CL. THE CONSTITUTION OF SPONGES.

I. THE COMMON BATH SPONGE, *HIPPOSPONGIA EQUINA*.

By VERNON JAMES CLANCEY.

(Received August 3rd, 1926.)

INTRODUCTION.

It is the belief of the author that the constitution of spongin varies with the source of its origin, and that the substances which form the main framework of the Euceratosa and which surround the spicules in the Pseudoceratosa are not identical, as appears to have been assumed by many physiologists. It is with the object of proving or disproving this theory that the present series of researches has been undertaken.

Many workers have published results for the constitution of spongin, usually obtained from either *Hippospongia equina*, the common bath sponge, or *Euspongia officinalis*, the "Turkey cup sponge." These results show remarkable differences due to defective methods of analysis and working with commercial sponges that have been variously macerated and bleached.

In this paper the author presents the results that he has obtained by applying the modern methods of protein analysis to the spongin from *H. equina*.

THEORETICAL AND METHODS.

The spongin for the following estimations was prepared by washing successively with water, dilute acid and alkali and then drying in a vacuum over P_2O_5 . This gave a substance containing 1.25 % ash and 0.8 % water.

The cause of the differences in the results of previous workers appears to be the presence of the iodine complex. Wheeler and Mendel [1909] isolated this complex from a barium hydroxide hydrolysis and showed that it was identical with the iodogorgonic acid first obtained from *Gorgonia cavolinii* by Drechsel [1896], the structure of which was shown to be that of 3 : 5-di-iodo-tyrosine by Wheeler and Jamieson [1905], and Wheeler and Johns [1910]. They also showed that it does not give all the reactions usually associated with tyrosine; for example, it gives the xanthoproteic reaction owing to the presence of the benzene ring, but the iodine in the ring apparently prevents it from reacting with Millon's reagent.

In order to obtain complete hydrolysis and comparative values for the amino-acids, the following method was adopted.

A weighed portion of the spongin, about 3 g., was introduced into boiling 20 % hydrochloric acid and boiled under a reflux for about 30 hours until no further increase of the amino-acids in the solution was shown. It is necessary to raise the acid to boiling before adding the protein in order to obtain concordant results [cf. Knaggs, 1923]. The resulting solution, after filtration from the "humin," was made up to 250 cc. A portion of 100 cc. was analysed by the methods of Van Slyke and Hausmann. The results show the discrepancies that arise by neglecting the iodine complex.

Another portion of the hydrolysed mixture was evaporated down, *in vacuo* at 40°, to about 1 cc. Water was added to bring it back to its original volume and the mixture again evaporated. This was repeated a third time to ensure that nearly all the hydrochloric acid had been removed. The resulting liquid was diluted to about 100 cc., and brought to boiling under a reflux condenser. A few drops of nitric acid were added, and then a solution of silver nitrate until no further precipitation took place. The liquid was filtered through a Gooch crucible and the precipitate well washed. The iodine in the precipitate was estimated by replacing it with chlorine and noting the loss of weight. A Kjeldahl determination was made to ascertain whether any of the nitrogen had been carried down with it. A control experiment showed that the addition of the nitric acid had no effect on the distribution of the nitrogen.

The filtrate from the silver precipitation was evaporated down and the nitrogen groups estimated in the ordinary way.

The total nitrogen was determined by Kjeldahl's method in an aliquot portion of the hydrolysed mixture.

The iodine was estimated by a combination of the methods of Kendall [1920], and Winkler [1915, 1916].

The individual amino-acids were estimated by the methods given in Plimmer's *Constitution of the Proteins*, using the "ester" separation.

RESULTS.

Table I gives the values for the "Hausmann numbers" obtained for spongin by the above two methods, and comparative values for iodogorgonic acid prepared from tyrosine by the method of Wheeler and Jamieson [1905].

Table I.

Substance	Humin-N %	Amide-N %	Diamino-N %	Monoamino-N %	Total N %
Spongin—					
Without removal of iodine	0.03	1.12	2.39	12.58	16.12
With removal of iodine	0.03	0.98	2.34	12.77	16.12
Iodogorgonic acid	0.00	2.99	0.00	0.24	3.23

The above values are calculated as percentages of the total protein.

Table II gives the values for the nitrogen in eight groups, calculated as percentages of the total nitrogen.

Table II.

Group	Without removal of iodine %	With removal of iodine %
Humin-N	0.18	0.18
Amide-N	6.95	4.91
Cystine-N	0.04	0.02
Arginine-N	11.16	10.90
Histidine-N	0.00	0.00
Lysine-N	4.37	4.39
Monoamino-N	77.43	80.01
Non-amino-N	2.86	2.78
	102.99	103.19

The following are the values for the individual amino-acids obtained by the ordinary methods, the tyrosine being estimated in a sample after removal of the iodine. They are given as percentages of the protein.

Glycine	14.0 %	Aspartic acid	4.5 %
Alanine	0.2 %	Glutamic acid	18.4 %
Leucine	7.9 %	Tryptophan	0.0 %
Tyrosine	2.8 %	Arginine	5.9 %
Cystine	trace	Lysine	3.6 %
Proline	5.7 %	Histidine	0.0 %
Hydroxyproline	0.0 %	Ammonia	0.8 %

The iodine content of the spongin was 1.46 %, whilst the silver precipitation carried down 1.41 %, the latter value being low, probably owing to the difficulty of getting an accurate estimation.

CONCLUSIONS.

Spongin on acid hydrolysis yields, besides the other amino-acids, iodogorgonic acid. This is liable to be overlooked owing to the fact that its solubility is 0.2 g. in 100 g. of water and that it is not insoluble as stated by Henze [1903]. It does not give the Millon reaction as it contains an iodine atom in both the 3 and the 5 positions according to Hofmeister [1898], and Blum and Vaubel [1898].

The iodogorgonic acid yielded on hydrolysis is converted into tyrosine and silver iodide by the treatment with acid silver nitrate. The yield of iodine is equivalent to 2.1 % tyrosine, whilst 2.8 % was found. This yield is equivalent to 4.7 % iodogorgonic acid in the protein.

By first removing the iodine it is possible to estimate the tyrosine in the ordinary way and to obtain true values for the nitrogen distribution. The tyrosine appears to be totally present as iodogorgonic acid, and varies with the percentage of the iodine.

On hydrolysis with dilute alkali iodogorgonic acid yields practically all its nitrogen as ammonia, *i.e.* it behaves as an amide. Consequently the iodine must first be removed in order to obtain correct values for the amino-acids.

Spongin yields principally glutamic acid and glycine on hydrolysis, with smaller quantities of leucine, aspartic acid, arginine, proline, lysine and tyro-

sine. It does not appear to be very closely related either to the chitinoids or to fibroin, as has been suggested by some authors, but is rather more closely related to the collagens, especially as there is some evidence that on prolonged heating with water a portion goes into solution which is reprecipitated by alcohol.

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