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Free Methionine, Occurence and Significance in Animal Tissues.

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An appreciable amount of the methionine found in muscle is present in a free state, viz. about 10 % of the total.[1,2] The residue is bound as a protein constituent. Under aseptic conditions, the content of free methionine in muscle is constant for at least three weeks at temperatures just above zero.[1,2]

As evidenced by several investigations, the rôle played by methionine in biological systems is as a methylating agent and as a metabolic precursor of cysteine. To be able to act in transmethylation reactions, however, methionine must be converted to S-adenosylmethionine, a phosphate-free sulphonium compound.[3,4] The conversion is performed by means of an activating enzyme, adenosine triphosphate, Mg^{++}, and reduced glutathione. The transmethylations are catalyzed by specific methylpherases. The product of transmethylation is usually the N-methyl derivative of the acceptor while S-adenosylmethionine is converted to S-adenosylhomocysteine. Examples of such transmethylations are glycine ⇆ sarcosine and betaine, ehtanolamine ⇆ choline, carnosine → anserine, dopa amine → adrenaline, glycocyamine → creatine.

The functional significance of methionine excites attention to the occurence of free methionine in various tissues. The presence of free methionine might indicate transmethylation activity and/or the conversion of methionine into cysteine.

Experimental

Free methionine was determined in a watery extract of various animal tissues after precipitating proteinous material with phosphotungstic acid and then applying the method of McCarthy and Sullivan[5] as modified by Horn, Jones and Blum[6].

The extract was prepared by heating 50 g finely ground material + 100 ml water for 20 minutes in a boiling water bath using reflux. After cooling to room temperature the mixture was agitated in a machine for 30 minutes and then centrifuged. Between 0.5 and 4 g phosphotungstic acid was added to 100 ml extract, the amount of phosphotungstic acid being dependent on the material; extracts of intestines, stomachs and certain organs require a larger addition than those of skeletal muscles. After having been kept in a refrigerator over night the extract was filtered to give a clear solution. Ground pig skin (Table I, sample No. 76) was extracted without preceding heat treatment.

Briefly, the determination was carried out by first adding 1 ml 5N NaOH to 4.5 ml extract and then by adding 0.5 ml of a 2 % Na-nitroprusside. After exactly 10 minutes at room temperature 2 ml of a 3 % solution of glycine was added and, after another 10 minutes period, 2 ml concentrated phosphoric acid. Ten minutes thereafter the extinction was read in a Beckman spectrophotometer model B at 510 mμ. A blank test and a series of standard solutions of methionine (0.25 to 1.00 ml of a solution of 100 mg methionine in 95 ml water + 5 ml 6N HCl were used) were measured for calibration. Two analyses were performed on separate extracts. Addition of known amounts of methionine to various extracts yielded complete retention.

For calculation the content of water and protein of the material was determined. The content of methionine was computed on a crude protein basis, assuming that the free methionine after agitating was evenly distributed in the added water and that contained in the tissue.

The method used is similar to the modification by Csonka and Denton[7] which has been found to give results in good agreement with those obtained by the microbiological method.[8]

Results and discussion

Table I shows the results obtained. The determinations were performed on the various tissues 1 to 4 days after slaughter of the animals. Although the content of free methionine is surprisingly constant in the refrigerated intact muscle during a long period post mortem - as was already remarked in the introduction - it was observed that the content generally increased considerably when ground samples were stored refrigerated or frozen. This was especially true for the organs. Increased enzyme activity due to cell disruption is, however, a well-known phenomenon.

Table I

The high content of free methionine in skeletal muscles - on an average 200 mg-% of the crude protein - is very striking. Assuming a total of 2.5 % methionine in the muscle protein (see ref.[9]), 6 to 11 % (average 8 %) of the methionine is present in a free state. Of course, this fact is no unambiguous proof of an outstanding functional significance of free methionine in the skeletal muscles, even less could conclusions be drawn about which functions it exerts. But the fact that the content of free methionine in skeletal muscle is essentially higher than in organs and other tissues investigated makes an interesting starting point for further studies on this particular subject.

