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5^{1}- Mono-, Di- and Triphosphates of Adenosine in Beef and Pork kept under Refrigeration

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Introduction and General Considerations.

To a great extent the qualities of meat are determined by its contents of nucleotides and especially of 5^1 phosphates of adenosine, particularly 5^1 - triphosphate of adenosine (ATP). According to Weber, whose theory is accepted by most scientists, working in this field, ATP exerts a double influence upon contractile proteins of muscles. When ATP is decomposed, contraction is caused. If ATP is not decomposed, a tender and strechable protein structure will result. The water binding properties of meat and generally the equilibrium between meat and water thus is determined by ATP and by the pH-value of the meat.

On this account ATP is a determining factor for the fitness of a meat for use in the manufacture of sausage, canned meat and so forth.

Up to the present no information has been given concerning the influence of different commercial methods of handling meat upon ATP. Of particular interest it seems to be to know the influence of storage under refrigeration immediately after slaughter and during so long time after the slaughtering of the animals that can be expected before use of meat. Our knowledge of ATP in meat is defective. To some extent this may depend on difficulties in carrying out quantitative determinations of ATP.

The importance of the water binding capacity of meat has interested several investigators to seek a test method which could measure the relative moisture binding properties of raw meat. Grau & Hamm (1953) measure the moisture expressed from a sample of meat when subjected to a certain pressure for a specified time. This method was modified by Wierbicke & Deatherage (1958). Wierbicke &al. (1957) also describe a method in which the moisture released during the cooking of a meat sample is measured. Urbin & al. (1962) modified the Grau & Hamm technique and give some interesting results showing the variation in " free moisture " especially within Longissimus dorsi of pork.

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Materials and Methods.

Cattle meat and pork were investigated. In total 25 carcasses of cattle and 10 of pork were used. Immediately after slaughter the carcasses to be investigated were taken into a refrigerated cooler kept at $\pm 0^{\circ}C$ to $+2^{\circ}C$. Samples of meat from the gracilis-muscle were collected immediately after slaughter and after 1, 3 and 5 days' storage respectively.

The temperature of the meat of the carcasses was on the surface after 24 hours $+2^{\circ}C$ to $+6^{\circ}C$ and in the centre of the thickest part of the carcasses $+5^{\circ}C$ to $+10^{\circ}C$. After 48 hours of storage the temperatures were $\pm 0^{\circ}C$ to $+2,5^{\circ}C$ resp. $\pm 0^{\circ}C$ to $+4^{\circ}C$. After 72 hours the temperatures were $\pm 0^{\circ}C$ to $+2^{\circ}C$ on the surface as well as in the centre parts of the carcasses.

The samples taken from the gracilis-muscle were at least 60 g, when the sample was taken immediately after slaughter as well as after 24 hours storage, whilst the samples taken after 3 resp. 5 days' storage weighed 150 and 300 g.

All cattle used in this investigation were premium grade and of an age between 5 and 10 years. The pork carcasses were from premium grade pigs.

Fat, connective tissue and tendons were cut away from the samples, which then were finely ground. To 60 g of finely ground sample 60 ml of 10 % cold ($\pm 0^{\circ}C$) perchloric acid was added and the mixture was vigorously shaken, then pressed by means of a citrus-press lined with three layers of gauze. The extraction was carried out once more with 30 ml of 5 % ice cold perchloric acid. Then the liquid was filtered through Hyflo supercel and the supercel-cake washed two times with 5 % perchloric acid. The pH-value of the filtered liquid was adjusted to 6,8 to 7,0 with ammonia 1:1. The quantity of filtrate was measured and the same quantity of ethanol was added. In order to have the precipitate formed well separated the mixture was kept at $+2^{\circ}C$ for 3 hours. After filtering through double filters so much of barium chloride was added that a complete precipitation of the barium salts of adenosine-mono, -di and -triphosphates was obtained. As a rule 5 ml of 1 M. barium chloride is sufficient. The mixture was kept overnight in refrigerator then filtered. Equal parts of

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Dowex 50 and precipitate were stirred for 15 minutes , then the mixture was filtered through a glass- filter. The pH - value of the filtrate was adjusted to 6,8 to 7,0, then the filtrate was diluted to 10 ml. The sample thus obtained can be kept at -25°C for about one month with no measurable decompostition. The ion exchanger must be washed with 1 N hydrochloric acid then with distilled water, until all acid is removed. All procedures are carried out in containers surrounded by crushed ice.

Paper ionophoresis. A modification of the method described by Bergkvist (1957) was used for the separation and estimation of adenosinemonophosphate (AMP) adenosinetriphosphate (ATP) and adenosinediphos - phate (ADP).

Whatman No. 1 filter papers were washed three times with N hydro - chloric acid and then with water to pH = 7. After drying papers were cut to 135 x 450 mm. A cross line was drawn 120 mm from the shorter side of the paper. The samples were applied to the dry paper at this cross line as lines and not as circular spots. The lines of applicated material varied from 2 x 8 to 3 x 11 mm. The samples were applied in very small portions and must dry under cold air for every portion in order to get a spot which i as concentrated and limited as possible. The distance between the different spots (lines) was not less than 20 mm. For applying Carlsberg pipettes were used. A total quantity of sample applied of 25 μ l was found suitable. Sodium acetate buffer of pH 4,15 (13,6 g NaAc, 3 H_2O + 18,0 ml HAc/1) and carbon tetrachloride was used as by Bergkvist.

The samples were freeze-dried to a convenient concentration.

