which splits glycyl-D or L-amino-acids and glycyldehydropeptides; glycylglycindipeptidase, which splits glycylglycin and sarcosylglycin; glycyl-leucindipeptidase, which splits glycin-L-leucin; carnosinase, which splits dipeptides of L-histidine and amides of these dipeptides; cysteinylglycindipeptidase, which splits L-cysteinylglycin; iminodipeptidase, which splits L-prolylglycin and L-oxyprolylglycin and a number of other enzymes.

In all probability a considerably greater number of enzymes take part in the autolytic processes occurring in the tissues than we know of at present.

After the death of the animal the splitting of proteins under the influence of tissue proteases does not set in at once. The tissue proteins are protected against protease action during the life of the cells by their alkaline reaction, which makes the basic proteases ineffective.

To enable the tissue proteases to act on their own proteins it is necessary for the latter to pass into a non-ionized state. In tissues at pH = 7.4 the proteins are in the state of salts and are bound with potassium, sodium and magnesium; protein salts resist protease autolysis. The acids which accumulate during autolysis bring the proteins to an unionized state, after which the autolysis proteases become effective.

In the presence of an acid reaction the proteins disaggregate completely if the decomposition products are removed (7).

Rona, studying tissue autolysis, arrived at the conclusion that when the products of autolysis are not removed, the autolytic manifestations are depressed. According to Rona's data (8) the quantity of forming nitrogen does not, in the absence of dialysis of the products of autolysis, exceed 50 p. c. of the nitrogen of uncoagulated proteins. The more products of autolysis are removed, the more elevated the decomposition of uncoagulated protein, attaining values of up to 91 p. c. nitrogen (9).

High-molecular peptides and a small quantity of amino-acids and ammonia are the principal substances appearing as a result of autolysis. An acid reaction is unfavourable for the action of peptase (10); hence, the pH is the chief factor determining the trend of autolysis.

Analysing these data we may draw the conclusion that the formation of lactic acid and reducing substances is more intense in unexsanguinated muscles than in exsanguinated.

The study of the processes occurring in muscle tissue is closely associated with the study of the processes of glycolysis.

It has long been known that, depending on the work done, the glycogen content differs in various muscles. At the end of the second day after the butchering of the animal glycogen usually disappears entirely, being transformed into lactic acid. The so-called cadaveric

rigidity sets in some time after death..The muscles become hard and opaque. The rapidity of the appearance of the cadaveric rigidity depends on the pH of the muscle and, to a great extent, on the glycogen content of the muscles. After some time the rigidity passes, and the muscles again become soft.

Table 5.

LACTIC ACID

Hours	Milligrams per 100 grams			
	Exsanguinated muscles	In p. c. of 1st hour	Un exsanguinated muscles	In p. c. of 1st hour
1	156.40	100	184.00	100
24	308.70	197	354.00	192
48	434.10	277	568.20	308
72	492.30	314	612.35	332

Table 6.

REDUCING SUBSTANCES

Hours	Milligrams per 100 grams			
	Exsanguinated muscles	In p. c. of 1st hour	Unexsanguinated muscles	In p. c. of 1st hour
1	94.3	100.0	85.54	100.0
24	106.4	112.8	112.00	129.4
48	132.3	140.2	128.16	148.0
72	142.7	151.3	136.12	157.2

According to modern ideas the main factor causing the appearance of cadaveric rigidity is the decomposition in the muscles of adenosine-triphosphoric acid. The elasticity of the muscle is preserved only in the presence of the necessary quantity of ATP. In living acid ATP decomposition is retarded chiefly under the influence of the Marsh-Bendal factor, which inhibits the adenosinetriphosphatase activity of myosin. When death sets in, the action of the Marsh-Bendal factor ceases and actomyosin begins to split adenosinetriphosphoric acid.

As a result of ATP decomposition the muscles acquire rigidity. But at the same time the splitting of glycogen with the formation of lactic acid causes resynthesis of ATP, which results in the vanishing of cadaveric rigidity. However, the mechanism of cadaveric rigidity has not as yet been completely ascertained.

It is known that during the lifetime of the animal a process of lactic acid formation occurs in the muscle tissue, but the lactic acid vanishes (11) as a result of the Meierhof reaction. The second phase of

the Meirhof reaction, the phase of lactic acid transformation, can occur only in the presence of oxygen. With the setting in of death the work of the heart ceases, and the circulation of blood stops as a result. In addition, when the animal is butchered, various methods are used to ensure complete bloodlessness of the carcass. When blood circulation ceases, the oxygen necessary for oxidizing part of the lactic acid is, naturally, no longer conveyed.

On the basis of Barcroft's researches the conclusion may be drawn that with the cessation of cardiac activity at the moment of butchering there is an almost complete cessation of the union of hemoglobin with the oxygen.

