CXLVI. THE EXTRACTIVE PURINES OF MUSCLE. I.

By ANTHONY DMOCHOWSKI.

From the Biochemical Laboratory, Faculty of Medicine, Warsaw University.

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DETERMINATIONS of the purine-nitrogen content of dog and rabbit skeletal muscle before and after extraction with boiling water show that the proportion of extractive purines is very considerable, amounting to over 50 % of the total muscle-purine content. The extractive purine content is fairly constant, and appears to be independent of the state of nutrition [Dmochowski, 1928, 1, 2].

In view of the fact that purine-nitrogen content has been taken as a base for researches on reserve protein, and also in view of the conflicting results obtained by previous workers in this field, it appeared necessary once again to investigate both quantitatively and qualitatively the purine substances present in skeletal muscle.

A review of the literature dealing with muscle-purines, in particular with extractive purines, shows a state of chaos. We have in another paper [Dmochowski, 1929] given a critical review of these papers; the discrepancies between the results of the various authors are illustrated by Table I, from which it will be seen that the purine-nitrogen content of muscle extracts as given by different authors varies from 2 to 129 mg. per 100 g.

Table I. *Purine-N content of whole muscle and of aqueous extracts in mg. per 100 g.*

Author	Whole muscle	Aqueous extract	Animal
Demant [1879]	—	(3-6)*	Starved pigeon
Kossel [1882, 1884]	(30-60)	—	Ox, dog, chicken
Salomon [1881]	—	(30)	Dog
Schittenhelm [1905, 1910]	—	40-50	Pig, ox
Burian and Hall [1903]	55-72	—	Ox, horse
Buglia and Costantino [1912]	68	—	Ox
Scaffidi [1910]	35	3	Frog
Scaffidi [1911]	61	9	Ox
Yoshimura [1911]	—	(2)	Wild rabbit
Suzuki and Yoshimura [1909]	—	(12)	Herring
Fürth and Schwartz [1910]	—	23-36	Dog, horse
Smorodincev [1913, 1914, 1922]	—	(3-50)	Horse, ox, sheep, pig
Offer and Rosenqvist [1899]	—	25-50	Ox, calf
Adler [1908]	—	14-129	Ox, calf
Bessau and Schmidt [1910]	—	15	Ox
v. Fellenberg [1918]	(50-55)	(28)	Ox, calf, frog
Kikuchi [1923]	58-83	—	Rabbit
Truszkowski [1927]	54-80	—	Dog, horse, calf, rat
Dmochowski [1928, 1, 2]	80-104	—	Dog, rabbit

* Values in brackets have been calculated by me as purine-N from the total purine content.

These discrepancies are, as will be shown in this paper, due to the presence in muscle extracts of purine-containing complexes.

EXPERIMENTAL.

Aqueous extracts from dog and rabbit skeletal muscle were taken as experimental material. The animals were killed by intracardial injection of chloroform, immediately after which muscle tissue from the hind limbs, as well as in some cases dorsal muscle tissue, was removed, passed with the least possible delay through a mincing machine, and 100 g. portions were thrown into 500–1000 cc. of boiling water. The mixture was boiled for 10–15 minutes, after which it was filtered, and the residue extracted a further two or three times. The united filtrates were feebly acidified with acetic acid, in order to coagulate proteins, and the protein-free filtrate was concentrated to about 400 cc. Its total nitrogen content was determined by the method of Kjeldahl, and the purine-N content before and after 4 hours' hydrolysis with 4 % sulphuric acid by the method of Krüger and Schittenhelm [1905]. The unhydrolysed extract contained on the average 5 mg. purine-N per 100 g. tissue, whilst after hydrolysis 30–50 mg. per 100 g. tissue were found.

Table II.

