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# Content of Creatine - an Index of the Quality of Meat Products?

By

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An approach to the estimation of the quality of comminuted meat products has been made by determining the content of tryptophan and hydroxyproline (1,2,3). The high-quality animal protein contained in expensive tissues like skeletal muscle and red organs are high in tryptophan and low in hydroxyproline, while the reverse is true for the low-quality protein contained in cheap tissues like connective tissue, tendons and pig skin. The protein in smooth muscles occupies an intermediate position. In this group lungs, pig stomach and intestines of pig and calf have the highest content of tryptophan and a moderate content of hydroxyproline, corresponding to that of meat, in which about 15 % of the protein originates from connective tissue.

The present studies were undertaken to investigate, whether the assessment of the content of creatine can serve as an analytical tool for making conclusions of the quality of meat products or whether it can supply additional information of the origin of the ingredients beyond those obtained from the content of tryptophan and hydroxyproline. Since such conclusions will rest upon the content of a water soluble substance, care must be taken when judging the quality of products manufactured from cooked meat without its cooking water, e.g. corned beef.

## Earlier analyses of creatine in animal tissues

Hunter (4) has made a thorough review of the literature up to the end of the year 1926. The method used after about 1904 was that based on Jaffe's test (1886) in various modifications. This method is still widely used. In Jaffe's test a deep red colour is produced from creatinine and picric acid in alkaline solution. Creatine is converted to creatinine by heating with diluted sulphuric or hydrochloric acid.

In Table I are means and ranges for the creatine content in muscles and various organs compiled from Hunter's (4) monograph for the period from approximately 1908 to 1926. In the monograph the contents are given in mg creatine per 100 g of wet weight. In Table I, however, the values have been recalculated to creatine as a percentage of crude protein. This has been made in order to facilitate a comparison with our own results.

Table I

It may be added that also the content of creatinine was determined in a few cases. In muscular tissue, only between 1 and 2 % (relative) of the sum creatine + creatinine was found to be creatinine. In liver the corresponding figure was about 10 % and in kidney ca 15 %.

Ennor and Rosenberg (5) have reported on the content of creatine in skeletal and cardiac muscle, liver, kidney, and spleen in experimental animals using a method of Ennor and Stocken (6), based on the Voges-Proskauer reaction (diacetyl yields a red colour with guanidino groupings in alkaline solutions) intensified by the addition of $\alpha$-naphthol according to the detection of Barritt (7). Ennor and Rosenberg's results are summarized in Table II. In this case, too, the contents were expressed as mg creatine per 100 g wet weight. For the same reason as already mentioned these values were also recalculated to give creatine as a percentage of crude protein.


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Table II

The values in Table II are essentially in agreement with those of the corresponding tissues in Table I. In both tables the ranges are wide. In some cases (Table I) this is due to the use of different modifications of the method. The extreme values are not connected with the beginning or the end of the investigation period (1908-1926).

In several instances specific muscles were analyzed. Probably the water content of the samples as well as the content of protein and lipids were different. Since creatine is biochemically related to protein rather than the other constituents of the tissues, the content of protein should have been known. The assumptions with regard to protein content made in calculating the values in Table I and II may have caused deviations from the true values of 10 % or in single cases perhaps even more. At any rate, many values of the content of creatine are very low. It may be that some determinations were made so short a time post mortem that the creatine phosphate had not been completely split up in creatine and inorganic phosphate. In addition, connective tissue has a significant influence on the content of creatine (see below). Nothing is known about the content of connective tissue in the samples of Table I and II. Finally, the two methods mentioned are influenced by certain substances present in the tissues (4,6,8). Therefore, it seems important to get an idea of the magnitude of the errors caused by interfering substances, e.g. by applying some modern technique of separation.

The factors of uncertainty referred to here make old data seem less reliable, particularly when the object is to investigate, whether it is possible to characterize different tissues by means of their content of creatine and, ultimately, draw conclusions of the quality of meat products. In addition, data of some tissues which are of interest in this connection are lacking.

Slaughter by-products are, with some exceptions, not allowed as ingredients in meat products in many countries, e.g. France and Sweden. It would be injurious, however, to discriminate against the by-products as foods, since many of them have a considerable nutritive value. But if they are used as ingredients without due allowance for their lower price than muscle meat, it is of importance to know about their occurence in meat products.

## Present investigations

### Experimental

Extract. An extract was prepared by shaking 2 g finely minced tissue with 100 ml water at room temperature for 30 min., using an agitating machine. The vessel containing the mixture was then fitted with a 50 cm. long glass tube to be used as a cooler. After heating in a boiling water bath for 5 min., the content was cooled and filtered to get a clear extract. Extracts of skeletal and cardiac muscle as well as those of tongue were diluted in the ratio 1:4 (20 ml to 100 ml volume) except when used for chromatographic separation. Two extracts were made of each sample. Extending the time of extraction to 60 min. had no effect on the results.

