Creatine Content as an Index of the Quality of Meat Products

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

An approach to the estimation of the quality of comminuted meat products has been made by determining the content of tryptophan and hydroxyproline (*2–4*). The high-quality animal protein contained in expensive tissues, such as skeletal muscle and red organs, is high in tryptophan and low in hydroxyproline, while the reverse is true for the low-quality protein contained in cheap tissues, such as connective tissue, tendons, and pig skin. The protein in smooth muscles occupies an intermediate position. In this group, lungs, pig stomach, and intestines of pigs and calves have the highest content of tryptophan and a moderate content of hydroxyproline, corresponding to that of such meat in which about 15% of the protein originates from connective tissue.

The present studies were undertaken to investigate whether the assessment of the creatine content can serve as an analytical tool for drawing conclusions as to the quality of meat products or whether it can supply additional information on the origin of the ingredients beyond those obtained from the tryptophan and hydroxyproline contents. Since such conclusions will depend 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 Analysis of Creatine in Animal Tissues

Hunter (*8*) reviewed the literature up to the end of 1926. The method used after about 1904 was that based on Jaffe's test (1886) in various modifications, and it is still widely used. In Jaffe's test, a deep red color is produced from creatinine and picric acid in alkaline solution. Creatine is converted to creatinine by heating with dilute sulfuric or hydrochloric acid.

Table I shows means and ranges for the creatine content in muscles and various organs as compiled from Hunter's (*8*) monograph for the period from

Table I. Creatine Content of Muscles and Various Organs According to Analyses during the Period 1908–26 (*8*)

In recalculating the values from mg. of creatine per 100 grams 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, Calcd. 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		1.12
5 weeks	1	Same litter	1.55
7 weeks	1		2.34
Adult skeletal muscle, rabbit	219	1.27–2.94	2.21
	46	Red muscles	1.60
	52	White muscles	2.71
Skeletal muscle, rabbit, 7 days	1		1.11
25 days	1	Same	1.74
46 days	1	investigator	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 muscles	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

approximately 1908 to 1926. Hunter gave the contents in mg. of creatine per 100 grams of wet weight. In Table I, however, the values were recalculated to creatine as a percentage of crude protein. This was done to facilitate comparison with the author's results.

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

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

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 (1908–26).

In several instances specific muscles were analyzed. Probably the water contents of the samples as well as the content of protein and lipides were different. Since creatine is biochemically related to protein rather than to the other constituents of the tissues, the protein content should have been known. The assumptions with regard to protein content made in calculating the values in Tables 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 for the creatine content are very low. Possibly some determinations were made too soon post-mortem so that the creatine phosphate had not been completely split up into creatine and inorganic phosphate. In addition, connective tissue has a significant influence on the content of creatine as shown below. Nothing is known about the content of connective tissue in the samples of Tables I and II. Finally, the two methods mentioned are influenced by certain substances present in the tissues (*5*, *7*, *8*). Therefore, it seems important to obtain 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 creatine content and, ultimately, to draw conclusions as to the quality

Table II. Creatine Content of Muscles and Organs (6)

The values have been recalculated to creatine as a percentage of crude protein using the protein content specified in the title of Table I

	Creatine, Calcd. as a Percentage of Crude Protein (Range)				
Animal	Skeletal muscle	Cardiac muscle	Liver	Kidney	Spleen
Rabbit	1.91–2.20 (3)[a]	0.62–0.78 (4)	0.03–0.11 (4)	0.03–0.18 (4)	0.03–0.07 (4)
Cat	0.97–2.31 (4)	0.65–1.20 (4)	0.17–0.28 (4)	0.09–0.25 (3)	0.05–0.08 (4)
Guinea pig	1.39–2.20 (2)	0.29–0.69 (2)	0.02–0.05 (3)	0.05–0.12 (4)	0.06–0.07 (2)
Dog	1.85 (1)	1.58 (1)	0.02 (1)	0.09 (1)	0.07 (1)

[a] Figures given in parentheses are number of samples.