Total N content %	Purine-N (as mg. per 100 g. tissue) content of				Remarks
	Extract		Whole muscle	Residue after extraction	
	Unhydro- lysed	Hydro- lysed			
0.56	8.7?	20.0?	109.75	39.07	Rabbit, fed
0.47	5.0	42.3	87.35	43.78	" "
0.44	6.7	—	—	—	" "
—	3.8	44.3	—	30.6	" "
—	3.4	44.2	85.8	—	" "
—	—	33.9	123.3?	74.19?	" fasting
—	5.0	—	95.5	46.46	" "
0.48	—	45.3	91.97	39.28	" "
0.65	5.1	—	101.5	47.22	" "
—	—	53.0	99.3	44.5	" fed
0.52	4.8	43.8	95.9	41.6	—
0.40	8.8	31.36	79.37	32.32	Dog, fed on meat
0.35	5.7	34.3	79.13	30.61	" fed on meat
—	5.6	27.5	—	—	" ill

The results, given in Table II, show that over 50 % of the purine-N of muscle tissue is found in the hydrolysed extract, whilst the remainder cannot be removed even by prolonged extraction. Free purines are present in very small quantity—about 5 % (of the total purine-N) in whole muscle, or 10 % for the extract alone. Slightly larger quantities are found in material which is not absolutely fresh.

With the object of investigating the influence of hydrolysis on the quantity of purines precipitated, or of the inhibitive influence of the colloids present on the precipitation of the various purine compounds, hypoxanthine, which is most probably the free purine present in muscle, or nucleotide (crystalline adenylic acid, prepared from Boehringer's sodium yeast nucleinate by the

method of Steudel and Peiser [1924]), was added to aqueous dog muscle extract, after which purine-N was determined by the method of Krüger and Schmidt.

It was found that hypoxanthine was in these circumstances practically quantitatively precipitated, adenylic acid hardly at all. Thus 27.6 mg. purine-N was found in an extract originally containing 16.2 mg. and to which a quantity of hypoxanthine equivalent to 10.4 mg. purine-N had been added, whilst in a different case the apparent purine-N content remained unchanged in spite of the addition of an amount of adenylic acid corresponding to 52.9 mg. purine-N.

Since adenylic acid is, as was previously shown by Thannhauser and Czoniczer [1920], readily precipitable from aqueous solution, and since this is not the case with aqueous muscle extracts, it would appear that here lies the cause of the low results obtained by many authors for extractive purines, as well as of the higher values obtained after hydrolysis.

Should it actually be the case that the nucleotides, adenylic and inosinic acids, are present in the aqueous extract, as would appear from a consideration of the solubilities of these acids and their salts to be extremely probable, then it might be expected that after the precipitation of these nucleotides with uranyl acetate according to Thannhauser and Czoniczer, much lower values for purine-N would be obtained for the hydrolysed filtrate.

This supposition was confirmed experimentally on rabbit muscle extract, which was found after hydrolysis to have a total content of 44.3 mg. of purine-N, whilst after precipitation with 1.5 % uranyl acetate and hydrolysis of the filtrate the content was only 12.4 mg. The residue, which contained 28 mg. purine-N, gave, after removal of uranium, the pentose reaction in its purine fraction. This reaction was negative in the case of the "free" purines precipitated from the unhydrolysed extract as copper compounds, as were also the murexide and Kossel's reactions. A positive Weidel reaction and a very feeble xanthine reaction were given in the latter case.

Having to a certain extent determined the proportions of purine-N in the various fractions of extractive purines, *i.e.* nucleotide, nucleoside and "free," as well as of those remaining after extraction, it remained to determine quantitatively the constituent purines, guanine, adenine, hypoxanthine and xanthine in the separate fractions.

Up to the present, aqueous extracts of rabbit and chicken muscle (300–1600 g.) have been analysed, as well as the residue after extraction [Dmochowski, 1929]. The nucleotide was separated from the nucleoside fraction and each was hydrolysed and analysed separately with certain modifications by the method of Steudel [1924] (double or triple precipitation as cuprous salt and single precipitation as silver salt).