In the case of rind (pig skin) gelatine goes into solution, thereby causing erroneous results when applying Method b (see below). Therefore, heating in the water bath was omitted in this instance.

Samples of specified muscles were freed from visible fat and connective tissue. In addition to creatine the content of water, crude protein (= N · 6.25) and fat (if necessary) were determined. In some cases also the content of hydroxyproline was established for correlating with the content of creatine. In calculating the content of creatine, due allowance was made for the water supplied to the extract by the sample. The content of creatine was calculated as a percentage of crude protein.

Method a: Based on Jaffe's colour reaction between creatinine and picrate. Measure 15-25 ml diluted extract of muscle, heart or tongue, or 10-15 ml original extract of fresh sausage, or 25 ml original extract of other tissues than those mentioned in a 50 ml volumetric flask. Adjust volume to 25 ml with water and add 10 ml 2 N HCl. Heat in an autoclave at 117°-120°C for 20 min. in order to convert creatine to creatinine. After cooling to room temperature add 9 ml 10 % NaOH and 3 ml saturated picric acid solution. Adjust to 50 ml volume. After 1 hour measure extinction at 5000 Å. Standard solutions containing 100, 200, 300, and 400 µg creatine are run simultaneously.

Method b: based on the colour reaction between water soluble guanidino compounds with a free guanidino-$NH_2$-group and diacetyl, intensified by $\alpha$-naphthol, in alkaline solution; method as modified by Rosenberg, Ennor & Morrison (9). Such guanidino compounds encountered in the material examined here are creatine and some free arginine. Others are present only in traces. Measure 2-4 ml (= v ml) diluted or original extract (see above) in a 25 ml Erlenmeyer flask, add (7-v) ml water, 1 ml 3 N NaOH, and 2 ml colour developing solution, which consists of 2.5 g $\alpha$-naphthol and 1.25 ml of an approximately 1 % watery solution of diacetyl (distilled from dimethylglyoxime and diluted (1:5) $H_2SO_4$) dissolved in n-propanol to a volume of 50 ml. The colour developing solution must be freshly prepared. The reaction mixture is left for 30 min. The extinction is then measured at 5350 Å. Standard solutions containing 10,20,30, and 40 µg creatine are run simultaneously.

Chromatographic separation. Creatine and arginine are easily separable on a column of Dowex 50 - X4 (sulphonated polystyrene of 4 % divinylbenzene cross-linking). Quantitative yields were obtained when running mixtures of these substances in the column. A 0.9 x 20 cm column of this cation exchange resin was used.

An extract was prepared as described above. However, of tissues other than skeletal and cardiac muscle and tongue 5 g were weighed instead of 2 g. None of the extracts was diluted. 10 ml of muscle extracts or 20-50 ml of extracts of other tissues were acidified with concentrated HCl to pH 2.0 - 2.2 and applied to the top of the column. The resin had previously been treated with a citrate buffer of pH 2.2 (10). After absorption of the bases and effluence of the liquid, creatine was displaced with 0.38 N (with respect to Na) Na-citrate buffer of pH 6.5 (see ref. (11)). Rate of flow was about 20 ml/hour. 50 ml of the effluent were collected in a 50 ml volumetric flask. This contained all the creatine. Then other guanidino compounds, which turned out to be traces or small amounts of arginine, were displaced with 0.35 N NaOH solution. In this case, too, 50 ml effluent were collected. Two separations were run on each sample.

For determination according to Method a, 15-25 ml of the creatine elution were used. It was established that the buffer solution did not influence the conversion of creatine to creatinine.

For determination according to Method b, 2-4 ml of the creatine elution and usually 8 ml of the arginine elution were used. In the latter instance the addition of 3 N NaOH was omitted. Since the method is influenced by the concentration of the reagents, also the concentration of NaOH, it is essential to run standard solutions of creatine and arginine in exactly the same way using the same quantities of buffer and 0.35 N NaOH solutions.

For comparison with the data obtained by Method b without chromatographic separation, the arginine values were converted to creatine values by multiplying by the factor 0.65 - arginine gives little less extinction at 5350 Å than creatine on a weight basis - and then added to the creatine values.

Solutions of pig skin were not suitable for chromatographic separation. The gelatine contained in them was displaced together with creatine and caused serious errors when applying Method b. If extracts of pig skin were not heated, this disturbance was not noted (Table IV).

Methods a and b applied in combination with chromatographic separation were designated c (a) and c (b), respectively.

Creatine (Merck) was purified according to Hunter (4) and its purity was established by the nitrogen content.

Arginine hydrochloride from California Corporation for Biochemical Research, Los Angeles, was used as a standard without further purification.

The content of hydroxyproline was determined in some cases for correlation with the content of creatine. Neuman & Logan's method as modified by Stegemann (12) was used.

## Results

In a series of analyses, the results of which are presented in Table III, it was established that, from a practical point of view, chromatographic separation can be omitted when analyzing skeletal and cardiac muscle, that is the tissues which beyond comparison are richest in creatine. Also, chromatographic separation is not necessary in analyzing other tissues, provided Method a is applied. However, such separation must be used in the case of milk powder.