Table III. Content of Creatine (plus Creatinine) and Other Water-Soluble Guanidino Compounds in Various Animal Tissues and Skim Milk As Determined without and with Chromatographic Separation of the Extracts

			Content Calcd. as a Percentage of Crude Protein					
			No Chromatographic Separation		Chromatographic Separation			
Sample No.	Tissue	Crude Protein (N × 6.25), %	Method A (creatine + creatinine)	Method B (water-soluble guanidino compounds calcd. as creatine)	Method C(A) (creatine + creatinine)	Method C(B) creatine	Method C(B) arginine	Method C(B) (water-soluble guanidino compounds calcd. as creatine)[a]
1′[b]	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′	cow, 8 years, grade 2+, aged 1 day	22.6	2.26	2.24	2.24	2.21	0.17	2.32
4′	cow, 8 years, grade 2+, aged 17 days	22.7	2.04	2.01	2.01	1.97	0.14	2.07
5′[b]	Heart, cow, 6 years, grade 1	16.8	2.01	1.99	1.98	1.88	0.14	1.97
6′[b]	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′	2 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′	6 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), 2 bacon pigs	14.6	0.56	0.64	0.55	0.52	0.17	0.63
14′	Chitterling (large intestine), 2 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)[c]	(0.56)[c]	0.25	0.22	0.02	0.23
17′	roller powder	33.6	(0.72)[c]	(0.49)[c]	0.29	0.24	0.02	0.25

[a] For conversion of the arginine values to creatine, the factor 0.65 should be applied. [b] Hydroxyproline content was also determined.
[c] Interfering substances in the extract may have caused the great difference between individual values.

of meat products. In addition, data for 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 occurrence in meat products.

Present Investigations

Experimental. EXTRACT. An extract was prepared by shaking on an agitator 2 grams of finely minced tissue with 100 ml. of water at room temperature for 30 minutes. The vessel containing the mixture was then fitted with a 50-cm. glass tube to be used as a cooler. After being heated in a boiling water bath for 5 minutes, the contents are cooled and filtered to obtain 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 extraction time to 60 minutes had no effect on the results.

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

Samples of the specific muscles were freed from visible fat and connective tissue. In addition to creatine, the water, crude protein (N × 6.25), and fat (if necessary) contents were determined. In some cases also, the hydroxyproline content 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 color reaction between creatinine and picrate). Measure in a 50-ml. volumetric flask 15 to 25 ml. of diluted extract of muscle, heart, or tongue, or 10 to 15 ml. of original extract of fresh sausage, or 25 ml. of original extract of tissues other than those mentioned. Adjust the volume to 25 ml. with water and add 10 ml. of 2*N* HCl. Heat in an autoclave at 117° to 120° C. for 20 minutes to convert creatine to creatinine. After cooling the solution to room temperature, add 9 ml. of 10% NaOH and 3 ml. of saturated picric acid solution. Adjust the volume to 50 ml. After 1 hour, measure the absorbance at 5000 A. Standard solutions containing 100, 200,

Table IV. Content of Creatine (Plus Creatinine) and Total Water-Soluble Guanidino Compounds in Various Animal Tissues As Determined without Chromatographic Separation of Extracts

