VI. THE AMINO-ACIDS OF FLESH.

THE DI-AMINO-ACID CONTENT OF RABBIT, CHICKEN, OX, HORSE, SHEEP AND PIG MUSCLE.

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A LONG series of food analyses has recently been made by Plimmer [1921, 1], who points out that by the ordinary routine method of analysis, in which the amount of protein is estimated by multiplying the nitrogen content by 6.25, no discrimination is made between the flesh of different animals. The protein of one animal is regarded as being the same as that of another. The work of Emil Fischer and Kossel and their pupils has definitely proved that the various proteins differ very widely in their composition as regards the amino-acids, and this difference is emphasised by the experiments on the food value of the individual amino-acids by Hopkins in conjunction with Willcock and Ackroyd, by Osborne and Mendel and other American investigators¹. These chemical and biological differences are sufficient evidence that quality of protein in nutrition must be taken into consideration.

Complete analyses of the protein of the muscle of the ox, chicken, halibut and scallop have been made by Osborne and Heyl [1908] and Osborne and Jones [1909], and Drummond [1916] has made some analyses of muscular tissue by Van Slyke's method. Both the more complete analyses by Osborne and co-workers and those by Drummond do not show any marked difference in the amino-acid content of the various muscle proteins. The flesh of various animals shows such distinct appearances, different both to the eye and palate, that it seems probable that greater differences may exist, and that there may be smaller differences in the flesh from various parts of the same animal's carcass, such as back and leg. Some further amino-acid analyses have therefore been made.

The methods of protein analysis are far from perfect: Fischer's ester method for the mono-amino-acids, as he pointed out, is not quantitative: Kossel and Patten's method for the di-amino-acids, in spite of the numerous manipulations, is generally considered to be fairly accurate, but it has been largely superseded by Van Slyke's method which gives higher values for these amino-acids. Van Slyke's method also possesses the advantage of requiring only small amounts of protein and is more rapidly carried out. This method of protein analysis has been used in these experiments, since it was chiefly

¹ See summary by Plimmer [1921, 2].

desired to compare the muscle protein of several animals with a view to more complete data at a later time. A comparison of these results with those by Kossel's method has been made in a few cases. The results indicate that differences exist in the amino-acid content of the various muscle proteins. Duplicate analyses were always carried out; frequently these analyses were not so concordant as was expected. This inconsistency of the results was under investigation by Plimmer [1916] who tested the arginine determination; other details of the method are now being studied.

EXPERIMENTAL.

In the case of the smaller animals (rabbit, chicken) opportunity was taken of comparing the flesh of different parts of the body of the same animal. In other cases the flesh was taken from the thigh. The mode of operation was the same throughout. The flesh (about 350 g.) was freed from inside fat, minced and put into about 2 litres of boiling water containing 0.1 % acetic acid and heated for about ten minutes so as to coagulate the protein and remove the extractives. The liquid was poured off and the coagulated protein squeezed dry in a cloth. This procedure was repeated twice. The coagulated protein (about 200 g.) was then digested with 1 g. pepsin in 2 litres of 0.1N HCl, so as to separate nucleins, indigestible matter, etc. After digestion, which usually occupied about ten days at 37° the liquid was filtered off and the total nitrogen estimated. A portion containing about 6 g. of protein was then hydrolysed by boiling with hydrochloric acid added to the liquid so as to make a concentration of 20 %. The hydrolysis was carried on for 36 hours. The hydrolysed solution was evaporated to dryness *in vacuo*, made up to 250 cc. and two samples of 100 cc. were analysed by Van Slyke's method. This was performed as described except for the arginine estimation which was effected by Plimmer's modification [1916]. In the earlier experiments it was impossible to make determinations of amide N owing to the facilities for vacuum distillation not being adequate. The analyses were made in duplicate and the percentage has been calculated from the average.

Table I. *Nitrogen percentages.*

	Amide	Humin N	Di-amino-acids						Mono-amino-acids			Total N of hydro- lysed solution
			Total N	Amino N	Non- amino N	Argi- nine N	Histi- dine N	Lysine N	Total N	Amino N	Non- amino N	
Rabbit, back	—	—	45.7	21.5	24.0	15	19	11.5	49	—	—	94.7
" fore limb	—	—	44.1	17.9	27.7	8.8	30.9	5.5	50.7	—	—	94.8
" hind limb	—	—	44.8	18.4	26.6	13	25	5.8	56.3	—	—	101.1
Chicken, breast	6.9	3	27	9	18	10	13	2	61.1	49.7	11.4	98.0
" legs	5.5	1.3	25.5	15	10.5	8	7	11	68.5	66.6	1.9	100.8
Beef	6.3	0.5	28.5	15	13.5	13.3	5	11.2	55	26.8	28.2	90.3
Horse	2.9	0.9	37.1	18.8	18.3	14.9	10.5	11.6	70	58	11.9	110.9
Mutton	6.5	0.5	38.3	22.3	15.6	15	18	4.3	54	52	2	99.3
Pork	6.4	1.2	28.2	13.3	15	14	7	7	57	53	4	92.8

Relatively little difference can be observed from the figures for the different meats. The amide N is almost similar, in each case averaging about 6.0 % of the total N.