Samples No. 4, 8, 9, 13, and 14 were taken from different parts of the same carcass. Evidently the content of free methionine in various beef skeletal muscles is fairly constant. The range was 181-202 mg-% free methionine in the crude protein and this content is obviously independent of the content of connective and fatty tissue (see below). These conclusions seem to be true also for horse muscles. Thus, the samples No. 29, 31, and 33, with contents of free methionine ranging between 200 and 225 mg-% of the crude protein, were taken from one horse carcass and the samples No. 30, 32, and 34 with contents of 152-183 mg-% were taken from another. In addition, these results indicate that there might be individual differences with regard to the level of free methionine.

As already pointed out, the content of free methionine in skeletal muscles seems not to be related to the content of connective tissue of the muscles. This is also evident from Fig. 1 in which the content of free methionine is plotted against the content of hydroxyproline, the latter taken as an index of the presence of connective tissue.[10,11] One per cent hydroxyproline corresponds to about $7\frac{1}{2}$ % connective tissue protein.[11]

Fig. 1

It has not been demonstrated whether connective tissue contains appreciable amounts of free methionine or not (skin has a low content, sample No. 76). If its content of free methionine is low, an explanation of the high levels of free methionine in skeletal muscles rich in connective tissue could be that these muscles have a larger working capacity and, consequently, also a greater metabolic activity. This could possibly be put in connection with a higher content of free methionine in the proper striated tissue of such muscles.

Among the organs particularly low contents of free methionine were found in udder, rumen, lungs, heart, kidney, and skin; blood and its fractions (samples No. 61-63 and 77-79) were also found to have very low levels of free methionine. Surprisingly enough striated muscles from the head (samples No. 16 and 17) showed extremely low values of free methionine. Also the striated muscle of diaphragm (samples No. 15 and 27) was found to contain significantly less free methionine than the skeletal muscles.

According to earlier investigations[12] head meat has an essentially lower content of creatine than skeletal muscles. This fact indicates some relationship between the content of free methionine and creatine and, with reference to what was mentioned in the introduction, may reflect the significance of methionine as a methylating agent in the formation of creatine from glycocyamine. In order to get a more clearcut conception of the relation between free methionine and creatine, the content of these constituents was analyzed for a number of samples of skeletal muscles, striated muscle from head and diaphragm as well as of organs. The results are presented in Fig. 2.

Fig. 2

Six separate groups can be distinguished. Group I has a high content of both free methionine and creatine; it includes skeletal muscles. Groups II and III have a moderate content of free methionine and a moderate to high content of creatine; this group is represented by striated muscle from diaphragm and tongue. Group IV (samples No. 24 and 28) has a low content of free methionine and a moderate to high content of creatine; it is represented by the cardiac muscle. Group V is characterized by a low content of free methionine and a moderate content of creatine; this group consists of striated muscles from the head. Finally, Group VI has a low to moderate content of free methionine and a low content of creatine; this group includes red organs (except heart and tongue), smooth (unstriated) tissues, skin, and blood.

The crude protein of the mucosa from beef bungs is much richer in free methionine than that of the bung tissue (Table I, samples No. 57-60). However, the mucosa protein was found to make only 22 to 26 % of the sum bung mucosa protein + bung tissue protein. Thus, it can be calculated that the crude protein of the bung with mucosa contained between 80 and 90 mg-% free methionine.

Also the crude protein of the mucosa from pig stomachs has a higher content of free methionine than that of the unstriated muscle tissue to which it is attached (Table I, samples No. 68-71). About 7 % of the sum mucosa protein + stomach tissue protein was mucosa protein. On the other hand, the free methionine contained in the crude protein of the mucosa from chitterling was found to be but a little higher than that in the crude protein of the chitterling tissue (Table I, samples No. 72-75).