Excludes data of 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 Calcd. as a Percentage of Crude Protein (No Chromatographic Separation) Method A	Method B
1	Longiss. dorsi, young bull, grade 1+, aged 3 days	21.0	2.40	2.53
2	old bull, grade 1−, aged 2 days	21.7	2.52	2.62
3	steer, 2 years, grade 1+, aged 1 day	20.1	2.47	2.49
4	heifer, 2 years, grade 1+, aged 1 day	20.2	2.51	2.53
5	heifer, 2 years, grade 1, aged 8 days	20.8	2.41	2.56
6	cow, 3 years, grade 1, aged 2 days	21.1	2.60	2.62
7	cow, 6 years, grade 1, aged 4 days	22.3	2.50	2.43
8	cow, 7 years, grade 1, aged 4 days	20.0	2.58	2.54
9[a]	Triceps brachii, cow, 7 years, grade 3+, aged 11 days	21.6	2.30	2.29
10	Longiss. dorsi, cow, 3 years, grade 1, aged 5 days	21.2	2.42	2.40
11	cow, 3 years, grade 1, aged 23 days	22.1	2.23	2.25
12	cow, 7 years, grade 1, aged 2 days	21.6	2.53	2.42
13	cow, 7 years, grade 1, aged 16 days	21.2	2.46	2.38
14	cow, 6 years, grade 1, aged 1 day	20.8	...	2.29
15	cow, 6 years, grade 1, aged 8 days	21.4	...	2.30
16	cow, 6 years, grade 1, aged 14 days	21.6	...	2.25
17	cow, 6 years, grade 1, aged 21 days	21.4	...	2.24
18[a]	Beef meat, assortment II[b]	20.1	2.16	2.15
19[a]	assortment III,[b] sample No. 1	17.9	1.79	1.72
20	assortment III,[b] sample No. 2	19.8	1.63	1.66
21[a]	assortment III,[b] sample No. 3	16.6	1.89	1.90
22[a]	Beef, head meat[b], sample No. 1	21.4	1.34	1.31
23[a]	sample No. 2	20.6	1.44	1.43
24[a]	Tongue, cow, 7 years	17.0	1.64	1.68
25	Heart, cow, 6 years	17.1	2.14	2.11
26	Liver, cow, 7 years, grade 1	20.5	0.14	0.08
27	Kidney, cow, 7 years, grade 1	14.6	0.18	0.22
28	Spleen, cow, 7 years, grade 1	18.4	0.22	0.36
29	Lungs, cow, 8 years, grade 1	16.9	0.18	0.29
30	Udder, cow, 8 years, grade 1	11.8	0.31	0.42
31	Rumen, cow, 4 years, grade extra prima	12.0	0.38	0.42
32	cow, 9 years, grade 1+	12.5	0.37	0.39
33	Diaphragm, 6 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
36[a]	Longiss. dorsi, newborn calf	17.9	2.15	2.29
37[a]	Veal, assortment II[b]	20.7	2.05	2.16
38[a]	Horse meat, assortment II,[b] frozen Argentinian	19.8	2.05	2.09
39[a]	assortment II,[b] Swedish	18.3	2.17	2.14
40[a]	assortment III,[b] Swedish	17.2	1.94	1.91
41	Longiss. dorsi, female bacon pig, normal	20.2	...	2.64
42	male bacon pig, muscle degenerated	20.5	...	2.60
43[a]	Ham, fore part, bacon pig, no visible fat or connective tissue	19.9	2.65	2.64
44[a]	hind part, bacon pig, no visible fat or connective tissue	20.2	2.56	2.46
45[a]	Shoulder, bacon pig, no visible fat or connective tissue	20.4	2.44	2.47
46[a]	Ham, bacon pig, connective tissue and part of the fat not removed	18.2	1.99	2.05
47[a]	Lean pork, assortment I[b]	19.4	2.38	2.46
48[a]	assortment II,[b] sample No. 1	15.7	2.05	2.06
49[a]	assortment II,[b] sample No. 2	18.3	2.12	2.19
50[a]	Tongue, bacon pigs	16.1	1.48	1.53
51	Heart, bacon pigs	15.6	1.50	1.45
52	Liver, bacon pig	20.4	0.19	0.15
53	Kidney, bacon pigs	16.2	0.23	0.39
54	Lungs, bacon pig, sample No 1	14.6	0.16	0.24
55	bacon pig, sample No. 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)[c]
58	bacon pig, sample No. 2	34.0	0.22	(0.42)[c]
59	bacon pig, sample No. 3	38.0	...	0.05[d]
60	bacon pig, sample No. 4	36.4	...	0.05[d]
61	Blood, pig	17.6	0.04	0.05

[a] Hydroxyproline content was also determined (Figure 1).

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

[c] Extracts were not clear. The results are influenced by the presence of protein.

[d] In these determinations, extracts were not heated; hence interfering heat-soluble protein did not get into solution.

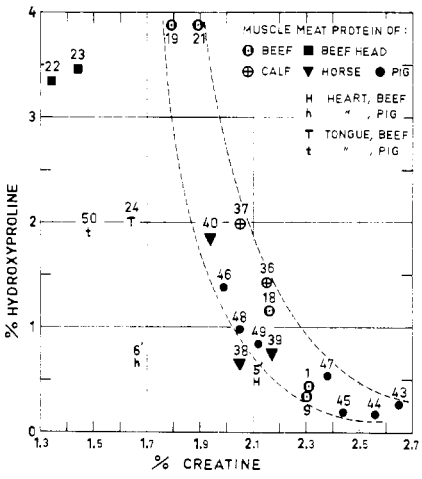

Figure 1. Relationship between creatine and hydroxyproline in muscle meat protein, heart and tongue protein. Figures refer to sample numbers in Tables III and IV

300, and 400 μg. of creatine are run simultaneously.

METHOD B [based on the color reaction between water-soluble guanidino compounds with a free guanidino-NH_2 group and diacetyl, intensified by α-naphthol, in alkaline solution; method as modified by Rosenberg *et al.* (*11*)]. Guanidino compounds encountered in the material examined here are creatine and some free arginine. Other such compounds are present only in traces. Measure 2 to 4 ml. (= *v* ml.) of diluted or original extract in a 25-ml. Erlenmeyer flask, add (7 − *v*) ml. of water, 1 ml. of 3*N* NaOH, and 2 ml. of color-developing solution, which consists of 2.5 grams of α-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 color-developing solution must be freshly prepared. Allow the reaction mixture to stand for 30 minutes, and measure absorbance at 5350 A. Standard solutions containing 10, 20, 30, and 40 μg. of creatine may be run simultaneously.