Table II. *Percentages of amino-acids. Giving the amount of amino-acids in 100 g. of protein.*

	Arginine	Histidine	Lysine	Total di-amino N
Rabbit, back	8	10	13	31
" fore limb	5	19	5	29
" hind limb	7	15	5	27
Chicken, breast	6	8	1	15
" legs	4	4	10	18
Beef	7	3	10	20
Horse	7	6	9	22
Mutton	7	11	4	22
Pork	7	4	6	17

Humin N shows a difference. It is, if anything, higher in the white meats, *e.g.* breast of chicken 3 %, legs 1.3 %, pork 1.2 %, than in the red meats, where the average is 0.5 %, except in the horse, where 0.9 % was found. The explanation of this slightly higher value may be that the animal was not properly bled on slaughter.

Lysine figures are, with the exception of mutton, higher for the red meats, averaging about 11 %, while, of the white meats, rabbit limbs show only 5.5 %, chicken breast 2 % and pork 7 %.

Gortner and Holm [1920, 1], working with mixtures of pure amino-acids, have shown that tryptophan, and in the presence of aldehyde also tyrosine, and their analogues are the only known amino-acids which go to form humin. There is therefore no connection between the humin content and the lysine content of the meats; this is exemplified especially in the chicken, where the humin is high, and the lysine is low in the breast; and humin is low and lysine high in the legs. It may perhaps be mentioned that in the preparation of the di-amino-acids by the method of Kossel and Patten a distinct yellowish colouring adheres to the lysine portion.

At the same time too much reliance must not be placed on the humin as an estimation of tryptophan and tyrosine. Gortner and Holm [1920, 2] and Thomas [1921] have shown that tyrosine and tryptophan which go to form humin are not necessarily the only substances giving a reaction with the phenol reagent of Folin and Denis. Estimations of substances giving the blue colour with this reagent were made during the progress of this work, both before the removal of the humin and afterwards. In the case of chicken breast, a white meat, the readings before removal of the humin represented 4 % "tyrosine" whereas after its removal the readings represented 3.5 %. In the case of beef however—a red meat—the difference was greater, the former reading being 3.5 % and the latter 2.1 %, yet the humin N was much lower in the case of beef.

The arginine figures are more constant at about 14 or 15 % except in rabbit fore limb and chicken legs where the average is 8 %.

The histidine figures are less satisfactory, and exhibit perhaps a weak point in the method. In this connection it is of interest to point out that in the cases of abnormally high histidine the figures for the non-amino N are lower than normal and *vice versa*, *e.g.* beef 5 % histidine, 28 % non-amino N,

mutton 18 % histidine, 2 % non-amino N, while in other cases this observation cannot be made. This may be due, either to incomplete precipitation of the histidine by the phosphotungstic acid, or to washing. Work in this connection is in progress.

It is not possible to draw any conclusions from the figures of the mono-amino fraction, which account for about 55 to 60 % of the total N.

The average percentage of the di-amino N is 35.

Comparison with former work on the hydrolysis of meat is difficult, because, with the exception of Drummond [1916] on chicken meat, the other figures relate to the method of Kossel, which generally gives lower results than the Van Slyke method.

The above figures for chicken breast agree in the main with those of Drummond, his total hexone bases N 27.26 being the same as that above. The arginine figures are within 1 % and he records having used the same modification of that process as mentioned above. The figures for histidine and lysine are discordant, Drummond finding 8.45 and 9.81 respectively, while the total N of the mono-amino fraction is 4 % higher than that found by Drummond.

In order to compare the figures of Osborne and co-workers with the above it is necessary to refer to the percentages not of total N but of actual arginine, histidine and lysine. The figures for arginine are generally constant within 1 %, those for histidine are higher than Osborne's, while the lysine figures, owing to the calculation in Van Slyke's method, are dependent on the histidine values. Apart from the arginine values, only the beef of the present sets has given results comparable with those of Osborne, who found 7.5 % arginine, 1.8 % histidine and 7.6 % lysine against 6.8 % arginine, 2.6 % histidine and 9.6 % lysine in this experiment.

SUMMARY.

1. Determinations have been made of the di-amino-acids of the protein of the flesh muscle of rabbit, chicken, ox, horse, sheep, pig by Van Slyke's method.
2. The red meats show a higher lysine content than the white meats.

I wish to take this opportunity of expressing my gratitude to Dr Plimmer, who suggested this work, for his kindness and guidance throughout the time I was under him, and also to Professor J. A. MacWilliam, F.R.S., for so kindly placing his laboratory at my disposal.

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