The results, given in Table III, indicate that very small quantities of pure guanine are present, and this exclusively in the residue after extraction. Xanthine is found only in traces.

Table III. *The individual purines of rabbit and chicken muscle tissues and their content as mg. purine-N per 100 g. tissue.*

Material	No. of animal	Quantity g.	Guanine	Guanine-N	Adenine picrate	Adenine-N	Hypoxanthine-N
Extracted rabbit muscle	I	300	10.0	4.6	13.4	2.6	>0.7
	II	1000	5.8	2.7	—	—	—
	III	950	>4.63	>2.13	16.38	3.24	1.0
	IV	950	8.0	3.7	16.5	3.3	—
	V	500	10.0	4.63	23.6	4.7	—
Total extract from same	I	300	0	0	103.5	19.9	>2.16
Uranyl acetate precipitate from extract of same	III	950	0	0	87.0	17.2	—
	IV	950	0	0	78.8	15.7	4.3
Filtrate from same	III	950	0	0	16.1	3.20	5.8
	IV	950	0	0	0	0	>2.9
Extracted chicken muscle	V	1600	7.9	3.6	41.6	8.2	2.0
Uranyl acetate precipitate from extract of same	V	800	0	0	38.5	7.6	10.26
Filtrate from same	V	800	0	0	9.1	1.8	>5.8

Adenine picrate, M.P. 278–280°, was precipitated in a fairly pure, crystalline state from the extracts, whilst that from the residue crystallised only after reprecipitation as silver salt. Hypoxanthine was at first determined as argentipicrate [Steudel, 1924], but as the crystallisation of hypoxanthine picrate does not take place quantitatively, and as the supposed silver precipitate of xanthine turned out to be in reality one of hypoxanthine, giving the characteristic nitrate, the quantity of hypoxanthine-N was calculated directly from the weight of the corresponding silver precipitate according to the formula $C_5H_2ON_4Ag_2, H_2O$.

DISCUSSION.

An examination of four different forms in which purines occur in muscle tissue shows that the proportion of free purines, extractible by hot or warm water and directly precipitable from the extract as copper compounds, is extremely small. In the case of rabbit muscle, containing on the average 80–100 mg. purine-N per 100 g., the mean free purine-N content is 5 mg. per 100 g. Nor is the value markedly increased by leaving the comminuted muscle for short periods of time, or by hydrolysing the extract during 2–3 minutes. Guanine, adenine, and uric acid (negative murexide reaction) are absent from this fraction.

The hydrolysis of the aqueous extract, using 4 % sulphuric acid, leads to a considerable increase in the amount of purines precipitated by copper sulphate in the presence of sodium hydrosulphite. The purine-N content now becomes 40 mg. per 100 g. for rabbit and 30 mg. per 100 g. for dog muscle extracts, an amount equivalent on the average to 50 % of the total quantity of purines present in muscle. This observation explains the cause of the low values obtained for extractive purines by many authors, such as Scaffidi [1910], who gives the purine-N content of frog muscle as 35 mg. per 100 g.,

and that of the extract as 3 mg., whilst our determinations for fresh muscles of *Rana esculenta* show that the respective figures are 54 and 23 mg. per 100 g. Similarly, the small quantities of purines precipitated by phosphotungstic acid from muscle extracts by Smorodincev [1913, 1914, 1922] may be ascribed to this cause.

The supposition that plant nucleic acid, which is similarly not precipitable under our conditions, occurs in muscle tissue extracts had to be abandoned in view of the absence of effect of gentle alkaline hydrolysis, as also of the absence of guanine from this fraction. The non-precipitability of adenylic acid added to muscle extract leads to the conclusion that considerable quantities of nucleotides, precipitable from aqueous solution but not from the extract, are present in muscle tissue.

