CIV. THE AMINO-ACIDS OF FLESH.

II. COMPARISON OF THE DIAMINO-ACID CONTENT OF SOME NORMAL AND PATHOLOGICAL TISSUES.

By JOHN LEWIS ROSEDALE (*Beit Memorial Research Fellow*).

From the Chemical Department, St Thomas's Hospital Medical School, London.

(Received April 20th, 1928.)

IN a previous paper [Rosedale, 1922] the diamino-acid contents of the muscle of various farm animals were compared. Very slight differences could be detected between the tissues analysed, although it was pointed out that available methods of analysis did not ensure complete accord even when duplicates were made upon the same hydrolysate. During the intervening years, fresh endeavours have been made to secure greater accuracy in the analysis of proteins. Plimmer [1924] studied more closely the quantitative reaction of the hexone bases with nitrous acid. Plimmer and Phillips [1924] estimated histidine and tyrosine, after hydrolysis, by bromination. Plimmer and Rosedale [1925] have investigated each step in the Van Slyke estimation method and published certain modifications which secure greater ease and accuracy in the procedure. More recently, Plimmer and Lowndes [1927] have devised a method by which cystine may be estimated in the diamino-fraction of the Van Slyke method. The work now presented was undertaken as an extension of the data previously obtained. With two exceptions, muscle had not been analysed from different parts of the same animal. It has also been thought desirable to compare the results of the Van Slyke method with the older procedure of Kossel. The latter method which includes so many manipulations cannot necessarily claim greater accuracy than other methods of estimation, although in the hands of Kossel and Osborne the results have proved sufficiently concordant to form the basis of our knowledge of the subject to-day. Certain modifications of Kossel's original procedure have been introduced which avoid some of the difficulties. The opportunity has been taken of comparing certain pathological with normal tissues, and of analysing the muscle tissue of animals which had been fed upon known proteins.

EXPERIMENTAL.

After removing as much fat as possible the tissues were minced. They were then extracted in 2 l. of boiling water containing 2 cc. glacial acetic acid in order to coagulate the protein and to separate it from extractives. The liquid was poured off and the tissue squeezed dry in a cloth. The procedure of extracting and drying was repeated twice. The tissues were then

digested for 8–12 days at 37° with 1 g. of pepsin in 2 l. of 0.1 N HCl. Indigestible matter was filtered off and the total nitrogen estimated by Kjeldahl's method. The filtrate was then hydrolysed with 25 % HCl for 36 hours, when it was evaporated to dryness *in vacuo* once or twice to remove as much HCl as possible. The hydrolysate was finally brought to such volume that 100 cc. represented approximately 0.5 g. of nitrogen. Portions of 100 cc. were then analysed by Van Slyke's method, according to the technique of Plimmer and Rosedale [1925], except in the cases of pig uterus and placenta when the original method was followed, although in these analyses also, the determinations of amide- and humin-nitrogen were carried out in accordance with the procedure of Plimmer and Rosedale, and the estimation of arginine-nitrogen followed the modification of Plimmer [1916] in every case.

The main difficulty in the Kossel procedure is that loss occurs at each precipitation. The problem has been therefore to ascertain whether any of the precipitates, especially those involving the formation of barium sulphate can be dispensed with. Various alterations have been attempted during the course of the work and the procedure finally adopted is as follows.

After removal of the portions for analysis according to Van Slyke, the bulk is acidified to 5 % with HCl and precipitated with phosphotungstic acid. The amount of phosphotungstic acid used was 15 g. for each 0.5 g. of nitrogen, the volume of the bulk being doubled in accordance with the Van Slyke procedure. The precipitation is carried out in a large flask which is heated in a water-bath to redissolve the precipitate. After standing for at least 48 hours, the precipitate, which contains, in addition to the diamino-acids, ammonia and humin, is filtered on a Büchner funnel and washed five times with 50 cc. of dilute HCl. The precipitate is then placed in a basin for the decomposition of the phosphotungstates, which is carried out by means of baryta gradually added with stirring until the solution is faintly alkaline. The precipitate of barium phosphotungstate, to which the humin adheres, is rapidly filtered off by suction and the filtrate which now contains the diamino-acids and ammonia, is freed from the latter by concentrating *in vacuo*. Plimmer and Rosedale [1925] showed that, in concentrating the alkaline diamino-fraction at this stage, loss occurred, which was presumably due to decomposition of the amino-acids. The above procedure has however been employed, as any loss at this stage is not greater than that sustained by an earlier removal of the ammonia which would entail the formation of an additional precipitate and washing of a large bulk of barium sulphate. The aim is to carry through the operations of decomposing the phosphotungstates, filtering and concentrating as rapidly as possible. The excess of barium is now removed quantitatively by means of sulphuric acid and filtered off. The filtrate is then acidified to 1.5 % with sulphuric or acetic acid and histidine precipitated by adding a saturated solution of silver sulphate or acetate to the hot solution. After standing for 24 hours, the precipitate of the silver salt of histidine is filtered off and treated in accordance with the usual Kossel method.

