SUMMARY

1. A comparison by electrophoretic analysis of extracts from normal and dystrophic ovine muscle has shown qualitative and quantitative differences in the protein composition between normal and affected muscle extracts.

2. There is an enhancement in two components which are thought to be actomyosin and myosin, and a marked decrease in a third component thought to be myogen in extracts from affected longissimus dorsi and quadriceps femoris muscles. These same extracts show an increase in a small, fast-moving component which is probably myoalbumin.

3. Electrophoresis differences were also observed in serum proteins from normal and affected animals. A marked increase in α -globulin and a decrease in the β -globulin and γ -globulin fractions were seen.

4. No increase in any serum component could be attributed to the decrease in the muscle-myogen fraction by the criterion of electrophoretic mobility. Similarly, muscle myoalbumin was not found to be the same protein as serum albumin by this criterion.

The authors wish to express their appreciation to Mr D. H. Fritts for the preparation of the photographs, and to Dr W. C. Marquardt for helpful criticism of the manuscript.

This investigation was supported in part by research grant A-4113 from the Institute of Arthritis and Metabolic Diseases, United States Public Health Service, and by a research grant from the Muscular Dystrophy Associations of America.

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Biochem. J. (1961) **81**, 98

Spectrophotometric Determination of β -Alanylhistidine Peptides in Ox-Muscle Extract

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(Received 26 January 1961)

Several workers have described chromatographic and microbiological methods for determining carnosiine in muscle and in muscle extracts from various animals (Williams & Krehl, 1952; Wood & Bender, 1957; Davey, 1957; McManus, 1957, 1960). However,

these methods either are too time-consuming and laborious for routine analyses or do not give reliable results. The present paper describes an accurate spectrophotometric method which is reasonably rapid and applicable to routine analyses.

EXPERIMENTAL

Meat extract. The commercial product known as 'no. 1 extract' or merely as 'meat extract' (Wood & Bender, 1957) was used.

Spectrophotometer. The spectrophotometric determinations were made with a Beckman model DU spectrophotometer in 1 cm. cells.

Chromatography. Paper chromatography was carried out by the ascending technique on Whatman no. 1 paper sheets (23 cm. $\times$ 25 cm.), with butan-2-one-propionic acid-water (75:25:30, by vol.) as solvent (Clayton & Strong, 1954).

The technique described by Hamilton & Anderson (1959) was employed for the column chromatography. The data for the column were: internal diameter, 0.9 cm.; length, 100 cm.; resin, Amberlite IR-120 II CG, fractionated according to the method of Moore, Spackman & Stein (1958) (fraction D consisting of particles $56 \pm 9 \mu$ in diameter was used); flow rate of buffers, 18.8 ml./cm.²/hr.

Precipitation of peptides. A solution containing about 0.2 g. of carnosine or β -alanylhistidine peptides (3.30 g. of meat extract) in about 50 ml. of water was mixed with 5 ml. of 10% (w/v) *m*-digallic acid and 1–2 drops of silicone antifoaming agent. It was shaken for 10 min. in a mechanical shaker and then placed in a refrigerator for 2 hr. It was made up to 100 ml. with water and a portion centrifuged at 2500 rev./min. The supernatant liquid was filtered through Whatman no. 42 filter paper and 2 ml. of the solution transferred to a 50 ml. centrifuge bottle. With stirring by a magnetic stirrer were added in this order: 5 drops of *N*-HCl, 10 ml. of a saturated aq. HgCl_2 containing 47% (w/v) sodium acetate trihydrate and 6.7% (w/v) NaCl, and 5 ml. of aq. 4% (w/v) $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$. It was set aside at room temperature until the precipitate had settled and then centrifuged at 2500 rev./min. for 30 min. All the supernatant liquid was sucked off through a thin capillary tube, and 5–6 drops of conc. HCl were allowed to fall on the precipitate. After stirring with a magnetic stirrer, about 5 ml. of water was added and the mixture saturated with H_2S . The precipitate was filtered off on Whatman no. 41 filter paper and the filtrate and washings were evaporated to dryness on a boiling-water bath. The residue was taken up in 6 ml. of 6*N*-HCl and after addition of a granule of Hengar silica (supplied by The Hengar Co., Philadelphia, Pa., U.S.A.) boiled for 8 hr. under reflux. The hydrolysate was dried on a boiling-water bath and allowed to cool, and the residue taken up with 10.0 ml. of water.