The addition of uranyl acetate to the extracts leads to the precipitation of pentose nucleotides, which constitute about 75 % of extractive purines. The filtrate from the uranyl acetate precipitate contained free and combined purines, the latter probably as nucleosides.

The values found for the purine-N contents of the nucleotide and nucleoside fractions agree fairly well with those of other authors. Thus Gudzent and Keeser [1922] found in human and chicken muscle 3-7 mg. of non-nucleotide purine-N per 100 g. tissue and a large quantity, not determined, of nucleotides.

The highest value given by Parnas [1928] for loss in adenine of rabbit muscle after trituration amounts to 32 mg. purine-N per 100 g. tissue, a value agreeing fairly well with those obtained in this research.

Grund's [1902] value for the pentose content of ox muscle is somewhat low (23 mg. per 100 g., corresponding to only 11 mg. of nucleotide- and nucleoside-N); it has, however, been shown by Schmidt [1928] that the pentoses of adenylic acid of muscles readily escape detection.

Apart from the early work of Kossel [1884] no accurate figures for the individual purines of muscles are available.

Our preliminary researches show that guanine is absent from the extract, and is present with an equal amount of adenine only in small quantity (6-10 mg. per 100 g.) in the residue after extraction, probably representing exclusively nuclear nucleic acid. By far the greater part (20 mg. adenine-N per 100 g.) of the adenine present in rabbit muscle is to be found in the extract, mostly as adenylic acid (17 mg. adenine-N), whilst the hypoxanthine of fresh muscle is present preponderatingly in the free state.

Under the experimental conditions applied in this research (immediate immersion of comminuted muscle in boiling water) deamination of adenylic acid does not take place, as is shown by the figures obtained for inosinic acid hypoxanthine. The small quantity of adenine remaining sometimes in the extract after precipitation of nucleotides may possibly be due to adenosine. The traces of hypoxanthine found in the extracted residue are probably due to incomplete extraction.

An important fact emerging from our researches is that the sum of

guanine-, adenine- and hypoxanthine-N found (xanthine was not found) corresponds to only two-thirds of the total purine-N content of muscle. A purine fraction was isolated, giving a soluble picrate, but not giving Weidel's or the xanthine reaction, or the characteristic nitrate of xanthine. A part of these purines is, in the case of rabbit muscle, precipitable by ammonia as a gelatinous mass from which a crystalline sulphate can be prepared. This sulphate is not identical with that of hydroxyadenine, since it possesses water of crystallisation. Research on this new fraction is now being prosecuted.

The above research was, with the exception of that part of it dealing with the individual purines, commenced in the spring of 1927; the results obtained were communicated to the Polish Biological Society on June 20th, 1928, and a summary of this paper has been published [Dmochowski, 1928, 1]. Parnas's [1928, 1929] important papers and that of Embden and Wassermeyer [1928] were published at a later date, the results of these authors being partly concordant with ours.

Parnas relegates nearly the whole purine-N content of frog muscle of about 30 mg. per 100 g. tissue to nucleotides, whilst Embden and Wassermeyer [1928] find considerably larger quantities of ammoniagenic nucleotide-N precipitated by copper sulphate and calcium hydroxide, viz. 40–80 mg. purine-N per 100 g. tissue for frog muscle. The values obtained by this author, as compared with those of Parnas and of Dmochowski, would indicate that adenylic acid and adenine are not the only bodies present in muscle capable of giving rise to ammonia; possibly the unidentified purine substances found by us are the source of this extra ammonia.

SUMMARY.

1. It has been shown that about 50 % of the purines present in muscle are to be found in the aqueous extract, which, however, contains only traces of "free" purines, precipitable as cuprous salts from the unhydrolysed solution (5 mg. purine-N per 100 g. tissue for dog and rabbit muscle).