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From these results it seems evident that, among other things, the mucosa of beef bung and pig's stomach are sites for intense metabolic turnover. Whether the high values of free methionine are merely a sign of proteolytic activity or of transmethylation, or both, must be disclosed by separate studies.

Earlier analyses of characteristic constituents of various animal tissues have revealed that the content of tryptophan and particularly of hydroxyproline in the tissue protein can be used as an index of high and low quality animal protein, respectively.[10,11] Blood and its fractions are exceptions; although their proteins are high in tryptophan, they are deficient in the essential amino acids isoleucine and methionine. To distinguish striated muscular tissue from organs and smooth muscles the content of creatine, calculated on a crude protein basis, is very useful.[12] The present investigations have disclosed that the content of free methionine in the crude protein is also a feature of skeletal muscle. However, as far as a suitable index of the quality of meat products is concerned, the determination of the content of free methionine in the crude protein offers no advantage over establishing the content of hydroxyproline and/or creatine. In addition, nothing is hitherto known about changes of the content of free methionine which may occur during the processing of meat products and this may cause erroneous conclusions.

Summary

Between 6 and 11 % of the total methionine present in skeletal muscle was found to be free methionine. The content of free methionine in other animal tissues as well as in blood and its fractions is low. Intestinal mucosa is rich in free methionine. The findings are discussed in view of the functional significance of methionine. The determination of the content of free methionine offers no advantage over the analysis of the content of hydroxyproline and/or creatine for the purpose of getting an index of the quality of meat products.

Résumé

De 6 à 11 % de la méthionine totale du muscle squelettique se trouve sous forme de méthionine libre. La teneur en méthionine libre des autres tissus de même que celle du sang et de ses fractions est faible. La muqueuse intestinale est riche en méthionine libre. Les résultats sont

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discutés quant à la signification fonctionnelle de la méthionine. La détermination de la teneur en méthionine libre n'offre pas d'avantage, par rapport à la détermination de la teneur en hydroxyproline ou/et en créatine, pour établir un index de la qualité des produits carnés.

Zusammenfassung

Zwischen 6 und 11 % vom Gesamtmethionin im Skelettmuskel wurden als freies Methionin gefunden. Der Gehalt an freiem Methionin in anderen tierischen Geweben sowie im Blut und in Blutfraktionen ist niedrig. Die Schleimhaut der Eingeweide ist reich an freiem Methionin. Die Befunde werden im Hinblick auf die funktionelle Bedeutung des Methionins erörtert. Die Ermittlung des Gehalts an freiem Methionin bietet keinen Vorteil über die Bestimmung des Gehalts an Hydroxyprolin und/oder Kreatin, um ein Qualitätsmass für Fleischwaren zu erhalten.

References:

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Table I. Content of free methionine calculated on a crude protein basis (crude protein = N x 6.25)

Sample No.	T i s s u e	Free methionine mg-% of crude protein
1	Beef, <u>M. longissimus dorsi</u>	257
2	" , skeletal muscles from whole carcass, no visible connective and fatty tissue	197
3	" , " " " " " " " " " " "	234
4	" , " " " " " " " " " " "	194
5	" , " " " " " with connective tissue but no visible fat	165
6	" , " " " " " " " " " " " "	150
7	" , " " " " " " " " " " " "	195
8	" , " " " " " " " " " " " "	197
9	" , " " " " " " " " " " " "	181
10	" , " " " " " " " and fatty tissue	195
11	" , " " " " " " " " " "	208
12	" , " " " " " " " " " "	206
13	" , " " " " " " " " " "	188
14	" , " " " " " " " " " "	202
15	" , striated muscle of the diaphragm	135
16	" , " muscles from the head, with connective tissue	21
17	" , " " " " " " " "	14
18	Calf, <u>M. longissimus dorsi</u>	239
19	" , skeletal muscles from the whole carcass, with connective tissue	177
20	Pig, ham muscles, no visible connective tissue	233
21	" , skeletal muscles from whole carcass, no visible connective and fatty tissue	230
22	" , " " " " " " " " " " "	167
23	" , " " " " " " " " " " "	182
24	" , " " " " " with connective tissue and some fat	185
25	" , " " " " " " " " " " "	178
26	" , " " " " " " " " " " "	244
27	" , striated muscle of the diaphragm	118
28	Horse, <u>M. longissimus dorsi</u>	268
29	" , " " "	220
30	" , " " "	183
31	" , skeletal muscles from whole carcass, with connective tissue but no visible fat	200
32	" , " " " " " " " " " " " "	183
33	" , " " " " " " " and fatty tissue	225
34	" , " " " " " " " " " "	152

