Analysis of Tissue Constituents

COMMERCIAL OX-MUSCLE EXTRACT

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Meat extracts have been known for nearly 150 years, and during that time various analyses have been carried out. These analyses are incomplete (Embry & Henley, 1919; Rees & Salway, 1947; *Allen's Commercial Organic Analysis*, 1932) and the present work was undertaken to develop methods for the separation, identification and determination of the constituents of muscle extracts and similar material. A preliminary account of this work has been published (Wood, 1956*b*) and also a more general review of the subject of meat extract (Bender & Wood, 1956).

The term 'meat extract' is not specific, and some confusion has arisen in the literature over the different types of extracts that can be prepared. Many authors quote Liebig's original statement that 34 lb. of trimmed meat yields 1 lb. of extract. In practice two very different types of extract have been prepared commercially. The first is known as 'direct extract' or, according to the report of the Society for Analytical Chemistry, Meat Extract Sub-Committee (1951), 'essence of beef'. This is defined in the report as the product of an extraction sufficiently prolonged to produce a jelly on cooling; in other words, a considerable amount of gelatin has been extracted. Approximately 13–14 parts of meat are needed to yield 1 part of direct extract, containing about 34 % of moisture.

The second product is known as no. 1 extract or, in the Society for Analytical Chemistry report, merely as 'meat extract'. This is a by-product from the manufacture of corned beef, and involves the immersion of chopped beef in boiling water for a limited period. Very little gelatin is extracted and the product is the normal article of commerce today. It requires 50 parts or more of trimmed meat to produce 1 part of this extract, containing 17 % of moisture. In the present paper no. 1 extract has been used.

EXPERIMENTAL

Materials

Meat extract. The commercial extract used was a concentrate of the liquor obtained when chopped beef was immersed for 30 min. in water at 95–100° during the initial stages of corned-beef manufacture. The flesh used con-

sisted entirely of muscle and connective tissue, the skin, viscera and head of the animal being removed. The first stage of concentration of the liquor to 45 % of solids was carried out under reduced pressure, the following final stage at atmospheric pressure and 75° over a period of 7–14 hr. During this final stage of concentration, at which there is free access of air, the commercially desirable flavours and colour are developed. Thus commercial extract may be expected to show certain chemical differences from fresh muscle extracts. The chemical manipulations during the analysis were carried out at temperatures below 60°.

Dialysed extract. The extract, contained in a Visking bag, was dialysed exhaustively against distilled water with chloroform as a preservative. The dialysis was judged to be complete when no trace of colour was visible outside the bag after dialysing against fresh water for 12 hr.; this took about a week. The diffusates obtained each day were combined and concentrated under reduced pressure.

An alternative method was treatment under reduced pressure at 40–45° in a circulating dialyser made by modifying a Soxhlet extractor. The existing siphon was replaced by one of wider bore. A tap was fused to the top of the condenser and could be closed when the apparatus was functioning satisfactorily under reduced pressure. The diffusate siphoned over and collected in the receiver below. The dialysis of 3 g. of beef extract was complete after 14 hr.

The material remaining in the bag was dried to constant weight over P_2O_5 *in vacuo* and weighed to obtain the percentage of protein in the extract. The nitrogen and ash of the protein were determined.

Electrodialysed extract. The dialysed extract was electro-dialysed in the apparatus described previously (Wood, 1956*a*), with water flowing through the electrode compartments. After electrodialysis colourless solutions were obtained from the anode and cathode compartments, and the centre fraction was pale brown. For some purposes of chromatography an electro-desalted extract was required, in which case the passage of current was continued only until the resistance of the cell began to rise rapidly. At this point the contents of the centre compartment were withdrawn.

Analytical methods

Dry weight. This was determined both by drying for 16 hr. at 100° and *in vacuo* over P_2O_5 at room temperature. *Ash.* The sample was ignited at 550°.

Nitrogen. This was determined by the semi-micro-Kjeldahl procedure.

Phosphate. This was determined on a dialysed sample by the method of the Association of Official Agricultural Chemists (1955*a*) before and after ashing; the difference gave the organic phosphate.

Potassium. This was determined by precipitation as the cobaltinitrite (Association of Official Agricultural Chemists, 1955b).

Creatine and creatinine. The Jaffe reaction was used as detailed by the Society for Analytical Chemistry (1951).

Allantoin. Larson's method of determination (Hawk, Oser & Summerson, 1947) showed none to be present.