Pure AMP-, ADP- and ATP- substances were dissolved in water. The solutions were treated with ethanol and precipitated with 1 M barium chloride, then stirred with Dowex and pH adjusted quite as has been described for the samples. The resulting solutions were always run parallell with the samples.

It must be pointed out that in order to get exact results the ionophoresis must be run over the cooling tubes of the apparatus. The ionophoresis was

carried out with 1.000 V and about 20 mA. The temperature of the carbon tetrachloride must be kept low and the voltage high (1.000 V). If not, the spots of the different adenosinephosphates will not be distinct.

After ionophoresis and drying of the papers the nucleotides were revealed by taking an ultraviolet contact print using light of a wavelength of 2540 A.

Results

Table 1 gives results from some series of ionophoresis, carried out on samples collected and prepared as described.

Table I.

Contents of adenosine-monophosphate (AMP), -diphosphate (ADP) and -triphosphate (ATP) in meat from cattle and pork immediately after slaughter of the animals and after 1, 3 and 5 days' storage under refrigeration.

Animal	Date of slaughter	Sample taken: Immediately				24 h. after slaughter				72 h. after slaughter				120 h. after slaughter			
		Quantity of sample	AMP	ADP	ATP	Quantity of sample	AMP	ADP	ATP	Quantity of sample	AMP	ADP	ATP	Quantity of sample	AMP	ADP	ATP
Cattle	7/12 -59									300 g - 15 ml	1	1	2				
	8/12 -59									300 g - 5 ml	1	1	2				
	2/3 - 60									600 g - 1 ml 600 g - 2 " 600 g - 5 "	3 3 2	3 3 2	3 3 2				
	4/3 - 60									600 g - 1 ml 600 g - 2 " 600 g - 5 "	3 3 2	3 3 2	3 3 2				
	15/3 - 60	300 g - 5 ml	3	3	3	300 g - 5 ml	2	2	2	300 g - 5 ml	2	2	2				
	30/3 - 60	60 g - 5 ml	2	2	2	60 g - 5 ml	1	2	2	150 g - 5 ml	1	3	3	300 g - 5 ml	1	2	2
	5/10 - 60					60 g - 5 ml	0	2	2	150 g - 5 ml	2	1	2	300 g - 5 ml	3	3	3
	7/10 - 60					60 g - 5 ml	2	1	2	150 g - 5 ml	2	1	3	300 g - 5 ml	2	2	2
	12/10 - 60	60 g - 5 ml	0	2	2	60 g - 5 ml	1	1	2	150 g - 5 ml	1	1	2	300 g - 5 ml	2	2	2
Pig	10/10 - 60	60 g - 5 ml	2	1	3	60 g - 5 ml	1	0	3	150 g - 5 ml	1	1	3	300 g - 5 ml	1	1	3
	17/10 - 60	60 g - 5 ml	0	0	2	60 g - 5 ml	1	0	2	150 g - 5 ml	2	2	3	300 g - 5 ml	2	2	3
	19/10 - 60	60 g - 5 ml	2	1	2	60 g - 5 ml	1	1	2	150 g - 5 ml	1	1	2	300 g - 5 ml	2	1	2

In column AMP, ADP, ATP used figures indicate:
0 = no spot
1 = weak but definite spot
2 = rel. strong definite spot
3 = very strongly coloured, definite spot

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Quantitative measurements of the colour intensity of the spots gave the results contained in table 2.

Table 2.

Relative contents of AMP, ADP and ATP in meat from cattle and in pork as measured after ionophoresis on samples taken immediately after slaughter and after storage under refrigeration.

Kind of meat	Quantity of adenosinephosphates after storage in per cent of quantity present immediately after slaughter			
	Storage Time Hours	AMP	ADP	ATP
Cattle	24	100	75	100
	72	60	30	50
	120	40	23	23
Pork	24	77	50	100
	72	31	50	35
	120	19	30	17

From the results found by means of ionophoresis the interesting conclusion must be drawn, that, in contrast to what has been supposed hitherto, adenosinetriphosphate is rather stable in meat which is handled under commercial conditions using refrigeration, which is applied immediately after slaughter as is the case to-day in most western countries.

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Summary.

Results obtained from paper ionophoresis involving separation, estimation and determination of adenosinemonophosphate, adenosinediphosphate and adenosinetriphosphate in meat from cattle and in pork are given.

Since particularly adenosinetriphosphate is a determining factor for the fitness of meat for use in the manufacture of sausage and canned meat as well as for use as fresh meat in preparing meals, special emphasis has been paid to the influence of commercial methods for handling meat on the decomposition of adenosinetriphosphate. Therefore the contents of the different phosphates of adenosine have been determined immediately after slaughter of the animals and after storage under refrigeration.

The methods used for collecting and treating of samples, which were all taken from the gracilis-muscle of the carcasses, are described in detail, as is the method used for ionophoresis.

The results indicate that, under the conditions described, adenosinetriphosphate is much more stable in meat than has been supposed hitherto. In meat from cattle as well as in pork the contents of adenosinetriphosphate are practically the same 24 hours after slaughter of the animal as immediately after slaughter, if the meat is handled as described, which means i. a. that it is cooled and kept under refrigeration under conditions given in detail in the paper.

After 5 days' storage there is still about 23 % of adenosinetriphosphate originally present in meat from cattle and about 17 % in pork. After 3 days of storage the figures are for meat from cattle 50 % and for pork 17 %. Also the di-and monophosphates of adenosine show a similar slow rate of decomposition. The figures are given in the paper. Generally it was found that decomposition of adenosinephosphates occurs with greater speed in pork than in meat from cattle under the conditions given.

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