Method b combined with chromatographic separation gives interesting information about guanidino compounds present in organs like spleen, chitterling, pig's stomach and kidney, and cow's udder.

The accuracy of the values in Table III, IV and V can be estimated to $\pm$ 0.05.

Table III

As a consequence of these results a larger number of samples were analyzed without chromatographic separation of the extracts but applying both Method a and b. The results are presented in Table IV. The samples of beef muscle were chosen in order to get an idea of the possible influence of the age of the animals and the aging of the meat on the content of creatine.

Table IV

The meat assortments I, II and III of beef, pork, veal, and horse used for sausage making contain increasing amounts of connective and fatty tissue. When analyzing, these tissues were not removed in these cases. From Table IV it is seen that connective tissue is practically devoid of creatine. For this reason it is to be expected that the assortments II and III have a lower content of creatine than pure muscle meat. That is also evident from Table IV. In fact, there exists an inverse relationship between the content of creatine and connective tissue. This is shown in Fig. 1, in which the content of creatine is plotted against that of hydroxyproline. The latter was used as an index of the content of

connective tissue (1,2,3). Also the values for beef head meat, beef and pig heart muscle, and tongue appear in the diagram (Fig. 1). However, these values lie outside the area for muscle meat marked by dotted lines. A possible explanation for this is discussed below.

Fig. 1

To check in practice the usefulness of the content of creatine as an index of the quality of meat, a number of fresh sausages from whole Sweden were analyzed according to both Method a and b without preceding chromatographic separation. The results are listed in Table V. In addition, the content of hydroxyproline was determined and the correlation between this content and the content of creatine (mean of the results of the two methods)was calculated.

Table V

From the data in Table V it can be calculated that there is no significant difference between the values of the two methods.

## Discussion

As has already been pointed out, the results presented in Table III suggest that there is no need for a chromatographic purification of heated extracts of animal tissue to assess the content of creatine in animal tissues, if the conventional picrate method (Method a) is applied. When analyzing milk powder - which could be used as an ingredient in sausage - however, serious errors occur, unless the extracts are purified.

Applying of Method b, by which the total content of guanidino compounds having a free guanidino-$NH_2$-group is determined, disclosed that the main part of the soluble guanidino compounds in beef and pig spleen is arginine, the content of which is about 3 times as high as that of creatine. The absolute content of arginine in this organ, calculated as a percentage of crude protein, is approximately 0.5 %. Also chitterling, pig's stomach, pig's kidney, and cow's udder contain significant amounts of arginine, viz. about 0.2-0.4 %, calculated as a percentage of crude protein. Separate analyses revealed, that the content of arginine was somewhat higher in the mucosa of pig stomach (0.50 % of the crude protein) but lower in that of chitterling (0.07 % of the crude protein) as compared with the whole organs.

With the exception of the organs just mentioned, the two methods give very similar results. This is evident from Table III, IV, and V. When Method b is applied, however, the determination of creatine in collagenous matter like pig skin (back rind) causes troubles due to the disturbance by water soluble protein (gelatine). This is not so, if heating is avoided during extraction (Table IV). Pig skin (rind) is to some extent used as an ingredient in sausage, since it is adhering to the pork (sometimes also extra rind is mixed in). Analyses of sausages from various manufacturers showed, however, that the rind contained in these products does not cause any significant difference between the two methods. This is evident from Table V, which also reveals a highly significant correlation between the content of creatine and hydroxyproline (inverse relationship).

Skeletal and cardiac muscle as well as tongue are the tissues which have by far the highest content of creatine, roughly $2\frac{1}{2}$, $1\frac{1}{2}$-2, and $1\frac{1}{2}$ % respectively, calculated as a percentage of crude protein. They are followed by pig's stomach

and chitterling (approx. 0.3-0.6 % creatine, calculated as a percentage of crude protein). Blood, skin, connective tissue, liver, and lungs are extremely poor in creatine (0.0-0.2 %, calculated as indicated).

No decisive conclusions can be made with regard to the influence of aging of meat on the content of creatine (samples No. 3´ and 4´, Table III; Nos. 10-17, Table IV), although there seems to be a tendency to decomposition of creatine during aging.

These findings permit certain conclusions of the quality of the ingredients in meat products from the content of creatine as a percentage of crude protein. One limitation, however, is the evaluation of the quality of products like liver paste and liver sausage. In addition, erroneous results will be obtained in the case of products manufactured from cooked meat without the cooking water, e.g. corned beef. In these instances the content of tryptophan can be used as guidance (cf. introduction to this paper). From an analytical point of view, however, the determination of water soluble guanidino compounds according to Method b, when applicable, is to be preferred because of its simplicity. The overestimation by this method of the quality, due to the presence of guanidino compounds other than creatine, which might occur in rare cases, is generally not serious.