Urea and ammonia. A microdiffusion method was used (Conway, 1950).

Colouring matter. Anode and cathode Permaplex ion-exchange-resin membranes (The Permutit Co. Ltd., London, W. 4) were dried over P_2O_5 *in vacuo* until their weights did not alter by more than 10 mg./24 hr. (0.5% of their weight). The membranes were then arranged in the electrodialysis apparatus on either side of the centre compartment containing the sample of dialysed extract. A compartment containing water separated the centre compartment from each electrode compartment and its appropriate membrane. Water was passed through the electrode compartments. Electrodialysis was commenced with a current of 0.20 A and continued until the current had fallen to approx. 0.08 A. At this stage the solutions in the outer compartments were withdrawn and replaced by water. Further passage of current resulted in the deposition of the colouring matter on the centre membranes and the solution of meat extract became pale yellow in colour. Finally, all the solutions were removed and replaced by water and the current was passed for a few minutes to remove traces of salts from the membranes. The membranes were then removed, blotted and dried as before.

Chromatography

The substances present were identified by ascending-paper chromatography of the dialysed, electrodialysed or desalted sample. The equipment used was the Universal Chromatography Outfit (Aimer Products Ltd., 56, Rochester Place, London, N.W. 1), in which 5 filter-paper sheets 10 in. square are mounted on 'dural' metal frames. Unless otherwise stated, Whatman no. 4 paper was used. A one-phase mixture of *n*-butanol-acetic acid-water was used as a 'standard' solvent, thus facilitating the comparison of R_F values for a large number of compounds.

When a compound had been identified by chromatography in several solvents it was determined quantitatively.

Solvents. All solvents are single phase.

(1) *n*-Butanol-acetic acid-water (60:15:25) (J. B. Jepson & I. Smith, personal communication).

(2) Phenol-aq. NH_3 soln.: 113 ml. of water was added to 450 g. of phenol; 1 ml. of aq. NH_3 soln. (sp. gr. 0.880) was added before use to each 200 ml. of solvent.

(3) Phenol-HCl: 100 ml. of the above phenol solvent was allowed to equilibrate overnight with a mixture of 60 ml. of conc. HCl and 40 ml. of water contained in a separate vessel.

(4) Ethanol-aq. NH_3 soln. (sp. gr. 0.880)-water (80:5:15) (Cheftel, Munier & Macheboeuf, 1952).

(5) Propan-1-ol-*n*-acetic acid (3:1) (Ames & Mitchell, 1952).

(6) Acetone-30% acetic acid (1:1) (Burrows, Grylls & Harrison, 1952).

(7) *tert*-Amyl alcohol-picric acid-water (80 ml.:4 g.:20 ml.).

(8) Methanol-90% formic acid soln.-water (80:15:5) (Bandurski & Axelrod, 1951).

Detection of spots. The papers were viewed under ultraviolet light from a Hanovia Chromatolite lamp (Hanovia Ltd., Slough, Bucks) before treatment with the location reagents described below. It was found possible to apply all the reagents by a 'dipping' procedure. Those reagents which had, of necessity, to be in aqueous solution, were applied by drawing the paper rapidly through the surface of the solution and blotting immediately.

Two types of location reagent were employed: 'general reagents' which showed the presence of a spot on the paper, with little or no regard to the chemical nature of the material, and 'specific reagents' which react with groups of related compounds.

The following general reagents were used to locate spots: iodine vapour, universal indicator, ammoniacal silver nitrate and bismuth tri-iodide.

Specific reagents were as follows: diazo reagent for iminazole compounds (Sanger & Tuppy, 1951); diacetyl reagent for guanidine derivatives (Foster & Ashton, 1953); silver nitrate-bromophenol blue for purines and pyrimidines and also for chloride (Wood, 1955); bismuth tri-iodide for quaternary ammonium compounds (Cromwell & Rennie, 1954); ninhydrin for amino acids and peptides; Ehrlich reagent for substituted ureas (yellow colours) and indoles (red colours) (Smith, 1953); Jaffe reagent for creatinine (Block, Durrum & Zweig, 1955). The organic phosphates were detected by treatment of the paper with a modified (Burrows *et al.* 1952) reagent of Hanes & Isherwood (1949), followed by irradiation with ultraviolet light.

