CXLI. AMINO-ACIDS OF TISSUES.

IV. THE DIAMINO-ACID CONTENT OF MUSCLE TISSUE OF DIFFERENT CLASSES OF ANIMALS.

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(Received July 8th, 1930.)

A SERIES of experiments [Rosedale, 1922, 1928], by van Slyke's method, upon various mammalian tissues has shown but slight variation. The white meats of chicken and of pork indicated the possibility that the amount of lysine might be slightly lower than in the other tissues examined, but at the time of making those determinations it was realised that the experimental procedure was not perfect, so that too great reliance could not be placed upon the figures. The whole method was carefully examined by Plimmer and Rosedale [1925], who so modified the method that experience with their technique may now lead to results which at least may be considered comparative. With this improved technique carcinoma tissue was analysed [Rosedale, 1928], and a low figure for lysine (2.5 % of the total nitrogen) was found in this degenerate tissue. From analyses of certain fish tissues [Rosedale, 1929] it appeared that the amount of histidine was slightly, though definitely, higher in fish tissue than in most of the normal mammalian tissues which had been previously examined.

It was of interest, therefore, to extend this comparison of the diamino-acid content of tissues to lower animals including, as far as possible, one member from each of the main zoological families. The survey, which is by no means as complete as we should like, is reported in this paper, since certain differences seem to exist between the different tissues which have been analysed.

EXPERIMENTAL.

In order to avoid as much error as possible, the preparation of each tissue has been considered separately. The tissues include those of the crocodile, the cockroach, the larvae of the mosquito *Anopheles ludlowi*, the oyster, and a calcareous sponge (monaxonid). Of these, the crocodile tissue alone was considered to be susceptible of the same treatment as was employed with mammalian and fish tissues, which consisted in mincing and extracting in a boiling solution of 0.1 % acetic acid, to coagulate the protein and to remove the extractives. The tissue, which is a white meat resembling chicken flesh in appearance and taste, was extracted three times, the coagulated tissue being squeezed dry in

a cloth after each extraction. The extracted material was then digested with pepsin in 0.1 *N* HCl at 40° for 11 days. The filtrate from the pepsin digest was used for the determinations.

The cockroaches were broken up in a mortar, dried and thoroughly extracted with ether before treatment with 0.1 % acetic acid. A heavy extract was obtained necessitating four extractions with the acid. The coagulated material was then digested with pepsin. Although concordant results were secured in the duplicate van Slyke analyses, only about 90 % of the total nitrogen of this tissue was accounted for. It was unfortunately impossible to make any further experiments with this material.

The amount of mosquito larvae available was not large on account of the great difficulty in collecting a sufficient number of the same species. We used 16 g. of this material which had been preserved in alcohol during the period of collection. Since it was possible that some protein might have been extracted by the alcohol, we evaporated the alcohol and added the residue to the larvae which were ground in a mortar and extracted with ether. The whole was then treated with 0.1 % acetic acid and digested with pepsin.

The oysters offered considerable difficulty, but since this material was easily obtained a more thorough examination of the process of preparation has been made. When the material was dried, minced and extracted with acetic acid we found large increases in the amide- and humin-nitrogen values. Since this might be due to the presence of glycogen, after many trials we adopted the following procedure. The oysters, while fresh, were boiled in water which had been made slightly alkaline with sodium carbonate; the tissues were then dried, ground in a mill, and extracted with ether. They were then extracted several times with 0.1 % acetic acid and digested with pepsin.

The sponges were first broken up and cleaned by immersion in dilute HCl for several days. The solid pieces of sponge were then picked by hand, freed from any seaweed, washed with water, dried, ground and extracted with ether. They were then treated in the usual way with acetic acid and pepsin. The treatment with dilute HCl extracted a certain amount of protein from the material. This was precipitated with ammonium sulphate and dialysed. The material after dialysing was digested with pepsin before analysis by van Slyke's method.

After digestion with pepsin, all these tissues were hydrolysed with 25 % HCl and analysed by van Slyke's method exactly as described by Plimmer and Rosedale [1925].