The data obtained in this study compare essentially with the highest of those reported from earlier investigations (Table I and II). According to the present study the content of creatine in skeletal muscle is almost constant, while earlier investigators have found a very changing content. Very low values were recorded by Ennor and Rosenberg (5) for cardiac muscle of rabbit, cat and guinea pig.

From Fig. 1 the lowering effect of hydroxyproline, used as a measure of the content of connective tissue, on the content of creatine is evident. The content of creatine in particularly beef head meat, beef and pig tongue, pig´s heart, and to some extent also beef heart and frozen Argentinian horse meat (sample No. 38) is lower than could be expected from the content of hydroxyproline. Maybe creatine has been object to enzymatic decomposition in these tissues. In the case of Argentinian horse meat slight decomposition might be due to prolonged aging. Earlier investigators have shown that dark muscles have a lower content of creatine than light ones (4). If this holds, it may explain the said deviations. Anyhow, this must be object to special studies.

The content of creatine + creatinine in milk powder (Table III) is in fairly good agreement with that, which can be calculated from data reported by Shahani and Sommer (13). These authors found on an average 4.5 mg creatine + creatinine per 100 ml milk containing 493 mg total N in the same volume. This means 0.15 % creatine + creatinine as a percentage of crude protein. The corresponding maximum value can be calculated to 0.22 %.

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## SUMMARY

Analyses of various animal tissues and sausages have shown that the content of creatine, calculated as a percentage of crude protein, can roughly be used as an index of the quality of meat products with the exception of items such as liver paste and liver sausage as well as cooked products of the type corned beef.

Two different methods of analysis were compared. Among water soluble guanidino compounds other than creatine, arginine was found to be present in significant amounts in certain tissues.

## ZUSAMMENFASSUNG

Analysen verschiedener tierischer Gewebe und Würste haben dargetan, dass der Gehalt an Kreatin, berechnet als Prozent des Rohproteins, als Qualitätsmasstab von Fleischwaren verwendet werden kann, jedoch mit der Ausnahme von Leberpastete und Leberwurst sowie Corned Beef und ähnlichen Erzeugnissen mit gekochtem Fleisch.

Zwei verschiedene Analysenmethoden wurden verglichen. Unter den wasserlöslichen Guanidinverbindungen ausser Kreatin wurde Arginin in beträchtlichen Mengen in gewissen Organen gefunden.

## RÉSUMÉ

Les analyses de différents tissus animaux ont montré que la teneur en créatine exprimée en pourcentage des protéines, peut être un moyen d'estimer la qualité des produits de charcuterie, à l'exception des pâtés de foie, boudin blanc et de certains produits cuits, tels le corned beef.

L'auteur compare deux méthodes différentes. Parmi les dérivés guanidiques solubles dans l'eau, outre la créatine, il a été trouvé dans certains organes, quantité notable d'arginine.

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## Table I

Content of creatine in muscles and various organs according to analyses during the period 1908-1926 (4). In recalculating the values from mg creatine per 100 g wet weight to creatine as a percentage of crude protein (=N · 6.25), the following contents of protein have been assumed: skeletal muscle and liver 20 % (fish muscle 18 %), spleen and blood 18 %, heart 17 %, kidney 16 %, pig's stomach 15 %, and intestines 10 %.

| Tissue | Number of analyses | Creatine, calc. as a percentage of crude protein | |
|---|---|---|---|
| | | range | mean |
| Adult skeletal muscle, man | 16 | 1.26-2.58 | 1.83 |
| " " " , ox | 69 | 1.56-2.66 | 2.13 |
| " " " , sheep | 2 | 2.03-2.09 | 2.06 |
| " " " , horse | 8 | 1.59-2.25 | 2.02 |
| " " " , pig | 5 | 1.82-2.36 | 2.11 |
| " " " , dog | 97 | 0.74-2.44 | 1.78 |
| " " " , cat | 220 | 1.22-3.28 | 2.46 |
| Skeletal muscle, cat, 2 weeks | 1 | the same litter | 1.12 |
| " " , " , 5 " | 1 | | 1.55 |
| " " , " , 7 " | 1 | | 2.34 |
| Adult skeletal muscle, rabbit | 219 | 1.27-2.94 | 2.21 |
| " " " , " | 46 | red muscles | 1.60 |
| " " " , " | 52 | white " | 2.71 |
| Skeletal muscle, rabbit, 7 days | 1 | the same investigator | 1.11 |
| " " , " , 25 " | 1 | | 1.74 |
| " " , " , 46 " | 1 | | 2.17 |
| " " , " , adult | 1 | | 2.47 |
| Adult skeletal muscle, rat | 22 | 2.27-2.38 | 2.33 |
| " " " , guinea pig | 5 | 1.80-1.90 | 1.85 |
| " " " , domestic fowl | 20 | 1.31-2.86 | 2.12 |
| " " " , " " | 2 | leg (red) | 1.56 |
| " " " , " " | 2 | breast (white) | 2.11 |
| " " " , duck | 1 | - | 2.09 |
| " " " , goose | 2 | leg (red) | 1.83 |
| " " " , " | 2 | breast (white) | 2.35 |
| " " " , pigeon | 9 | 2.13-2.32 | 2.23 |
| " " " , turtle | 5 | 1.18-1.70 | 1.52 |
| " " " , frog | 314 | 0.99-2.56 | 1.73 |
| " " " , toad | 25 | 1.10-1.65 | 1.37 |
| Muscle, various fishes | 22 | 1.00-4.07 | 2.17 |
| " , " " | 2 | red muscles | 1.31 |
| " , " " | 2 | pale " | 2.64 |
| Heart, dog | 13 | 1.05-1.93 | 1.56 |
| " , rabbit | 13 | 0.82-1.71 | 1.17 |
| " , cat | 12 | 1.31-1.96 | 1.57 |
| " , sheep | 8 | 1.22-1.99 | 1.67 |
| " , ox | 6 | 1.41-1.73 | 1.51 |
| " , horse | 2 | 1.34-1.42 | 1.38 |
| " , fowl | 2 | 0.98-1.12 | 1.05 |
| Liver; dog, ox, pig, rabbit, cat, fowl, goat | 42 | 0.06-0.23 | 0.12 |
| Kidney; dog, ox, pig | 13 | 0.08-0.18 | 0.12 |
| Spleen; dog, ox | 8 | 0.09-0.17 | 0.12 |
| Stomach, pig | 2? | 0.61-0.72 | 0.67 |
| Small intestine, rabbit | 1 | - | 0.27 |
| Colon, rabbit | 1 | - | 0.38 |
| Blood; mammals, birds | 193 | 0.00-0.14 | 0.03 |

