XX. THE AMINO-ACIDS OF FLESH.

III. THE DIAMINO-ACID CONTENT OF FISH.

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PREVIOUS analyses [Rosedale, 1922, 1928] led to the broad conclusion that normal mammalian tissues showed but little variation in their diamino-acid composition. The methods employed, however, seemed sufficiently sensitive to detect certain differences in some pathological tissues. The work reported in this paper was undertaken as an extension of the study of food proteins and deals with types of fish which constitute a very important source of food in this part of the world. In some districts, owing to situation as well as to religious sentiment, fish forms almost the only source of protein. While the prowess of the Malay as a sea-fisherman is well known, the development of the culture of carp is a local industry which merits attention, not only from the point of view of increasing the food supply, but also on account of the fact that ponds which can be made to rear these fish successfully cease to be suitable breeding grounds for mosquitoes. Practically the whole of a fish is used as human food. Heads, skins, etc. are offered for sale in local markets and form a cheap commodity used by the poorer masses of the population. It has not been possible to analyse every species of fish which finds its way into Singapore markets, but a selection has been made of those which are most commonly found, and which serve as general types.

While slight differences may be noted between these tropical fish and mammalian tissue which was analysed in Europe, the results cannot, on the whole, be said to be very divergent. In the following list of the specimens which have been included in this work the local name is given in brackets: red snapper, *Lutianus roseus* (Ikan merah); horse mackerel, *Caranx rottileri* (Chencharu); tunny, *Thynnus* (Tongkol); thread fins, (1) *Polynemus indicus* (Kurau), and (2) *P. tetradactylus* (Senangin); carp, *Thynnichthys* (two species locally called Twatow and Song Hu respectively). The local names are taken for the most part from Maxwell [1921]. In addition to the muscle meat of the above, analyses have been made of the roes and liver of sting-ray (Pari beting), the skins of horse mackerel, and the heads of carp (Twatow). The horse mackerel and Kurau were specimens of dried fish, and the horse mackerel and tunny are red-fleshed varieties.

EXPERIMENTAL.

The preparation of the muscle tissues has followed exactly the procedure of Rosedale [1928], which consists in extraction with 0.1 % acetic acid and subsequent digestion with pepsin. The livers, skins and heads were ground, dried and thoroughly extracted with ether prior to carrying out the above treatment. The preparation of the roes is dealt with in another section. After digestion with pepsin, the protein was hydrolysed by 25 % HCl and analyses were carried out by Van Slyke's method as described by Plimmer and Rosedale [1925] and in certain cases also by a modification of the method of Kossel. The analyses by Van Slyke's method have been made at least in quadruplicate.

Irregularities of the Van Slyke method.

The several analyses of tunny and of the dried fishes were not so concordant as were those of the fresh white-fleshed varieties and compared less favourably in this respect with the former analyses of mammalian tissues. Kernot and Knaggs [1928] consider these discrepancies to be due to the temperature and mode of precipitating with phosphotungstic acid, and give directions towards improvement of this technique. Since however the temperature of this laboratory is higher than is usual in Europe, and since concordant results had been secured with fresh white-fleshed fish without resort to the precautions of these authors, it was felt that the cause of error must be sought elsewhere. It was decided to make a study of the hydrolysis by acid.

Table I. *Results of variation in time of hydrolysis.*

Percentage of total nitrogen.								
	Time (hours)	Amide- N	Humin- N	Diamino- fraction		Monoamino- fraction		% of N
				Total N	Amino- N	Total N	Amino- N	
Tunny	5	5	1	36	20	54	51	96
	10	8	1	39	20	55	54	103
	15	8	1	30	21	63	58	102
	40	9	1	27	20	62	60	99
	60	9	1	27	21	63	62	100
	80	9	1	26	20	67	61	103
	100	9	1	26	20	67	61	103
Carp (Twatow)	10	6	1	46	20	59	57	112
	25	7	1	33	16	62	58	103
	40	7	1	27	17	63	61	98
	100	7	1	27	18	63	62	98
Kurau	25	6	1	35	17	61	54	103
	40	6	2	31	21	61	54	100
	60	6	1	26	18	62	54	95
	100	8	2	27	18	63	54	100
Horse mackerel (flesh)	36	4	1	39	11	41	37	85
	60	6	1	27	15	59	51	93
	100	8	1	28	16	62	56	99
Horse mackerel (skins)	36	6	11	27	22	52	48	96
	100	6	12	20	11	64	61	102

Experiments were made with different varieties of tissue, in which the only alteration in the Van Slyke procedure was a variation in the time of hydrolysis with 25 % HCl. A duplicate analysis was made in each experiment, and the results are given to the nearest whole number in Table I.

These figures show that, when hydrolysis is incomplete, material which cannot correctly be considered as diamino-acids is precipitated by phosphotungstic acid. As hydrolysis proceeds, the proportion of the monoamino-acid fraction becomes greater. In the case of the fresh fishes, tunny and carp (Twatow), hydrolysis appears to be complete by the 80th and 40th hour respectively.

Experiments with Kossel's method.

In a former paper [Rosedale, 1928] a comparison was made between the Van Slyke and Kossel methods. No fundamental differences appeared in the results other than those due to losses sustained in the manipulation, and it seems evident that the Van Slyke method is capable of giving as accurate information of the diamino-acid composition of proteins as any available method. Vickery and Leavenworth [1928] published a modification of Kossel's method in which the separation of histidine from arginine is brought about by the exact neutralisation of the mixture, at which point the histidine is completely precipitated as the silver salt, arginine remaining in solution until it is made alkaline. This procedure differs from that of Rosedale [1928], who precipitates histidine from acid (1.5 %) solution. An experiment was therefore carried out on the flesh of Senangin in two portions, one portion being treated according to the directions of Vickery and Leavenworth, and the other according to the writer's modification [1928].