In carrying out the hydrolysis of fish tissues [Rosedale, 1929], discordant results were experienced in the van Slyke determinations if the tissues were hydrolysed for too short a time. Accordingly, we have made analyses of these tissues after different periods of hydrolysis. In the cases of the cockroach and the mosquito larvae this was not done, since, unfortunately, we had not sufficient material for more than a duplicate analysis. Our results confirmed the former work, that the time required for hydrolysis may vary according to the tissue. The results recorded in Table I are those secured after hydrolysing for the period which we found to be the most suitable, and the times are recorded.

Table I. *Percentage of total N.*

	Time of hydrolysis (hrs.)	Diamino-fraction							Monoamino-fraction		Total
		Amide-N	Humin-N	Total N	Amino-N	Arginine-N	Histidine-N	Lysine-N	Total N	Amino-N	
Crocodile ...	60	6.9	1.3	28.8	12.8	13.8	8.4	6.5	61.7	54.1	98.7
	60	6.9	1.2	28.9	12.9	13.8	8.5	6.6	61.8	54.0	98.9
Mean ...	...	6.9	1.25	28.85	12.85	13.8	8.45	6.55	61.75	54.05	98.8
Cockroach ...	40	16.9	4.3	19.4	7.5	4.9	12.2	2.2	48.9	44.5	89.5
	40	16.8	5.3	19.6	7.4	4.9	12.3	2.1	50.0	46.1	91.7
Mean ...	...	16.85	4.6	19.5	7.45	4.9	12.25	2.15	49.4	45.3	90.6
Mosquito larvae	100	9.4	3.9	23.3	10.3	11.4	7.3	4.6	55.9	52.9	92.5
	100	9.4	3.9	23.4	10.2	11.5	7.4	4.5	56.5	53.1	93.2
Mean ...	...	9.4	3.9	23.35	10.25	11.45	7.35	4.55	56.2	53.0	92.8
Oysters ...	100	7.8	9.9	22.7	6.8	8.4	12.7	1.5	61.5	40.8	101.9
	100	7.9	9.9	22.4	6.9	8.5	12.6	1.3	61.6	40.7	101.8
Mean ...	...	7.8	9.9	22.5	6.8	8.4	12.6	1.4	61.5	40.7	101.8
Sponge-protein precipitated by (NH ₄) ₂ SO ₄	100	10.5	3.1	25.2	8.4	11.6	12.3	1.3	63.3	59.3	102.1
	100	10.5	3.0	25.0	8.3	11.5	12.5	1.1	63.2	59.0	101.7
Mean ...	...	10.5	3.0	25.1	8.3	11.5	12.4	1.2	63.1	59.2	101.9
Sponge-protein (residue) ...	100	20.0	1.5	21.4	7.4	7.4	12.5	1.4	56.8	49.9	99.7
	100	19.8	1.4	21.7	7.6	7.5	12.8	1.3	57.0	50.1	99.9
Mean ...	...	19.9	1.4	21.5	7.5	7.4	12.6	1.3	56.9	50.0	99.8

The figures obtained (Table I) show a general tendency towards an increase of histidine-nitrogen and a decrease of lysine-nitrogen in the lower animals. The corresponding averages from the former work on mammalian tissues [Rosedale, 1922, 1928] were about 2 % for histidine-nitrogen and 8 % for lysine-nitrogen. In the examination of fish tissues [Rosedale, 1929], which was carried out by means of the same experimental procedure as used in the present work, the average histidine value was 4.5 %.

We intend to investigate these points further, using other methods when material is available.

SUMMARY.

1. Tissues of the crocodile, cockroach, mosquito larvae (*A. ludlowi*), oysters and sponge (monaxonid), have been analysed by van Slyke's method.
2. An increase in the amount of histidine-nitrogen and a decrease of lysine-nitrogen is found in the lower organisms.

We desire to express our deep gratitude to Prof. K. B. Williamson for his kindness in undertaking the tedious task of collecting the mosquito larvae for us, to Mr W. Birtwhistle, Superintendent of Fisheries, and to Mr L. A. S. Jermyn for kindly supplying sponges and the crocodile.

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