## Table III

Content of creatine (+creatinine) and other water soluble guanidino compounds in various animal tissues and skim milk as determined without and with chromatographic separation of the extracts.

| Sample No. | Tissue | Crude protein (N·6.25) % | Content calculated as a percentage of crude protein | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | No chromatographic separation | | Chromatographic separation | | | |
| | | | Method a | Method b | Method c(a) | Method c(b) | Method c(b) | Method c(b) |
| | | | creatine (+ creatinine) | water soluble guanidino compounds, calc. as creatine | creatine (+ creatinine) | creatine | arginine | water soluble guanidino compounds, calc. as creatine xxx) |
| 1 x) | Biceps femoris; cow, 7 years, grade 3+ | 22.3 | 2.31 | 2.27 | 2.23 | 2.23 | 0.10 | 2.30 |
| 2 | Longiss. dorsi; bacon pig, 6 months | 21.3 | 2.60 | 2.53 | 2.53 | 2.47 | 0.04 | 2.50 |
| 3 | Longiss. dorsi; cow, 8 years, grade 2+; aged 1 day | 22.6 | 2.26 | 2.24 | 2.24 | 2.21 | 0.17 | 2.32 |
| 4 | Longiss. dorsi; cow, 8 years, grade 2+; aged 17 days | 22.7 | 2.04 | 2.01 | 2.01 | 1.97 | 0.14 | 2.07 |
| 5 x) | Heart; cow, 6 years, grade 1 | 16.8 | 2.01 | 1.99 | 1.98 | 1.88 | 0.14 | 1.97 |
| 6 x) | Heart; bacon pig, 6 months | 17.0 | 1.66 | 1.63 | 1.62 | 1.58 | 0.14 | 1.67 |
| 7 | Kidney; cow, 7 years, grade 1 | 14.6 | 0.17 | 0.21 | 0.14 | 0.14 | 0.07 | 0.19 |
| 8 | Kidney; two bacon pigs | 14.3 | 0.18 | 0.34 | 0.15 | 0.20 | 0.24 | 0.36 |
| 9 | Spleen; cow, 7 years, grade 2+ | 18.5 | 0.26 | 0.48 | 0.17 | 0.17 | 0.50 | 0.50 |
| 10 | Spleen; six bacon pigs | 17.2 | 0.20 | 0.53 | 0.18 | 0.19 | 0.56 | 0.55 |
| 11 | Udder; cow, 6 years, grade 1 | 11.8 | 0.24 | 0.35 | 0.16 | 0.18 | 0.17 | 0.29 |
| 12 | Rumen; cow, 8 years, grade 1 | 14.2 | 0.37 | 0.43 | 0.38 | 0.36 | 0.09 | 0.42 |
| 13 | Stomach (maw); two bacon pigs | 14.6 | 0.56 | 0.64 | 0.55 | 0.52 | 0.17 | 0.63 |
| 14 | Chitterling (large intestine); two bacon pigs | 8.1 | 0.44 | 0.68 | 0.36 | 0.34 | 0.44 | 0.63 |
| 15 | Cracklings, from wet rendered pig fat | 28.1 | 0.06 | 0.14 | 0.05 | 0.04 | 0.10 | 0.11 |
| 16 | Dried skim milk, spray powder | 36.0 | (0.62) xx) | (0.56) xx) | 0.25 | 0.22 | 0.02 | 0.23 |
| 17 | Dried skim milk, roller powder | 33.6 | (0.72) xx) | (0.49) xx) | 0.29 | 0.24 | 0.02 | 0.25 |

x) Also the content of hydroxyproline was determined; see Fig. 1.
xx) Great difference between individual values. Interfering substances in the extract.
xxx) For conversion of the arginine values to creatine the factor 0.65 shall be applied.