Separation of β -alanine from the α -amino acids. A mixture of 1 g. of basic Al_2O_3 (chromatographic grade) and 1 g. of freshly prepared $\text{Cu}(\text{OH})_2$ was introduced into a glass adsorption column (28 cm. $\times$ 0.9 cm. diameter). The column was washed with 50 ml. of water by gentle suction. The hydrolysate (2 ml.) was then applied, followed by water so that the total volume of hydrolysate and water sucked through was just short of 50 ml. The volume was made up to 50 ml. The β -alanine in 25 ml. of this solution was oxidized with acidic KMnO_4 , and the 2:4-dinitrophenylhydrazones prepared (Szalkowski, Mader & Frediani, 1951). The dried dinitrophenylhydrazones were dissolved in successive 5 ml. portions of methyl Cellosolve (ethylene glycol monomethyl ether) and made up to 50 ml.

Colour development and spectrophotometric measurement. A sample (10 ml.) of the solution was transferred to a

Table 1. *Analyses of meat extracts by the spectrophotometric and column-chromatographic methods*

Values are given as percentages of β -alanylhistidine peptides.

Sample	No. of analyses	Range	Average
Spectrophotometric method			
Argentina 1955	9	6.61–6.90	6.73
Argentina 1959	2	7.19–7.35	7.27
Paraguay 1959 (1)	14	5.50–6.18	5.83
Paraguay 1959 (2)	2	6.60–6.90	6.75
Paraguay 1959 (3)	2	6.50–6.60	6.55
Paraguay 1959 (4)	2	6.53–6.60	6.56
Tanganyika 1959 (1)	2	5.10–5.25	5.17
Tanganyika 1959 (2)	2	5.82–5.85	5.83
Tanganyika 1959 (3)	1	6.10	6.10
Kenya 1959 (1)	4	5.90–6.00	5.95
Kenya 1959 (2)	2	6.70–7.00	6.85
Column-chromatographic method			
Argentina 1955	4	6.50–6.90	6.65
Argentina 1959	2	7.22–7.30	7.26
Paraguay 1959 (1)	2	5.44–5.69	5.56

100 ml. graduated flask, 50 ml. of water at 20° added, and the flask placed in a water bath maintained at 20° for 10 min.; 5 ml. of 5*N*-NaOH was added and the mixture was diluted to volume with water at 20°. After thorough shaking the absorption was read in a Beckman DU spectrophotometer at 570 $m\mu$ in a 1 cm. cell. The instrument was adjusted to give zero absorption with a solution obtained by placing 10 ml. of methyl Cellosolve and 5 ml. of 5*N*-NaOH in a 100 ml. volumetric flask and making up to volume with water at 20°. With a factor obtained from a standard solution of β -alanine, the amount of β -alanine present in the hydrolysate was calculated. A blank prepared by carrying out all the operations described above on 25 ml. of water must be deducted both from the reading of the standard carried out to obtain the factor and from the reading of the sample under examination.

RESULTS

To determine the accuracy and the specificity of the proposed method, we added various amounts of pure carnosine (F. Hoffmann-La Roche and Co. Ltd., Basle, Switzerland) to a commercial meat extract whose content of β -alanylhistidine peptides had previously been determined by column chromatography. The recovery was almost quantitative. For instance, the recovery of 15.54 mg. of carnosine added to 0.5 g. of meat extract containing 6.8% of carnosine was 15.22, 15.44, 15.66 mg., representing a mean recovery of 99.35% and a standard error of ± 1.15 ; the recovery of 77.7 mg. of carnosine added to 2.5 g. of the same meat extract was 75.0, 77.2, 78.3, 78.3, 79.4 mg. (mean recovery of $99.92 \pm 1.90\%$).

Analysis of 11 samples of meat extract from various sources yielded 5.6–6.6% (average 6.2%) of β -alanylhistidine peptides. Further, we found a

very good agreement between the results obtained by the proposed method and those obtained by the column chromatography (see Table 1) which showed that the peptides consisted of 90% of carnosine and 10% of anserine.

The precipitate obtained with the HgCl_2 reagent was dissolved in a few drops of 5% (w/v) NaCN solution and chromatographed on resin columns. Fig. 1 shows that the precipitate in question contains predominantly carnosine with anserine in much smaller amount, as well as neutral and acidic amino acids, which emerge first from the columns. To confirm the identity of the peaks, the precipi-

Fig. 1. Ninhydrin-positive components of the precipitate obtained from meat extract by means of the HgCl_2 reagent. The precipitate was dissolved in a few drops of 5% (w/v) NaCN solution and diluted to 10 ml. with 0.15M-citrate buffer, pH 5, and 2 ml. was put on the column. The eluting buffer for the first 180 ml. was sodium citrate (0.15M; pH 5) and sodium citrate (0.2M; pH 5) for the remainder.