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

An extract was prepared as described above. However, for tissues other than skeletal and cardiac muscle and tongue, 5 grams were weighed instead of 2 grams. None of the extracts was diluted. Ten milliliters of muscle extracts or 20 to 50 ml. of extracts of other tissues were acidified with concentrated HCl to pH 2.0 to 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 (*9*). Rate of flow was about 20 ml. per hour. Fifty milliliters of the effluent were collected in a 50-ml. volumetric flask. This contained all of 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. of effluent were collected. Two separations were run on each sample.

For determination according to Method A, 15 to 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 to 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 and also by 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 somewhat less absorbance at 5350 A. than creatine on a weight basis—and then added to the creatine values.

Solutions of pig skin were not suitable for chromatographic separation. The gelatin contained in them was displaced together with creatine and caused serious errors when Method B was used. 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 & Co.) was purified according to Hunter (*8*), and its purity was established by the nitrogen content.

Arginine hydrochloride (California Corp. for Biochemical Research, Los Angeles) was used as a standard without further purification.

Hydroxyproline content was determined in some cases for correlation with the content of creatine. The method of Neuman and Logan as modified by Stegemann (*13*) was used.

Results. In a series of analyses (Table III), it was established that, from a practical point of view, chromatographic separation can be omitted when analyzing skeletal and cardiac muscle—i.e., 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 such as spleen, chitterling, pig's stomach and kidney, and cow's udder.

The accuracy of the values in Tables III, IV, and V can be estimated to be ±0.05.

As a consequence of these results, a larger number of samples were analyzed without chromatographic separation of the extracts but by applying both Methods A and B. The results are presented in Table IV. The samples of beef muscle were chosen to show the possible influence of the age of the animals and the aging of the meat on the content of creatine.

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 analyzed, these tissues were not removed. Data in Table IV show that connective tissue is practically devoid of creatine. For this reason, assortments II and III may be expected to have a lower content of creatine than pure muscle meat, as 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 Figure 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 (*2–4*). The values for beef head meat, beef and pig heart muscle, and tongue are also shown in the diagram. However, these values lie outside the area for muscle meat as marked by dotted lines. A possible explanation for this is discussed below.

To check in practice the usefulness of creatine content as an index of meat quality, a number of fresh sausages from various parts of Sweden were analyzed according to both Method A and B without preceding chromatographic separation. The results are listed in Table V. In addition, the hydroxyproline content was determined, and the correlation between this content and that of creatine (mean of the results of the two methods) was calculated. From the data in Table V, it can be calculated that there is no significant difference between the values of the two methods. The quality of sausage products can be estimated with regard to the data given in Tables III and IV. If feasible, the products may be graded in two or three groups with creatine contents within fixed ranges.

Discussion. 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. However, when analyzing milk powder—which could be used as an ingredient in sausage—serious errors occur unless the extracts are purified.

Applying Method B disclosed that the main part of the soluble guanidino compounds in beef and pig spleen is arginine, the content of which is about three 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 to 0.4%, calculated as a percentage of crude protein. Separate analyses revealed that the arginine content 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 organ.

With the exception of the organs just mentioned, the two methods give very similar results. This is evident from Tables III, IV, and V. When Method B is applied, however, the determination of creatine in collagenous matter, such as pig skin (back rind), causes trouble due to the interference by water-soluble protein (gelatin). This is not the case if heating is avoided during extraction (Table IV). Pig skin (rind) is to some extent used as an ingredient in sausage, since it adheres to the pork (sometimes extra rind is also 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—approximately $2^1/_2$, $1^1/_2$ to 2, and $1^1/_2$%, respectively, calculated as a percentage of crude protein. They are followed by pig's stomach and chitterling (approximately 0.3 to 0.6% creatine, calculated as a percentage of crude protein). Blood, skin, connective tissue, liver, and lungs are extremely poor in creatine (0.0 to 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; 10 to 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 such as 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 tryptophan content can be used as a guide. From an analytical point of view, however, the determination of water-soluble guanidino compounds according to Method B, when applicable, is preferred because of its simplicity. Overestimation of the quality by this method, 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 (Tables I and II). According to the present study, the content of creatine in skeletal muscle is almost constant, while earlier investigators found a very changing content. Very low values were recorded by Ennor and Rosenberg (*6*) for cardiac muscle of rabbit, cat, and guinea pig.

From Figure 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, particularly in beef head meat, beef and pig tongue, pig's heart, and, to some extent also, beef heart and frozen Argentinian horsemeat (sample no. 38), is lower than could be expected from the content of hydroxyproline. Creatine may be the object of enzymatic decomposition in these tissues. In the case of Argentinian horsemeat, slight decomposition might be due to prolonged aging. Earlier investigators have shown that dark muscles have a lower content of creatine than light ones (*8*). If this is true, it may explain the deviations. However, this must be the object of special studies.