## Table IV

Content of creatine (+ creatinine) and total water soluble guanidino compounds in various animal tissues as determined without chromatographic separation of the extracts. Excludes data of the samples listed in Table III.

Aging of M. longissimus dorsi was performed at +2° to +5°C.

| Sample No. | Tissue | Crude protein (N·6.25) % | Content calculated as a percentage of crude protein (No chromatographic separation) | |
|---|---|---|---|---|
| | | | Method a creatine (+creatinine) | Method b water soluble guanidino compounds, calc. as creatine |
| 1 | Longiss. dorsi; young bull, grade 1+; aged 3 days | 21.0 | 2.40 | 2.53 |
| 2 | " " ; old bull, " 1- ; " 2 " | 21.7 | 2.52 | 2.62 |
| 3 | " " ; steer, 2 years, " 1+ ; " 1 day | 20.1 | 2.47 | 2.49 |
| 4 | " " ; heifer, " " , " 1+ ; " 1 " | 20.2 | 2.51 | 2.53 |
| 5 | " " ; " , " " , " 1 ; " 8 days | 20.8 | 2.41 | 2.56 |
| 6 | " " ; cow, 3 " , " 1 ; " 2 " | 21.1 | 2.60 | 2.62 |
| 7 | " " ; " , 6 " , " 1 ; " 4 " | 22.3 | 2.50 | 2.43 |
| 8 | " " ; " , 7 " , " 1 ; " 4 " | 20.0 | 2.58 | 2.54 |
| 9x) | Triceps brachii; " , 7 " , " 3+ ; " 11 " | 21.6 | 2.30 | 2.29 |
| 10 | Longiss. dorsi; " , 3 " , " 1 ; " 5 " | 21.2 | 2.42 | 2.40 |
| 11 | " " ; " , " " , " " ; " 23 " | 22.1 | 2.23 | 2.25 |
| 12 | Longiss. dorsi; " , 7 " , " 1 ; " 2 " | 21.6 | 2.53 | 2.42 |
| 13 | " " ; " , " " , " " ; " 16 " | 21.2 | 2.46 | 2.38 |
| 14 | Longiss. dorsi; " , 6 " , " 1 ; " 1 day | 20.8 | - | 2.29 |
| 15 | " " ; " , " " , " " ; " 8 days | 21.4 | - | 2.30 |
| 16 | " " ; " , " " , " " ; " 14 " | 21.6 | - | 2.25 |
| 17 | " " ; " , " " , " " ; " 21 " | 21.4 | - | 2.24 |
| 18x) | Beef meat, assortment II xx) | 20.1 | 2.16 | 2.15 |
| 19x) | " " , " III xx) ; sample No. 1 | 17.9 | 1.79 | 1.72 |
| 20 | " " , " III xx) ; " " 2 | 19.8 | 1.63 | 1.66 |
| 21x) | " " , " III xx) ; " " 3 | 16.6 | 1.89 | 1.90 |
| 22x) | Beef, head meat xx) ; sample No. 1 | 21.4 | 1.34 | 1.31 |
| 23x) | " , " " " xx) ; " " 2 | 20.6 | 1.44 | 1.43 |
| 24x) | Tongue; cow, 7 years | 17.0 | 1.64 | 1.68 |
| 25 | Heart; cow, 6 " | 17.1 | 2.14 | 2.11 |
| 26 | Liver; cow, 7 years, grade 1 | 20.5 | 0.14 | 0.08 |
| 27 | Kidney; " , " " , " " | 14.6 | 0.18 | 0.22 |
| 28 | Spleen; " , " " , " " | 18.4 | 0.22 | 0.36 |
| 29 | Lungs; " , 8 " , " " | 16.9 | 0.18 | 0.29 |
| 30 | Udder; " , " " , " " | 11.8 | 0.31 | 0.42 |
| 31 | Rumen; " , 4 " , " expr | 12.0 | 0.38 | 0.42 |
| 32 | " ; " , 9 " , " 1+ | 12.5 | 0.37 | 0.39 |
| 33 | Diaphragm; six cows | 19.7 | 0.14 | 0.14 |
| 34 | Achilles tendon; steer, 2 years, grade 1 | 29.6 | 0.07 | 0.03 |
| 35 | Blood, beef | 18.0 | 0.03 | 0.02 |
| 36x) | Longiss. dorsi; newly born calf | 17.9 | 2.15 | 2.29 |
| 37x) | Veal, assortment II xx) | 20.7 | 2.05 | 2.16 |
| 38x) | Horse meat, assortment II xx) ; frozen Argentinian | 19.8 | 2.05 | 2.09 |
| 39x) | Horse meat, assortment II xx) ; Swedish | 18.3 | 2.17 | 2.14 |
| 40x) | " " , " III xx) ; " | 17.2 | 1.94 | 1.91 |