A further quantity of silver salt is added to the filtrate which is made alkaline with baryta to secure precipitation of arginine. The filtrate from the silver-arginine compound is freed from silver by acidifying to 5 % with HCl and filtering. Lysine is precipitated from the filtrate by phosphotungstic acid and treated from this point in accordance with the Kossel method.

RESULTS.

In order to compare the results of the Kossel and Van Slyke methods, the figures have all been reduced to the percentage of the total nitrogen which they represent. As already stated, during the Kossel procedure the amide- and humin-nitrogens were not estimated, so that these amounts have been secured only during the Van Slyke estimations. The average of the results obtained by Kossel's method are given in Table I.

Table I. *Results by the modified Kossel method.*

	Percentages of total nitrogen.				
	Pork muscle	Pig uterus	Human arm	Human foot	Breast carcinoma
Histidine-N	1.5	2.0	2.0	2.0	2.5
Arginine-N	7.0	13.0	5.0	7.0	7.0
Lysine-N	6.5	3.0	6.0	6.0	3.0
No. of exps.	3	5	4	4	3

While the above figures have been presented to the nearest 0.5 %, divergencies have occurred in some of the determinations composing the average. The most noteworthy concern arginine, where the figures varied in the "foot" experiments from 6.1 to 8.2 %, and in the "arm" experiments from 4 to 6.1 %. The most notable figure in the above table is that of lysine in carcinoma, but in this case the figures for the three determinations, 3.3, 3.8, 3.0 % show no very marked divergence from one another. As will be seen from Table II, the Van Slyke estimation gives a very low figure.

Table II. *Results by Van Slyke's method.*

Percentages of total nitrogen.											
			Diamino-N					Mono-amino-nitrogen			
			Amide-nitrogen	Humin-nitrogen	Total	Amino-nitrogen	Arginine-nitrogen	Histidine-nitrogen	Lysine-nitrogen	total	Total
Pig muscle	...	...	8.0	6.8	18.4	11.1	8.1	1.9	8.4	65.7	98.9
Pig uterus	...	...	8.5	8.7	18.0	9.6	8.3	3.2	6.5	64.2	99.4
Pig placenta	...	...	8.2	3.2	24.6	9.0	11.3	9.4	3.9	59.8	95.8
Pig foetus	...	...	7.5	14.8	15.4	8.8	8.5	0.3	6.6	58.0	95.7
Human arm	...	...	4.2	5.2	19.0	10.9	8.7	2.5	7.8	67.8	96.2
Human foot	...	...	4.4	5.0	18.3	9.9	9.0	2.5	6.8	68.1	95.8
Breast carcinoma	...	...	7.0	14.5	14.6	7.9	9.1	3.0	2.5	64.7	100.8
Chicken feathers	...	...	8.5	8.7	18.0	9.6	8.3	3.2	6.5	64.2	99.6
Maize-fed chicken flesh	...	...	8.5	8.7	13.4	7.6	8.7	2.6	2.1	66.8	97.4

In general, it will be seen that the Van Slyke method gives slightly higher results than the Kossel method. At the same time the difference if calculated in terms of the actual amino-acid is negligible. The conclusion may be drawn that normal muscle tissue does not vary to any extent in the various animals

or in different tissues of the same animal, provided the animal secures normal protein in the diet. The maize-fed chickens were taken from a group of experimental birds, which had received no protein other than that contained in maize. While maize contains other proteins than the deficient zein, it would appear that these are not present in sufficient quantity to produce normal flesh.

In Table III the amounts of the three diamino-acids in 100 g. of flesh are given.

Table III. *Diamino-acids, g. per 100 g. tissue.*

	Arginine	Histidine	Lysine
Pig muscle	1	0.27	1.7
Pig uterus	1	0.5	1.3
Pig placenta	1.2	1.1	0.9
Pig foetus	0.6	0.26	0.8
Human arm	0.75	0.25	0.8
Human foot	1	0.27	1
Breast carcinoma	0.63	0.24	0.3
Chicken feathers	3.71	1.7	4.9
Maize-fed chicken flesh	0.75	0.26	0.3

SUMMARY.

1. The diamino-acid content of several animal tissues has been determined by Van Slyke's method and by a modification of Kossel's method.
2. No fundamental differences occur in the two methods used.
3. It appears that the diamino-acid contents of the tissues of different animals, and of different parts of a normal animal are similar.
4. Low content of lysine has been found in carcinoma, and in chickens which have derived the whole of their food-protein from maize.

In conclusion, I desire to thank Prof. R. H. A. Plimmer most heartily for his guidance and advice throughout the course of this work, and also the Dean of this School and Dr M. J. Rowlands for the supply of material used.

REFERENCES.

- Plimmer (1916). *Biochem. J.* **10**, 115.
 — (1924). *Biochem. J.* **18**, 105.
 Plimmer and Lowndes (1927). *Biochem. J.* **21**, 247.
 Plimmer and Phillips (1924). *Biochem. J.* **18**, 312.
 Plimmer and Rosedale (1925). *Biochem. J.* **19**, 1004.
 Rosedale (1922). *Biochem. J.* **16**, 27.