Inorganic ions. To avoid spots produced by inorganic ions being attributed to unknown compounds, reagents were required for the common inorganic ions. The ions K^+ and NH_4^+ were detected with cobaltinitrite (Steel, 1954), Na^+ ion with violuric acid or from its fluorescence after treatment with uranyl acetate, Ba^{2+} and Ca^{2+} ions with rhodizonic acid (Burma, 1953), Cl^- , Br^- and I^- ions with silver nitrate-bromophenol blue (Wood, 1955), SO_4^{2-} by the procedure of Burma (1953) and PO_4^{3-} with the organic phosphate reagent described below.

Amino acids. These were identified on paper chromatograms of the desalted, dialysed extract and of an amino acid concentrate obtained as follows: the desalted, dialysed extract was treated with the hydrogen form of Zeo-Karb 225, the resin washed with water and the adsorbed amino acids were eluted with 2*N*-aq. NH_3 soln. and concentrated *in vacuo* over H_2SO_4 . The quantitative estimation was carried out on a dialysed extract with a resin column, and the identity of the peaks was checked by paper chromatography (Moore & Stein, 1951) (Fig. 1).

Two-dimensional chromatograms were run in butanol-acetic acid and in phenol-aq. NH_3 soln.

Carnosine and anserine. Chromatograms were run in phenol-HCl to separate carnosine and anserine. The sum of these two compounds was measured with the resin column and the amount of carnosine was estimated by comparison of the diazo colour on the paper with that from known amounts of pure carnosine. The difference between these two figures gave the amount of anserine.

Urea and citrulline. These two compounds were detected with Ehrlich reagent. Citrulline was determined on the resin column and urea as described above.

Guanidine compounds. The trace amounts of guanidine and methylguanidine were estimated by visual comparison of the spot intensity on paper with known amounts.

Quaternary ammonium compounds. The bismuth tri-iodide reagent of Cromwell & Rennie (1954) was used, with the modification that the background colour was removed by rinsing in ethanol. Carnitine was estimated by comparison of the spot with those due to known amounts of the pure compound.

Purines and pyrimidines. Hypoxanthine was estimated directly from its spot intensity after development with silver nitrate-bromophenol blue. Inosine could not be measured in this way because of variation in the depth of colour of its spot. Since only hypoxanthine and its riboside (with a trace of adenine and inosinic acid) were present, the inosine content could be calculated if the amount of combined hypoxanthine was known. The total hypoxanthine, after hydrolysis, was obtained by precipitation of its silver complex (Embry & Henley, 1919), and the percentage of inosine was calculated from the results. This figure includes the small amount combined as inosinic acid.

Organic phosphates. A dialysed sample of the extract was treated with baryta in the cold to remove inorganic phosphate, and excess of barium was removed with H_2SO_4 . Chromatograms were run on Whatman no. 541 (acid-washed) paper in several solvents. The use of metal equipment was avoided and a trace of ethylenediaminetetra-acetic acid was added to the solvents to remove traces of metal ions which have been shown to interfere with the chromatography of phosphoric esters (Isherwood, 1954).

Organic acids. A dialysed sample of the extract was fractionated by electro dialysis as previously described (Wood, 1956a). The colourless solution from the anode compartment of the cell contained the free acids. The mixture of acids was treated with baryta to remove the considerable amounts of phosphoric and sulphuric acids, which otherwise tended to interfere with the running of the chromatograms. Since oxalic acid would also have been removed by this procedure it was tested for by adding a strong solution of calcium chloride to a portion of the original dialysed extract made acid with acetic acid; as there was no precipitate, and since small amounts of added oxalic acid were detectable under these conditions, oxalic acid was presumed to be absent.

After removal of excess of barium with H_2SO_4 , the solution was concentrated to a thick syrup *in vacuo*. The syrup was extracted four times with ether and the ether removed. The ether extract and the extracted material were dissolved separately in a small amount of water and chromatographed on Whatman no. 4 paper, the standard solvent being used in one direction and ethanol-aq. NH_3 soln.-water in the other.