-continued

Table IV - continued

| | | | | |
|---|---|---|---|---|
| 41 | Longiss. dorsi; female bacon pig, normal | 20.2 | - | 2.64 |
| 42 | " " ; male " " , muscle degenerated | 20.5 | - | 2.60 |
| 43[x] | Ham, fore part; bacon pig; no visible fat or connective tissue | 19.9 | 2.65 | 2.64 |
| 44[x] | " , hind " ; " " ; no visible fat or connective tissue | 20.2 | 2.56 | 2.46 |
| 45[x] | Shoulder; bacon pig; no visible fat or connective tissue | 20.4 | 2.44 | 2.47 |
| 46[x] | Ham; bacon pig; connective tissue and part of the fat not removed | 18.2 | 1.99 | 2.05 |
| 47[x] | Lean pork, assortment I[xx]) | 19.4 | 2.38 | 2.46 |
| 48[x] | " " , " II[xx]); sample No. 1 | 15.7 | 2.05 | 2.06 |
| 49[x] | " " , " II[xx]); " " 2 | 18.3 | 2.12 | 2.19 |
| 50[x] | Tongue; bacon pigs | 16.1 | 1.48 | 1.53 |
| 51 | Heart; " " | 15.6 | 1.50 | 1.45 |
| 52 | Liver; " pig | 20.4 | 0.19 | 0.15 |
| 53 | Kidney; " pigs | 16.2 | 0.23 | 0.39 |
| 54 | Lungs; " pig; sample No. 1 | 14.6 | 0.16 | 0.24 |
| 55 | " ; " " ; " " 2 | 12.1 | 0.20 | 0.34 |
| 56 | Stomach (maw); bacon pig | 15.0 | 0.54 | 0.91 |
| 57 | Pig skin (back rind), bacon pig; sample No. 1 | 29.0 | 0.11 | (0.52)[y] |
| 58 | " " ( " " ), " " ; " " 2 | 34.0 | 0.22 | (0.42)[y] |
| 59 | " " ( " " ), " " ; " " 3 | 38.0 | - | 0.05[yy] |
| 60 | " " ( " " ), " " ; " " 4 | 36.4 | - | 0.05[yy] |
| 61 | Blood, pig | 17.6 | 0.04 | 0.05 |

x) Also the content of hydroxyproline was determined; see Fig. 1.

xx) The content of connective tissue increases from assortment I to III. This is reflected by the content of hydroxyproline (Fig. 1). Assortment I has only an insignificantly higher content of hydroxyproline than pure muscle. According to Fig. 1 the content of connective tissue in head meat of beef is slightly lower than that of assortment III of beef.

y) The extracts were not clear. The results are influenced by the presence of protein.

yy) In these determinations the extracts were not heated; hence interfering heat soluble protein did not get into solution.