Fig. 2. Ninhydrin-positive components of the precipitate obtained from meat extract by means of the HgCl_2 reagent. The precipitate was dissolved in 6 ml. of 6N-HCl, hydrolysed for 8 hr., dried on a boiling-water bath and taken up in 10 ml. of 0.15M-citrate buffer, pH 5, and 2 ml. was put on the column. The eluting buffer for the first 180 ml. was sodium citrate (0.15M; pH 5) and sodium citrate (0.2M; pH 5) for the remainder.

tate was hydrolysed with 6N-HCl and the hydrolysate chromatographed on a resin column. Fig. 2 shows the β -alanine, histidine and 1-methylhistidine peaks.

Substances that, after oxidation with KMnO_4 , can form insoluble dinitrophenylhydrazones with 2:4-dinitrophenylhydrazine interfere in the determination of β -alanine. Histidine and the neutral and acidic amino acids that were present in our hydrolysate did this. A weighed amount (100 $\mu\text{g.}$) of each amino acid tested or of a mixture of them gave values corresponding to 10 $\mu\text{g.}$ of β -alanine. These interfering substances are, however, removed by passing the hydrolysate through the $\text{Cu}(\text{OH})_2\text{-Al}_2\text{O}_3$ column. The presence of alumina not only facilitates the filtration, but also serves to retain any traces of other interfering substances, such as carbohydrates. Fig. 3 shows the disappearance of the interfering amino acids, after passage through the column; only trace amounts of neutral and acidic amino acids are still present (peak no. 1), and peaks nos. 2 and 3 have been identified, as creatinine and NH_3 , respectively, which do not interfere.

The blue colour produced by the dinitrophenylhydrazone in alkaline medium quickly turned reddish on solution of the dinitrophenylhydrazone precipitate in acetone; it remained unchanged for at least 2 hr., even on exposure to light, when the precipitate was dissolved in methyl Cellosolve.

It is very important that the concentration of H_2SO_4 in the solution from which the dinitrophenylhydrazone is precipitated should be constant, since it markedly affects the precipitation. It is also advisable that, for each new batch of reagents, tests with known amounts of β -alanine should be carried out.

Fig. 3. Ninhydrin-positive components of the aq. eluate from the $\text{Cu}(\text{OH})_2\text{-Al}_2\text{O}_3$ column. This was concentrated to 10 and 2 ml. put on the resin column. The eluting buffer for the first 180 ml. was sodium citrate (0.15M; pH 5) and sodium citrate (0.2M; pH 5) for the remainder. Peak 1 corresponds to a trace amount of neutral and acidic amino acids, peaks 2 and 3 to creatinine and NH_3 respectively.

DISCUSSION

The method described is specific for β -alanylhistidine peptides, since, of the substances containing the histidine group that are precipitable by the mercuric chloride reagent, only those which yield β -alanine or subsequent hydrolysis are determined. In biological products this group includes only carnosine and anserine.

SUMMARY

1. A reasonably rapid and accurate method is described for the determination of β -alanylhistidine peptides in biological materials.

2. The peptides are precipitated with a mercuric chloride reagent, the precipitate is hydrolysed with 6N-hydrochloric acid, and the β -alanine formed is oxidized to a product which forms a dinitrophenylhydrazone.

3. The recovery of the carnosine added to a meat extract was $99 \pm 1.90\%$.

4. Meat extract from various sources contains 5.6–6.6% (average 6.2%) of β -alanylhistidine peptides containing 90% of carnosine and 10% of anserine.

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Biochem. J. (1961) **81**, 101

Rutilantin: an Antibiotic Substance with Antiphage Activity

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(Received 21 March 1961)

Asheshov, Strelitz, Hall & Flon (1954) examined 1000 actinomycete cultures for antiphage activity to determine whether bacterial viruses could be used as simulants of animal or plant viruses, since antiphage tests can be handled more easily and give more rapid results than antiviral tests. Of these cultures, 201 showed antiphage activity. One of them, which was bright red in colour (Serial No. A220), had a wide range of activity against phages of various strains of staphylococci, enterococci, *Bacillus cereus*, *Bacillus megatherium*, *Escherichia coli*, *Salmonella paratyphi* and *Vibrio cholerae*. Extracts prepared from cultures of this organism were also active against the viruses of Eastern and Western Equine encephalomyelitis, PR8 influenza, mumps and Y-SK poliomyelitis.

It was of interest to investigate the chemistry of the active substance, to which the name

'rutilantin' was given. It was recently shown by Ollis, Sutherland & Gordon (1959) that mild acid hydrolysis of rutilantin picrate, or of the purified base, yielded a bright-red crystalline aglycone ('rutilantinone'), and a water-soluble basic carbohydrate fraction, neither of which showed antibacterial or antiphage activity. Chemical investigations led to structure (I) for rutilantinone.

(I)

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This constitution was of particular interest because it was later established by Brockmann *et al.* (1960) that it is identical with the structure of