2. By far the greater part of extractive purines (40 mg. purine-N per 100 g. tissue) are precipitable only after hydrolysis of the aqueous extract with 4 % sulphuric acid, and 75 % of this fraction is contained in pentose nucleotides, precipitated by uranyl acetate; the filtrate contains, apart from "free" purines, probably also nucleosides.

3. Hunger and different conditions of alimentation do not appear to affect the distribution of the various purine fractions.

4. Quantitative determinations of the individual purines indicate the absence of guanine from rabbit muscle extracts, and the presence in the residues of 2.7–4.6 mg. of guanine-N per 100 g. tissue, with the same adenine-N content. These purines probably therefore originate from nuclear nucleic acids. Most of the adenine present in fresh muscle occurs in the nucleotide fraction of extracts, whilst the hypoxanthine is found in the nucleoside fraction, probably as the free purine. Xanthine is found only in traces.

5. A considerable purine fraction giving a soluble picrate was isolated both from the extracts and residues of rabbit muscle. The purines of this fraction are precipitable by ammonia and yield a crystalline sulphate different from those of guanine and of hydroxyadenine.

REFERENCES.

- Adler (1908). *Berlin. klin. Woch.* **8**, 393.
 Bessau and Schmidt (1910). *Therap. Monatsh.* 116.
 Buglia and Costantino (1912). *Z. physiol. Chem.* **81**, 120.
 Burian and Hall (1903). *Z. physiol. Chem.* **38**, 336.
 Demant (1879). *Z. physiol. Chem.* **3**, 381.
 Dmochowski (1928, 1). *Biochem. J.* **22**, 1555.
 — (1928, 2). *Compt. Rend. Soc. Biol.* **99**, 1148.
 — (1929). *Acta Biol. Exp. (Warsaw)*, **4**, 63.
 Embden and Wassermeyer (1928). *Z. physiol. Chem.* **179**, 226.
 v. Fellenberg (1918). *Biochem. Z.* **138**, 323.
 Fürth and Schwartz (1910). *Biochem. Z.* **30**, 413.
 Grund (1902). *Z. physiol. Chem.* **35**, 111.
 Gudzent and Keeser (1922). *Z. klin. Med.* **94**, 1.
 Kikuchi (1923). *J. Biochem. (Japan)*, **2**, 409.
 Kossel (1882). *Z. physiol. Chem.* **7**, 7.
 — (1884). *Z. physiol. Chem.* **8**, 404.
 Krüger and Schittenhelm (1905). *Z. physiol. Chem.* **45**, 14.
 Offer and Rosenqvist (1899). *Berlin. klin. Woch.* **44**, 968.
 Parnas (1928). *Acta Biol. Exp. (Warsaw)*, **1**, No. 3.
 — (1929). *Biochem. Z.* **205**, 16.
 Salomon (1881). *Jahresber. Thierchem.* **11**, 108.
 Scaffidi (1910). *Biochem. Z.* **30**, 473.
 — (1911). *Biochem. Z.* **33**, 247.
 Schittenhelm (1905). *Z. physiol. Chem.* **46**, 354.
 — (1910). *Z. physiol. Chem.* **66**, 53.
 Schmidt (1928). *Z. physiol. Chem.* **179**, 243.
 Smorodincev (1913). *Z. physiol. Chem.* **87**, 12.
 — (1914). *Z. physiol. Chem.* **92**, 214, 221.
 — (1922). *Z. physiol. Chem.* **123**, 115.
 Steudel (1924). Hoppe-Seyler—Thierfelder. *Physiol. Pathol. Chem. Anal.* p. 875. (Berlin, Springer).
 — and Peiser (1924). *Z. physiol. Chem.* **127**, 262.
 Suzuki and Yoshimura (1909). *Z. physiol. Chem.* **62**, 1.
 Thannhauser and Czoniczer (1920). *Z. physiol. Chem.* **110**, 307.
 Truszkowski (1927). *Biochem. J.* **21**, 1040.
 Yoshimura (1911). *Biochem. Z.* **37**, 477.