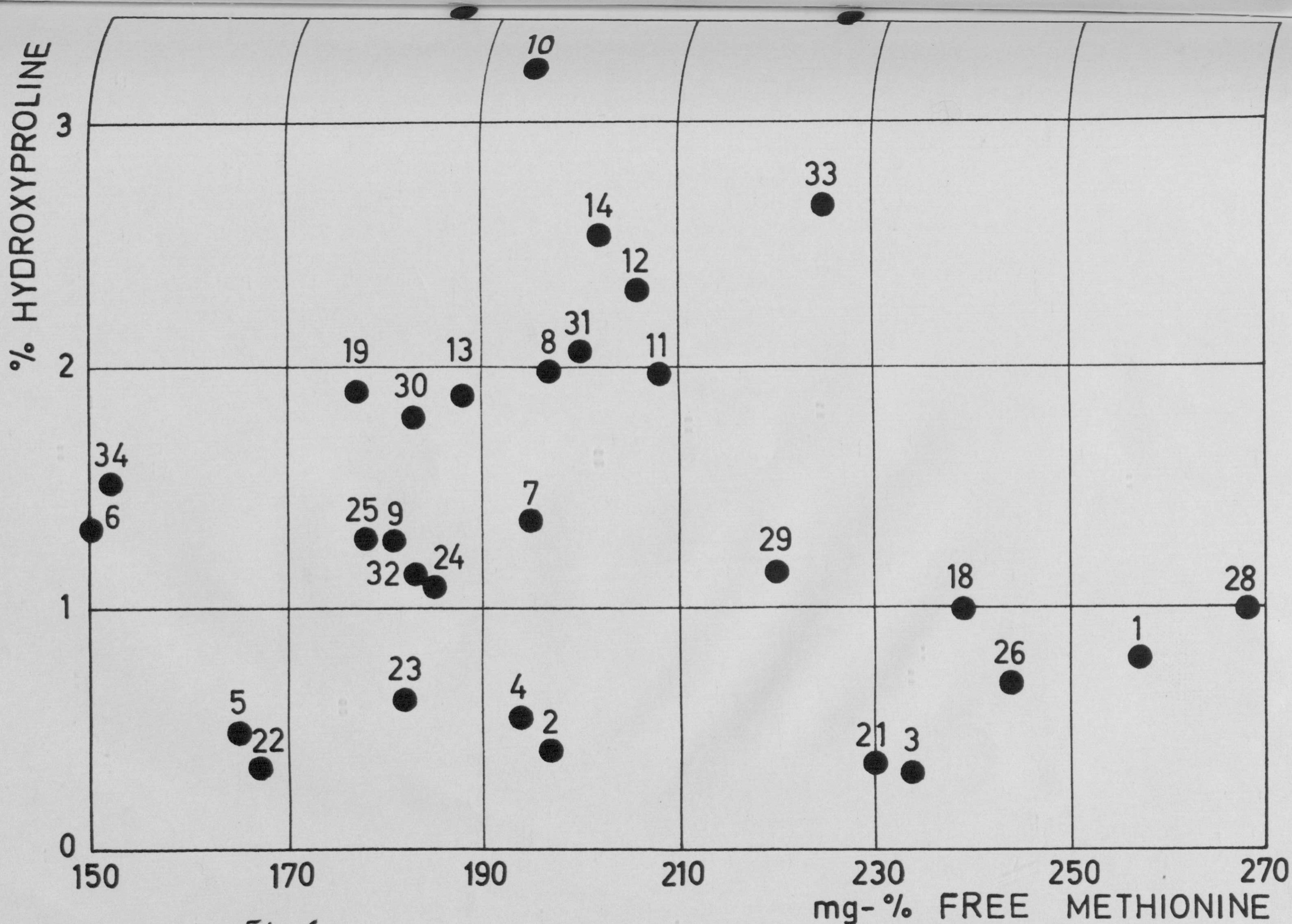
(continued)