Either solvent mixture may be used first but the choice of developing reagent depends upon the form in which the acids are present at the end of the run, whether as ammonium salts or in the free state. Thus aniline-xylose reagent (Saarnio, Niskasaari & Gustafsson, 1952) or potassium iodide-iodate-starch (Feigl, 1954) was used to reveal the

Fig. 1. Elution curve of diffusate of 80.2 mg. of meat extract chromatographed on a Moore & Stein (1951) resin column. (A) Neutral and acidic amino acids: the buffer was changed from 0.1M-citrate, pH 3.42 at 37.5°, to 0.1M-citrate, pH 4.25 at 50°, after 220 ml. had been collected, and the temperature raised to 75° after 278 ml. (B) Basic amino acids: 0.1M-phosphate buffer, pH 6.8 at 25°, was changed to 0.2M-citrate buffer, pH 6.5 at 25°, after 105 ml. (X denotes the unidentified peptide.)

free acids and Nessler's solution to show up the ammonium salts. In addition a dilute solution of ferric chloride sprayed on to the paper served to reveal hydroxy acids as yellow spots.

A map of the spots was prepared and is shown in Fig. 2. The map was constructed by taking a number of acids which might possibly be present and running them singly and in mixtures of six or seven in the two solvents described above. R_F values and the relative positions of the acids to each other were checked on both one-dimensional and two-dimensional chromatograms. The relative positions on a two-dimensional chromatogram were also checked when either

solvent was used first. It would appear that acids of a similar chemical nature tend to travel to the same region of the paper, and this is illustrated by the distribution of the homologous dicarboxylic acids.

The pattern of spots obtained from the muscle extract was compared with the chromatographic chart of the acids (Fig. 2), and the following acids were identified in the extract from their R_F values and colour reactions: lactic, glycollic, succinic and β -hydroxybutyric.

Lactic acid. Lactic acid was determined by the method of Furth & Charnass (1910). When the determination was carried out on a deproteinized extract, the distillate contained considerable amounts of formaldehyde along with the acetaldehyde arising from oxidation of the lactic acid; the determination was therefore repeated on the anode fraction from an electrodialysed sample. Glycollic acid when oxidized by the same method gave a figure one-tenth of that obtained from the same weight of lactic acid; interference from the small amount of glycollic acid present in the extract was consequently adjudged negligible.

Glycollic acid. Glycollic acid was confirmed on the chromatogram by the violet colour produced when the spot was eluted and the eluted material was allowed to react with 2:7-dihydroxynaphthalene. The quantity present could not be estimated with this reagent since lactic acid interferes; it was therefore determined on deproteinized meat extract by the method of Dagley & Rodgers (1953).

Succinic acid. Succinic acid was determined on the anode fraction fluorimetrically after heating with resorcinol and H_2SO_4 (Barr, 1948).

RESULTS

Identification on paper chromatograms. R_F values of the various compounds for whose presence meat extract was examined, and their colour reactions, are shown in Tables 1-4.

Meat extract. The full analysis is presented in Table 5.

Colouring matter. A brown substance was deposited on both the anode membrane and the cathode membrane during electrodialysis. That on the anode membrane contained 16 % N and on the cathode 28 % N. The combined materials were hydrolysed with 6N-HCl for 4 hr. and then chromatographed on paper. β -Alanine and histidine were found and no sugars could be detected.

Fig. 2. Chromatographic chart of acids. The chromatograms were developed with ethanol-aq. NH_3 soln. and butanol-acetic acid, the order of development depending on the location reagent (see text). A, Aconitic; Ad, adipic; C, citric; F, fumaric; Gl, glycollic; Gt, glutaric; G, glyceric; Gp, glycerophosphoric; Gy, glyoxylic; H, hippuric; HCl, hydrochloric; β -Bu, β -hydroxybutyric; Hp, hydroxypyruvic; I, itaconic; α -K, α -oxoglutaric; L, lactic; Me, maleic; Ma, malic; Mo, malonic; Oa, oxaloacetic; Ox, oxalic; P, pyruvic; Pc, pyrrolidone-carboxylic; Pp, phenylpyruvic; Pl, phenyl-lactic; Ph, phosphoric; S, sulphuric; Su, succinic; T, tartaric; U, uric.

Table 1. R_F values of anserine and substances reacting with the diazo reagent

Solvents: 1, butanol-acetic acid; 3, phenol-HCl; 5, propanol-acetic acid.

	Solvent no.			Colour with diazo reagent
	1	3	5	
Histidine	0.21	0.19	0.07	Red
Histidine methyl ester	0.44	0.62	0.17	Red
1-Methylhistidine	0.21	0.36	0.07	None
Histamine	0.24	0.40	0.24	Red
Carnosine	0.20	0.36	0.07	Red
Anserine	0.20	0.58	0.07	None
Thiolhistidine	0.27	0.22	0.15	Yellow
Urocanic acid	0.61	0.77	0.67	Red
Ergothioneine	0.32	0.88	0.24	Yellow
Unidentified peptide	0.33	0.71	0.17	Red

Quaternary ammonium compounds. The only members of this group present were carnitine and a trace of choline. The identity of the latter was confirmed with phosphomolybdic acid (Block *et al.* 1955).