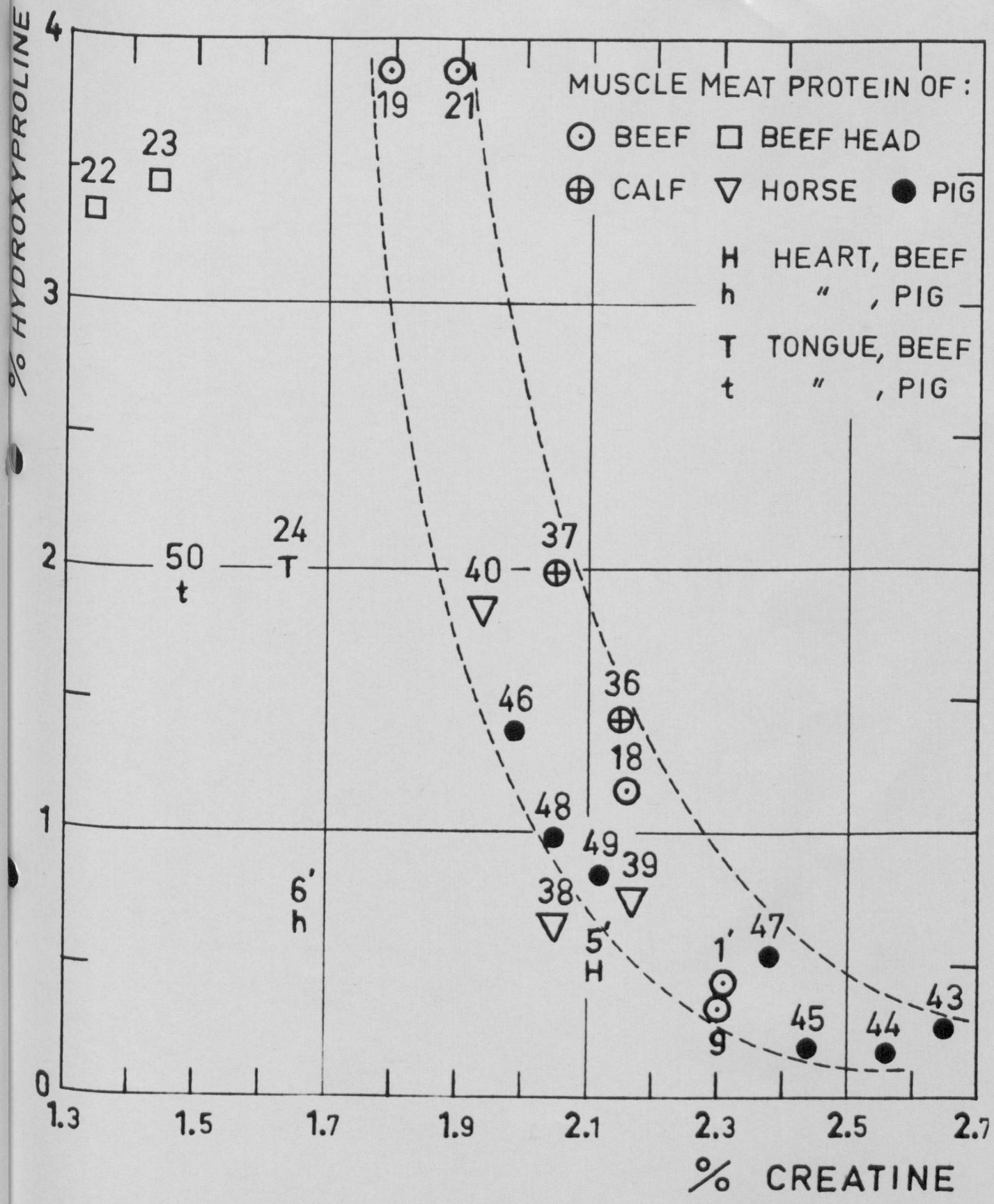


Fig. 1. Relationship between creatine and hydroxyproline in muscle meat protein, heart and tongue protein. The figures refer to sample numbers in Table III and IV.

Table V

Content of creatine(+ creatinine), total water soluble guanidino compounds and hydroxyproline in fresh sausages: Bologna (Falukorv) and Luncheon sausage (Frukostkorv).

| Sample No. | Sausage | Content calculated as a percentage of crude protein (N·6.25) | | |
|---|---|---|---|---|
| | | Method a creatine (+ creatinine) | Method b water soluble guanidino compounds, calc. as creatine | Hydroxy-proline |
| | | No chromatographic separation | | |
| 1 | Bologna | 1.55 | 1.60 | 3.43 |
| 2 | " | 1.40 | 1.44 | 4.16 |
| 3 | " | 1.44 | 1.52 | 3.88 |
| 4 | " | 1.78 | 1.72 | 2.59 |
| 5 | " | 1.78 | 1.74 | 2.44 |
| 6 | " | 1.83 | 1.77 | 2.66 |
| 7 | " | 1.74 | 1.68 | 3.26 |
| 8 | " | 1.61 | 1.66 | 3.79 |
| 9 | " | 1.54 | 1.56 | 2.97 |
| 10 | " | 1.63 | 1.71 | 2.92 |
| 11 | " | 1.56 | 1.51 | 2.63 |
| 12 | " | 1.64 | 1.62 | 2.72 |
| 13 | " | 1.53 | 1.58 | 3.27 |
| 14 | " | 1.68 | 1.67 | 3.31 |
| 15 | " | 1.76 | 1.77 | 2.30 |
| 16 | Luncheon sausage | 1.76 | 1.78 | 3.22 |
| 17 | " " | 1.71 | 1.70 | 3.62 |
| 18 | " " | 1.48 | 1.54 | 3.42 |
| 19 | " " | 1.92 | 1.91 | 2.53 |
| 20 | " " | 1.89 | 1.89 | 2.49 |
| 21 | " " | 1.77 | 1.72 | 2.63 |
| 22 | " " | 1.91 | 1.93 | 2.99 |
| 23 | " " | 1.64 | 1.72 | 3.25 |
| 24 | " " | 1.58 | 1.58 | 2.99 |
| 25 | " " | 1.50 | 1.58 | 2.98 |
| 26 | " " | 1.54 | 1.58 | 2.82 |
| 27 | " " | 1.59 | 1.57 | 2.70 |
| 28 | " " | 1.71 | 1.68 | 2.70 |

No significant difference exists between the results obtained by Method a and b. When correlating the content of creatine (mean of the results of Method a and b) with the content of hydroxyproline, the following results were obtained (C = creatine, H = hydroxyproline):

Coefficient of correlation $r = -0.55$ (Significance: $P < 0.01$, $P > 0.001$)

Regression equation $H = -2.1C + 6.3$