The content of creatine plus creatinine in milk powder (Table III) is in fairly good agreement with that calculated from data reported by Shahani and Sommer (*12*). These authors found, on an average, 4.5 mg. of creatine plus creatinine per 100 ml. of milk containing 493 mg. of total N in the same volume. This means 0.15% creatine plus creatinine as a percentage of crude protein. The corresponding maximum value can be calculated as 0.22%.

Table V. Content of Creatine (plus Creatinine), Total Water-Soluble Guanidino Compounds, and Hydroxyproline in Fresh Sausages—Bologna (Falukorv) and Luncheon Sausage (Frukostkorv)[a]

	Content Calcd. as a Percentage of Crude Protein (N × 6.25)		
	No Chromatographic Separation		
Sample No.	Method A, (creatine + creatinine)	Method B, (water-soluble guanidino compounds, calcd. as creatine)	Hydroxyproline
	Bologna		
1	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
	Luncheon Sausage		
16	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

[a] When correlating the content of creatine (mean of the results of Methods 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: $H = -2.1C + 6.3$

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SELENIUM SUPPLEMENTATION

A Survey of Selenium Treatment in Livestock Production

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Reports of the striking results of selenium supplementation in correcting disease and increasing livestock production in New Zealand prompted a survey of selenium work at each experiment station in the U. S. and at the principal agricultural centers abroad. The survey showed that such selenium deficiency diseases as white muscle disease of cattle and sheep are widespread, though often unrecognized. Selenium therapy prevents these diseases and many cases of scours and general ill thrift. Correction of subacute deficiencies gives striking increases in weight gain, fertility, and survival rates. Recommended methods of treatment include injection and oral drenching. As dosages are well below toxic levels, the safety factor is high. While a number of research problems still await solution, better diagnosis of known deficiency diseases and wider recognition of available methods of treatment can bring immediate practical benefits in livestock production.

Selenium, long considered toxic to livestock, has recently been found to be a trace element essential for animal life. In 1957 it was identified as a key factor in nutrition (*46, 49*). Already more than one hundred publications have appeared describing fundamental investigations or practical applications of selenium in animal nutrition.

Particularly striking reports on selenium treatment have appeared from New Zealand (*19, 22–25, 40, 50*), where large areas are deficient in selenium. Applications of minute doses of selenium compounds have cured such disorders as white muscle disease in cattle, sheep (stiff-lamb disease), horses, and swine, and exudative diathesis in poultry. Selenium treatment has also corrected such poorly defined conditions as ill thrift, infertility, and chronic scouring. Perhaps most important, even in areas where no clinical symptoms of deficiency were observed, selenium treatment has often given marked increases in weight gain, reproductive rate, and wool yield.

To determine the extent of selenium deficiency in soils and feeds and the potential significance of selenium treatment in world livestock production, a survey was conducted among the state agricultural experiment stations in the United States and in the principal agricultural research centers abroad. Replies were received from several federal agencies, all 50 states, Puerto Rico, the six Australian states, Canada, New Zealand, South Africa, Turkey, and the United Kingdom. Besides completing a questionnaire, many respondents sent summaries of unpublished work, and several New Zealand workers sent detailed reports of both experimental and practical field results. Sources of such unpublished data are listed in section A of the Literature Cited. The survey was conducted in the period September 1961 to January 1962.

Selenium as a Nutritional Factor

The research that eventually led to the discovery of selenium as a nutritional factor originated in studies of brewer's yeast as a protein supplement in Europe during World War II. German research workers found that rats on a yeast diet developed liver necrosis (*26*). Wheat germ and wheat bran showed protective activity against this disorder. On fractionation, vitamin E (α-tocopherol) was identified as the main protective agent (*45*).

Attempts by American scientists to duplicate the necrotic liver syndrome by feeding rats a diet of brewer's yeast were unsuccessful. However, with a diet based on torula yeast, Schwarz reproduced the results obtained in Europe (*44*). He gave the name Factor 3 to the unidentified material present in American brewer's yeast but not in torula yeast or European brewer's yeast. L-Cystine was erroneously considered to be Factor 2 which, like vitamin E, appeared to provide protection against necrotic liver damage.

A second connection between vitamin E and Factor 3 was established when Scott and coworkers discovered that brewer's yeast would prevent exudative diathesis in chickens. This condition was recognized as a vitamin E deficiency which could be induced with a torula yeast diet (*47*).

Selenium was finally identified as the key component of Factor 3 in 1957

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