Organic phosphates. Only small amounts of these compounds were present, and inosinic acid was the only one to be identified with certainty. Others were tentatively identified from their R_f values as

Table 2. R_f values of phosphates

Solvents: 1, butanol-acetic acid; 6, acetone-acetic acid; 7, *tert.*-amyl alcohol-picric acid; 8, methanol-formic acid.

	Solvent no.			
	1	6	7	8
Orthophosphate	0.28	0.80	0.52	0.85
Pyrophosphate	0.09	0.63	0.11	0.73
Glucose 1-phosphate	0.09	0.61	0.09	0.55
Glucose 6-phosphate	0.09	0.68	0.11	0.66
Ribose 5-phosphate	0.17	0.80	0.25	0.76
Adenosine 5-phosphate	0.17	0.61	0.41	0.53
Adenosine diphosphate	0.02	0.83	0.14	0.73
Inosine 5-phosphate	0.13	0.62	0.17	0.50
α -Glycerol phosphate	0.15	0.70	0.50	0.87

Table 3. R_f values of acids

Solvents: 1, butanol-acetic acid; 4, ethanol-aq. NH_3 soln.

Acid	Solvent no.	
	1	4
Aconitic	0.79	0.04
Adipic	0.92	0.34
Citric	0.49	0.05
Fumaric	0.83	0.30
Glycollic	0.70	0.60
Glutaric	0.85	0.23
Glyceric	0.17	0.73
Glycerophosphoric	0.15	0.00
Glyoxylic	0.66	0.11
Glucuronic	0.25	0.30
Hippuric	0.96	0.80
Hydrochloric	0.19	0.65
β -Hydroxybutyric	0.75	0.68
Hydroxypyruvic	0.28	0.55
Itaconic	0.81	0.24
α -Oxoglutaric	0.55	0.27
Lactic	0.78	0.78
Maleic	0.54	0.20
Malic	0.55	0.13
Malonic	0.65	0.12
Oxaloacetic	0.78	0.05
Oxalic	0.26	0.08
Pyruvic	0.65	0.44
Pyrrolidone-carboxylic	0.63	0.46
Phenylpyruvic	0.94	0.80
Phenyl-lactic	0.95	0.89
Orthophosphoric	0.27	0.00
Pyrophosphoric	0.18	0.00
Sulphuric	0.26	0.06
Succinic	0.82	0.25
Tartaric	0.39	0.15
Uric	0.26	0.13

ribose 5-phosphate, α -glycerol phosphate and there was an unidentified compound moving at a slower rate in all solvents than the compounds listed in Table 2.

Carbohydrate. Tests with ammoniacal AgNO_3 , *m*-phenylene diamine, silver hydroxide, permanganate-periodate and other reagents (Block *et al.* 1955) failed to reveal any carbohydrate other than ribose 5-phosphate.

Organic acids. A small amount of β -hydroxybutyric acid was found to be present but was not determined. From the size and intensity of its spot relative to that due to the lactic acid it was estimated that the amount of β -hydroxybutyric acid was less than 1%. Oxalic acid was absent.

Unidentified compounds. An unknown peptide giving a positive diazo reaction was found to be present (see Fig. 1). This compound, together with carnosine, was adsorbed by the hydrogen form of Zeo-Karb 226. The eluted material was chromatographed in quantity on Whatman 3MM paper, and the zone containing this material, which ran slightly faster than carnosine in the standard solvent, was cut out and eluted with water. The solution was evaporated to dryness over H_2SO_4 *in vacuo* and colourless crystals were obtained. The compound was then run onto the resin column and found to occupy the position previously assigned to it. Another portion of the compound was hydrolysed for 6 hr. with 6*N*-HCl and the hydrolysis products were run on the column and on paper chromatograms. This work is still in progress but the compound appears to be a peptide containing histidine.

DISCUSSION

As the total of the compounds estimated accounts for approximately 100% of the meat extract, and the nitrogen fractions of the separate components amount to 97% of the nitrogen present, it would seem that apart from errors in the determinations it is unlikely that any major constituent has been overlooked.