Sample No.	Tissue	Free methionine mg-% of crude protein
35	Beef, tongue	70
36	" , heart	48
37	" , "	30
38	" , liver	72
39	" , "	77
40	" , "	50
41	" , spleen	102
42	" , "	96
43	Pig, tongue	69
44	" , heart	14
45	" , "	14
46	" , liver	70
47	" , "	30
48	" , spleen	64
49	" , "	53
50	" , kidney	37
51	" , "	41
52	Beef, lungs	24
53	" , udder	0
54	" , "	0
55	" , rumen	18
56	" , "	26
57	" , bung (Caecum), without mucosa	30
58	" , " " " "	43
59	" , mucosa from bung	266
60	" , " " "	197
61	" , blood	19
62	" , blood plasma	30
63	" , erythrocytes	0

Sample No.	Tissue	Free methionine mg-% of crude protein
64	Pig, lungs	40
65	" , "	52
66	" , stomach, with mucosa	101
67	" , " " "	106
68	" , " without mucosa	91
69	" , " " "	80
70	" , " " "	98
71	" , mucosa from stomach	216
72	" , chitterling (large intestine), without mucosa	97
73	" , " " "	57
74	" , mucosa from chitterling	95
75	" , " " "	105
76	" , skin (rind)	47
77	" , blood	10
78	" , "	4
79	" , "	13

Fig. 1

Content of free methionine and total hydroxyproline in the crude protein of skeletal muscle from beef, calf, pig, and horse. The figures refer to sample numbers given in Table I.


% HYDROXYPROLINE
0
1
2
3
150
170
190
210
230
250
270
mg-% FREE METHIONINE
10
33
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1
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23
4
2
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22
21
3

Fig. 1

Fig. 2.

Content of free methionine and creatine calculated on a crude protein basis. The figures refer to the following samples:

Sample No.	Tissue
1	Beef, M. longissimus dorsi
2	" , skeletal muscles from whole carcass, no visible connective and fatty tissue
3	" , " " " " " " " " " "
4	" , " " " " " with connective tissue but no visible fat
5	" , " " " " " " " and fatty tissue
6	Calf, M. longissimus dorsi
7	" , skeletal muscles from whole carcass, with connective tissue
8	Pig, " " " " " no visible connective and fatty tissue
9	" , " " " " " " " " " " "
10	" , " " " " " with connective tissue and some fat
11	Horse, M. longissimus dorsi
12	" , " " "
13	" , skeletal muscles from whole carcass, with connective tissue but no visible fat
14	" , " " " " " " " and fatty tissue
15	" , " " " " " " " " " "
16	Beef, striated muscle from the diaphragm
17	Pig, " " " " "
18	Horse, " " " " "
19	Beef, striated muscles from the head, with connective tissue
20	" , " " " " " " " "
21	" , " " " " " " " "
22	Pig, " " " " " " " "
23	Horse, " " " " " " " "
24	Beef, heart
25	" , tongue
26	" , liver
27	" , spleen
28	Pig, heart
29	" , tongue
30	" , liver
31	" , spleen
32	" , kidney
33	Beef, lungs
34	" , udder
35	" , rumen
36	" , blood
37	Pig, lungs
38	" , stomach, with mucosa
39	" , chitterling (large intestine), with mucosa
40	" , skin (rind)
41	" , blood