Table II. *Results comparing the two modifications of Kossel's method.*

Total nitrogen of each portion = 7.224 g.		
	V. and L. method	R. method
Total N of diamino-ppt.	—	2.1 g. = 29 %
„ histidine portion	0.458 g. = 6.3 %	0.301 g. = 4.1 %
„ arginine portion	0.612 g. = 8.4 %	0.717 g. = 9.9 %
„ lysine portion	0.419 g. = 5.7 %	0.421 g. = 5.8 %

The histidine portion of the Vickery and Leavenworth method was made up to 1 litre and arginine estimations according to the directions of Plimmer [1916] were carried out upon 20 cc. portions. These gave an average value of 0.0981 g. arginine-nitrogen, which corresponds to 21 % of this fraction. Plimmer [1916] states that in using glass flasks, as was done in this case, the extent of the decomposition of histidine is from 1.5 to 3 % of its nitrogen. Consequently, in spite of great care used in adjusting the bulky solution to p_H 7.0 before precipitation of the histidine, it appears that some arginine was carried down with the silver salt of histidine and that it was not excluded by the subsequent reprecipitation with mercuric sulphate. A few of the other tissues have been analysed by Kossel's method and the results are given in Table III.

Table III. *Results by Kossel's method.*

	Percentage of total N.		
	Senangin	Horse mackerel	Carp heads (Twatow)
Histidine-N	4	3	1
Arginine-N	10	9	11
Lysine-N	6	8	5
No. of exps.	3	2	2

Comparison with the Van Slyke results (Table IV) shows that these figures are as usual lower than the Van Slyke figures.

Experiments with roes.

Male and female roes were worked up separately. They were treated in accordance with the directions of Kossel and Dakin [1904] for the preparation of protamine sulphate, by precipitating the minced tissue with acetic acid, boiling the precipitate, and reprecipitating with alcohol after treatment with dilute H_2SO_4 . A large precipitate was thus obtained, but in the case of the female roes much of the precipitate redissolved on standing at room temperature. The remaining portion of this precipitate was filtered off, and formed fraction I (see Table IV). An attempt to reprecipitate the filtrate by the use of more dilute H_2SO_4 and alcohol produced a small quantity of material which formed fraction II (Table IV). In the case of the male roes, the first precipitate was filtered off without being allowed to stand. This forms fraction I of the male roes (Table IV). The residual tissues of both male and female roes were coagulated in the same way as the ordinary flesh tissues, forming fraction II of the male and fraction III of the female roes (Table IV). Digestion with pepsin was followed in every case by hydrolysis with 25 % HCl and the usual Van Slyke procedure. The general results by Van Slyke's method are presented in Table IV.

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

Percentage of total N.											
	Time of hydrolysis (hours)	Amide-N	Humine-N	Diamino-fraction					Monoamino-fraction		Total
				Total N	Amino-N	Arginine-N	Histidine-N	Lysine-N	Total N	Amino-N	
Male roes, fraction I	40	15	9	49	14.5	41	6	1	31	29	104
" II	40	14	1	24	15	12	0	12	58	57	97
Female roes, fraction I	40	15	6	48	27.5	37	7	4	32	31	101
" II	40*	19	3	40	14.5	12	16	12	38	31	100
" III	40	7	1	28	15	14	4	10	65	63	101
Red snapper (Ikan merah)	40	8	1	31	23	16	2	10	65	62	105
Thread fin (Senangin)	40	7	1	28	22	14	5.5	8	62	60	98
Thread fin (Kurai)	100	8	2	27	18	12.5	7	11	63	54	100
Raia livers	40	10	2	30	19	12	3	12	60	58	102
Horse mackerel (flesh)	100	8	1	28	16	12	5	11	62	56	99
Horse mackerel (skins)	100	6	12	20	11	9	4	7	64	61	102
Tunny	80	9	1	26	20	10	5	6	67	61	103
Carp (Twatow)	40	7	1	27	17	12	6	7	63	61	98
Carp (Song Hu)	40	6	1	29	16	12	7	10	67	60	103
Carp heads (Twatow)	100	7	2	24	18	14	2	7	66	60	99

It is clear that no protamine has been obtained from the roes. Fractions I and II of the female and I of the male roes appear to contain proteins

resembling the histones. This may depend upon the maturity of the milt [Kossel and Staudt, 1927]. The flesh muscles show slight differences from the analyses of mammalian tissues in that the total diamino-acid content is generally higher. This appears to be due to the presence of a greater amount of histidine, the most notable exceptions being found in the red snapper and the carp heads. General support of these statements is given by the results with Kossel's method (Table III).

SUMMARY.

1. The diamino-acid content of several fish tissues has been determined by Van Slyke's method, and in a few cases by a modification of Kossel's method.

2. The value for diamino-acid nitrogen in the flesh muscles of fish appears to be higher than that previously obtained for mammalian tissues. This seems to be due mainly to an increased amount of histidine-nitrogen.

3. Some evidence is brought forward showing that incomplete hydrolysis leads to error in the Van Slyke analysis. Different tissues appear to require different lengths of time to ensure complete hydrolysis with 25 % HCl.

4. In the separation of histidine and arginine as silver salts, it appears that unless the mixture is kept slightly acid there is a danger of precipitating some of the arginine together with the histidine.

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