Only one new compound has been discovered in the course of this work, the histidine-containing peptide described above, which is possibly identical with the avertabrin described by Kutscher & Ackermann (1926). Small amounts of various fluorescent substances were noted on the chromatograms viewed under ultraviolet light, but they were not identified.

The composition of the extract corresponds fairly well with the figures available in the literature (Embry & Henley, 1919; Rees & Salway, 1947; Allen's *Commercial Organic Analysis*, 1932). The carnitine content agrees with that found by biological assay (Carter, Bhattacharyya, Weidman & Fraenkel, 1952). The quaternary ammonium

Table 4. *R_F* values of further compounds

Means of detection: UVF, ultraviolet fluorescence; UVD, dark spot under ultraviolet light; B, bismuth tri-iodide reagent; D, diacetyl reagent; J, Jaffe reagent; SB, silver nitrate-bromophenol blue reagent; NI, ninhydrin; MP, *m*-phenylenediamine reagent; EH, Ehrlich reagent; C, cobaltinitrite; R, rhodizonic acid. The letter G denotes that one of the general reagents described in the text was used.

Guanidines	<i>R_F</i> in solvent no. 1	Means of detection	Colour
Arginine	0.16	D	Rose
Arcaine	0.36	D	Rose
Agmatine	0.08	D	Rose
Creatine	0.39	D	Violet
Creatinine	0.47	J	Orange
Glycocylamine	0.36	D	Rose
Guanidine	0.45	D	Rose
Methylguanidine	0.53	D	Rose
<i>as</i> -Dimethylguanidine	0.63	G	—
<i>s</i> -Dimethylguanidine	0.64	D	Rose
Quaternary ammonium compounds			
Betaine	0.37	B	Orange
Choline	0.47	B	Brown
Carnitine	0.45	B	Brick red
Carnitine ethyl ester	0.45	B	Brick red
Crotonbetaine	0.45	B	Yellow
Neurine	0.53	B	Brown
Purines and pyrimidines			
Adenine	0.60	SB	Blue
Guanine	0.38	SB	Blue
Hypoxanthine	0.48	SB	Blue
Xanthine	0.43	SB	Blue
Thymine	0.67	UVD	—
Cytosine	0.46	SB	Royal blue
Uracil	0.52	UVD	—
Adenosine	0.49	SB	Blue
Inosine	0.36	SB	Royal blue
Xanthosine	0.33	SB	Blue
Guanosine	0.38	SB	Blue
Cytidine	0.38	SB	Blue
Uridine	0.41	UVD	—
Adenylic acid	0.21	SB	Blue
Guanlylic acid	0.16	UVD	—
Cytidylic acid	0.21	UVD	—
Inosinic acid	0.13	SB	Blue
Uridylic acid	0.19	UVD	—
Adenosinediphosphoric acid	0.21	SB	Blue
Miscellaneous			
Ammonium	0.11	C	Yellow
Barium	0.16	R	Red
Calcium	0.18	R	Red
Sodium	0.36	—	—
Potassium	0.25	C	Yellow
Chloride	0.19	SB	Violet
Bromide	0.19	SB	Violet
Iodide	0.19	SB	Violet
Sulphate	0.08	—	—
β -Alanine	0.43	NI	Purple
Glucosamine	0.29	NI	Purple
Glutathione	0.14	NI	Purple
Citrulline	0.32	EH	Yellow
Ethanolamine	0.46	NI	Purple
Taurine	0.27	NI	Purple
Urea	0.55	EH	Yellow
Uric acid	0.30	G	—
Glucose	0.23	MP	Brown
Ribose	0.30	MP	Brown
Kynurenine	0.50	UVF	Blue
Kynurenic acid	0.62	UVF	Blue-green
Riboflavin	0.44	UVF	Yellow

compounds in muscle have been investigated and carnitine and choline were found (Bregoff, Roberts & Delwiche, 1953; Friedman, McFarlane, Bhattacharyya & Fraenkel, 1955). Glutathione was reported in meat extract by Rees & Salway (1947) but not by other investigators and none was found in the present analysis.

Apart from the high percentages of colouring matter the constituents listed in Table 5 are mostly those which would be expected from a knowledge of

the compounds occurring in muscle. It was somewhat surprising that no carbohydrates were found; it would seem that these compounds had been converted completely into lactic acid after the death of the animal. It is likely, too, that some sugar had been degraded to 'Maillard' type compounds (Hodge, 1953; Sharp, 1957).