Barcroft established the fact that in a pure hemoglobin solution there is a definite equilibrium between oxyhemoglobin and hemoglobin. This equilibrium is defined by the following formula:

$$\frac{H(HbO_2)}{[H(Hb)]\cdot(O_2)} = K$$

Designating the degree of oxygen saturation of hemoglobin by y, then y will equal unity under the condition of complete saturation. The hemoglobin concentration equals $1 - y$. If the oxygen pressure is taken as unity, the above equilibrium formula may be written in the following form:

$$\frac{y}{1-y} = Kx \text{ or } y = \frac{Kx}{1+Kx}$$

The curve for this equation is an equilateral hyperbola.

The system is more complex in the blood than in a pure hemoglobin solution; the equation of the relation of oxyhemoglobin to hemoglobin is, therefore, also more complex.

Carbonic acid is known to hinder the union of oxygen with hemoglobin. By its chemical properties hemoglobin is a weak acid. The accumulation of some stronger acid lowers the capacity of the blood to absorb oxygen, since the ion of reduced hemoglobin has a greater affinity for oxygen than the undissociated molecule (12).

Owing to the presence of carbonic acid in the blood, as well as salts, the equilibrium curve deviates from the hyperbola and acquires an S-shaped form. At the moment of butchering of the animal a strong contracture of the muscles occurs, a considerable quantity of lactic acid being formed.

As a result of the elimination of buffer systems with the outflowing blood the relative free lactic acid content is still further raised.

Disintegration of glycogen occurs under the influence of muscle amylase, but it has not yet been ascertained how amylase and glycogen exist together in the cells of a living organism without the latter being decomposed, and why it is that after the death of the animal the effect of muscle amylase is so strongly manifested. Without ignoring the specific adaptation of living cells, tending towards dynamic equilibrium, the possibility of the coexistence of amylase and glycogen may

be partly explained by the physico-chemical properties of muscle tissue.

As is known the pH of the normal cell fluctuates around 7.5; at this pH value the action of amylase is limited. After the death of the animal the pH changes toward an acid value, owing to the accumulation of acids. Optimal conditions set in for the action of tissue amylase, which energetically splits glycogen.

Lesser proved experimentally that the glycogen disintegration process may be intensified in fresh tissue by acidifying the latter.

We established the fact that introducing into the muscles lactic acid or a whey concentrate accelerates the autolytic processes in the muscles and preserves them for some time from putrefaction (13).

According to Lesser the principal cause of the inactivity of the amylase of the cells during the life of the organism is that in all cells amylase is separated in space from glycogen, being absorbed by the «inert substances — colloids of boundary surfaces». Lesser confirmed his thesis by the fact that on washing tissues with surface active substances — as, for instance, alcohols inducing ruptures of absorption bonds — an increase in glycogen decomposition is observed.

Proceeding from this viewpoint glycogen decomposition in the muscles during the post-mortem period may be represented in the following sequence.

During the lifetime of the organism the protoplasm of the muscle cells and its inclusions constitute a highly disperse system. As lactic and other acids accumulate the pH tends to become acid. When the pH attains a value of 6.3, myogen passes into the isoelectric state, in which it is extremely unstable, and myogen coagulation therefore sets in. As a result of the formation of aggregates of particles, the total surface decreases; amylase passes from the adsorbed state into solution and begins to split glycogen. The intensification of glycogen decomposition may also be due to the elimination of the inhibiting effect of insulin.

Table 7.

TOTAL PHOSPHORUS

Hours	Milligrams per 100 grams	
	Exsanguinated muscles	Unexsanguinated muscles
1	104.20	116.60
24	102.42	113.00
48	108.88	115.18
72	107.35	118.63

As can be seen from the above table the increase in inorganic phosphorus occurs somewhat more rapidly in unexsanguinated muscles than in exsanguinated.

Table 8

INORGANIC PHOSPHORUS

Horus	Milligrams per 100 grams			
	Exsanguinated muscles	in p. c. of 1st hour	Unexsanguinated muscles	in p. c. of 1st hour
1	60.18	100.0	54.75	100.0
24	67.36	111.9	59.23	108.1
48	68.52	113.8	64.36	117.5
72	76.65	127.3	72.00	131.5

In glycolytic processes occurring in muscle tissue a considerable part is played by the transformations of the adenyl system consisting of adenosine triphosphoric (ATP), adenosinediphosphoric (ADP) and adenylic (AA) acids.

During the post-mortem period the process of splitting off phosphoric acid from the components of the adenylic system occurs in muscle tissue.

$$ATP + H_2O \rightleftarrows ADP + H_3PO_4$$
$$ADP + H_2O \rightleftarrows AA + H_3PO_4$$

Introducing inorganic phosphorus not only fails to retard the hydrolysis of the adenylic system but, on the contrary, accelerates the hydrolysis.

CONCLUSIONS

On the basis of the results of the investigations the conclusion can be drawn that in sterile unexsanguinated muscles, as compared with sterile exsanguinated muscles, the rise in residual nitrogen, in free amino-acids, as well as in lactic acid, reducing substances and inorganic phosphorus occurs more rapidly. This indicates that the presence of blood in the muscles stimulates the autolytic processes occurring in them.

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