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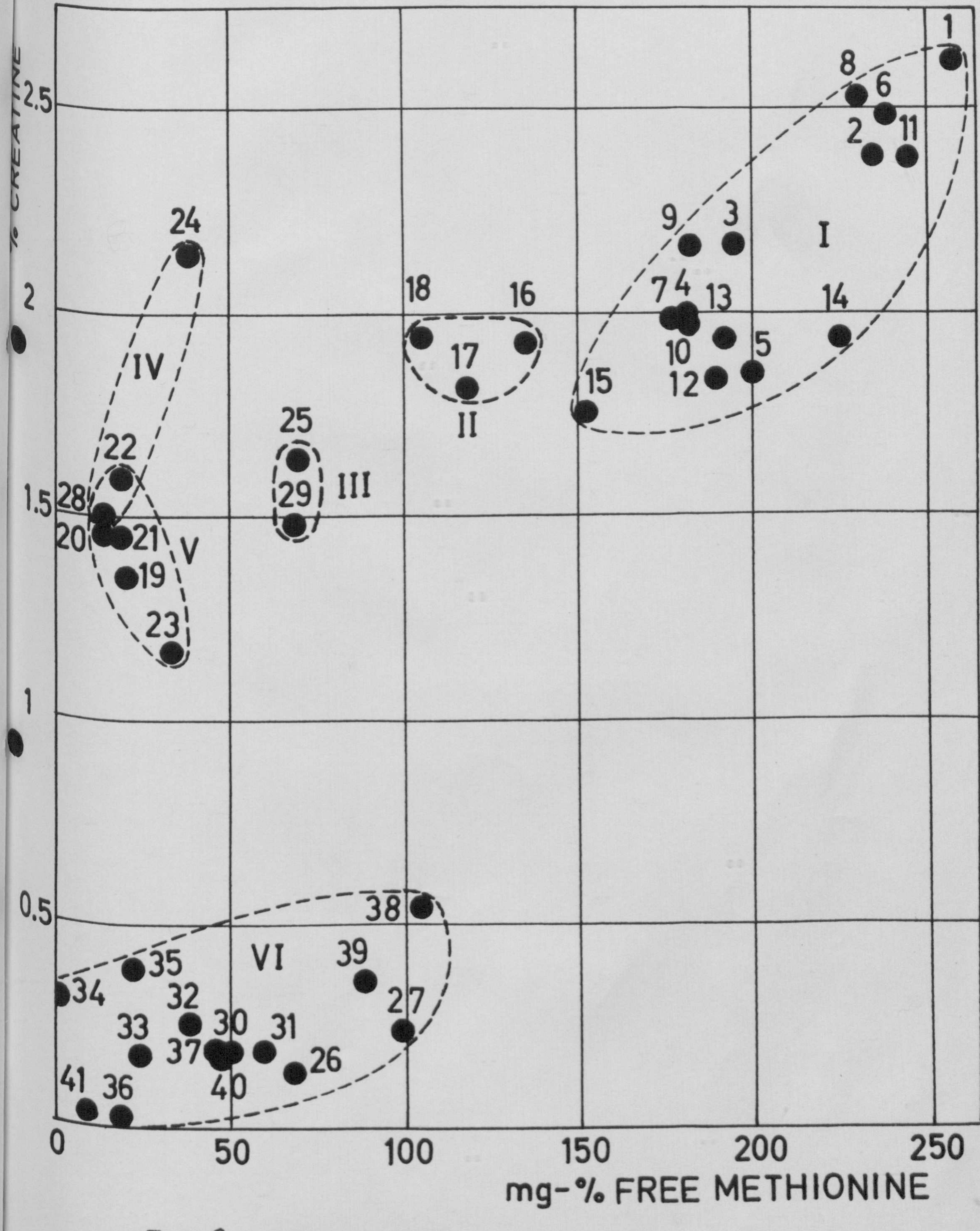
% CREATINE
2.5
2
1.5
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50
100
150
200
250
mg-% FREE METHIONINE
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Fig. 2