Citrulline, glycollic acid and β -hydroxybutyric acid have not previously been reported in ox muscle. The glycollic acid apparently does not arise from the disproportionation of glyoxal since no oxalic acid could be detected in the extract.

In terms of commercial value, that is mainly flavour and texture, different batches of meat extract show small differences. These differences are partly due to varying qualities of the meat and there is a marked difference in the method of preparation from factory to factory. Nevertheless, it is reasonable to suggest that the analyses presented here are typical of meat extracts of the grade examined.

SUMMARY

1. A scheme for the separation, identification and determination of the compounds present in muscle extracts is outlined, and applied to commercial meat extract.

2. Protein was removed by dialysis and inorganic salts were removed by electrodialysis. A preliminary fractionation was made by electrodialysis.

3. The substances present were identified by paper chromatography and a range of colour reagents.

4. R_f values of compounds likely to be present in such extracts were determined, including a wide range of peptides, organic acids, organic phosphates, guanidines, quaternary ammonium compounds, purines, pyrimidines and inorganic ions.

5. A new procedure for the identification of the organic acids is described, consisting of a preliminary separation by electrodialysis followed by two-dimensional paper chromatography.

6. When identified the constituents were determined quantitatively or, where suitable methods were not available, by direct estimation of the colour intensity on the chromatograms.

7. The weight of colouring matter was determined by deposition on ion-exchange membranes after electrodialysis.

8. A hitherto undescribed compound and several compounds not previously reported in ox muscle were detected in the extract.

9. The sum of the constituents that have been determined in a sample of meat extract approximates to 100 % of the material.

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Table 5. *Composition of no. 1 meat extract*

All figures are based on the dry weight of the extract, which contained 17.28 % moisture and 13.21 % N on a dry basis. The carnosine, methylguanidine, guanidine, carnitine and hypoxanthine figures were based on the comparison of the colours on chromatograms with known amounts.

	Amount (% of total)	N (% of total wt.)
Amino acids		
3-Methylhistidine	(Trace)	—
α -Alanine	1.32	—
Serine	0.10	—
Methionine	0.01	—
Isoleucine	0.08	—
Leucine	0.08	—
Histidine	0.03	—
Taurine	0.32	—
Citrulline	0.26	—
(Total)	2.20	0.22
Peptides		
Carnosine	3.70	0.92
Anserine	0.75	0.16
Iminazole peptide	1.88	0.47
Guanidines		
Creatine	4.80	1.54
Creatinine	5.51	2.04
Methylguanidine	(~0.1)	—
Guanidine	(~0.1)	—
Purines, etc.		
Hypoxanthine	1.90	0.78
Inosine	0.70	0.15
Inosinic acid	(Trace)	—
Protein		
(14.9 % N; 0.1 % ash)	10.92	1.65
Organic acids		
Lactic acid	14.60	—
Glycollic acid	0.98	—
Succinic acid	1.26	—
β -Hydroxybutyric acid	(<1)	—
Carnitine	3.3	0.29
Choline	(Trace)	—
Urea	0.11	0.05
Ammonia	0.42	0.35
Inorganic matter (8.95 % K; 7.3 % P₂O₅)	29.40	—
Colouring matters	18.30	4.23
	100.73	12.85

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Studies on the Aerobic Degradation of Glucose by *Saccharomyces cerevisiae*

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The anaerobic degradation of glucose by yeasts has been thoroughly studied (Lardy, 1949; Meyerhof, 1951). However, much less is known about the metabolic pathways involved in the oxidation of glucose by yeasts. Based on studies of various strains of yeast and also by analogy with mechanisms discovered in other organisms, two major routes have been described for glucose oxidation: (1) direct oxidation, i.e. oxidation of the C-1 of glucose as the primary step, with or without preliminary phosphorylation of the molecule, and (2) oxidation

only after an obligatory stage of fermentation via the classical Embden-Meyerhof pathway.

The presence in *Saccharomyces cerevisiae* of enzymes involved in fermentation can be readily demonstrated, and the enzymic equipment necessary for the oxidation of glucose via a hexose monophosphate (HMP) pathway is almost as well documented (Horecker & Mehler, 1955). Recent attempts to assess the relative importance in yeast of these two routes during glucose oxidation have been performed by allowing intact cells to oxidize [¹⁴C]glucose labelled in different positions and analysing the products. For example, Blumenthal, Lewis & Weinhouse (1954) collected the acetate formed during oxidation of labelled glucose and,

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