THE COMPOSITION OF CHINESE EDIBLE BIRDS' NESTS AND THE NATURE OF THEIR PROTEINS.*

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(Received for publication, September 26, 1921.)

Appearance and Origin.

The edible birds' nests are gelatinous substances produced by certain swifts, the *Collacalia*, natives of Malaya (1) and Ceylon. The nests, constructed in caves on the seashore, are collected while they are still moist and made into various shapes. The lowest grade is sold in the form of coarse powder. The higher the grade, the whiter the color and fewer the feathers and twigs. Owing to their high price, their use is limited to a delicacy at the feasts of the wealthy and a food for convalescents and the aged.

The source from which the birds make the nests has been uncertain. Green (2) gives three suggestions: in the algæ found in caves where the swifts make their nests, fish spawn, or a secretion from the swifts themselves. The algæ theory is disproved by the lack of vegetable cells shown by microscopic examination of the nests. The secretion theory is believed by most of the natives and has the support of Home (3) and Bernstein (4). The latter author found in the birds two large salivary glands which secreted much viscous mucus. The observation given in this paper shows that the nests consist largely of a mucin-like substance and, therefore, is in accord with the latter hypothesis.

Review of Literature.

The literature on the subject is limited. Descriptive statements concerning chiefly the occurrence and appearance of the nest may be found in Encyclopedias, China year books, and some semiscientific articles written during the early part of the 19th century. Green (2) and Krukenberg (5) are the first to give a report of a scientific study of the nest. Their work

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* The work reported in this article was conducted at the Nutrition Laboratory, Department of Home Economics, University of Chicago. It forms part of the thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Chicago.

covers the solubilities, the response to protein tests, and some observations on hydrolysis products. Their results prove that the birds' nest contains both the carbohydrate and the protein radicle, belonging to the class of mucin-like substances, the glycoproteins.

Scope of the Present Work.

The present work covers the study of the general properties, the chemical composition, the artificial digestion, the carbohydrate radicle, and the biological value of the proteins. Comparison is made with other work on mucin, especially Lothrop's, Müller's, and Levene's.

The material used for this work was supplied by the Hoo Loong Edible Birds' Nest Store, Chicago, which imported it directly from China. It was of the highest grade, having somewhat the appearance of agar-agar, but it was extremely crisp and had tiny feathers interwoven with the mucilaginous material. For quantitative analysis, the material was ground and sifted. On sifting, most of the feathers cling together and may be removed, but some go through the sieve so that it is difficult to obtain a pure sample.

General Properties.

A sample boiled in distilled water for 3 hours and left there for several days, swells like a piece of sponge, but shows no tendency to dissolve. The filtrate responds to neither protein nor carbohydrate tests. 5 per cent sodium hydroxide dissolves it on standing 2 hours in the cold. The colorless solution responds to Millon's, the biuret, xanthoproteic, and Hopkins-Cole tests. The last reaction shows only faintly. It also has a slight reducing power with Fehling's reagent. A dilute acid, such as 3 per cent hydrochloric acid, dissolves the birds' nests only on heating. The solution acquires a purplish brown color, gives both protein and carbohydrate tests, and has a strong reducing action.

So far the properties agree with those reported by Green (2) in every respect except that he found the nest insoluble in dilute sodium hydroxide in the cold. They also agree with the commonly recognized properties of mucin.

Chemical Analysis.

Samples between 1.5 to 2 gm. were taken for the determination of moisture and ash. Neumann's method given in Mathews (6) was used for the estimation of phosphorus, and Denis's method (7) for that of sulfur. An attempt was made to determine the ether-soluble substance, but the results were too small to be of significance, only 0.3 per cent. The estimation of total nitrogen by the Kjeldahl-Gunning method was made on samples treated in three different ways: (1) original birds' nest, (2) ground birds' nest with feathers partially removed, and (3) a sample hydrolyzed for 13¼ hours in 20 per cent hydrochloric acid. Results are given in Table I.

TABLE I.

Chemical Composition of Chinese Edible Birds' Nests.

No.	Moisture.	Ash.			Phosphorus.	Sulfur.	Total nitrogen.		
		Water-soluble.	Water-insoluble.	Total.			Original birds' nests.	Ground, and feathers partially removed.	Hydrolyzed 13¼ hrs. in 20 per cent HCl.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	11.88	0.75	1.74	2.49	0.035	1.10	8.81	9.16	10.57
2	11.41	0.74	1.80	2.54	0.034	1.10	8.75	9.14	10.24
3	11.52	0.74	1.78	2.52					10.33
4									10.31
Average....	11.60	0.74	1.77	2.51	0.035	1.10	8.78	9.15	10.29

The total ash, 2.51, is almost seven times as high as that of submaxillary mucin, 0.37 per cent, reported by Lothrop (8). This high percentage of ash shows that the birds' nest is not a pure mucin, but more probably dried saliva. Of the total ash 29.48 per cent is insoluble in water, but none insoluble in acid. Hence no sandy material is present.

The sulfur content, 1.10 per cent, is in agreement with the figures given by Müller (9) for salivary mucin, 1.40 per cent, but it is higher than that reported by Lothrop (8), 0.55 per cent. The discrepancy may be due to the different methods used or in case of birds' nests, the presence of feathers. The phosphorus, 0.035 per cent, is too small to be of any significance.

The different values for nitrogen found in birds' nests treated in three different ways may be explained by the variation in the feathers present. The original sample containing the most feathers, had the lowest figure, 8.78 per cent, while that hydrolyzed with the least feathers, the highest or 10.29 per cent. The ground and sifted sample gave 9.15 per cent. Some feathers were removed during grinding and sifting, but more of them separated out by clinging to the walls of the vessel on hydrolysis. They could then be easily removed. The percentage of nitrogen of the hydrolyzed material, 10.29, agrees with the value given by Müller (9) for salivary mucin, 10.70 for total nitrogen, but it is lower than that reported by Lothrop (8), 12.49 per cent.

Artificial Digestion.

Artificial digestion experiments carried out in comparison with hard boiled egg white showed that the birds' nests were digested by both pepsin hydrochloric acid and trypsin though not so quickly as the egg. The speed of digestion was determined by Sörensen's titration (6). Comparison was made of the increase during 24 hours in the volume of 0.1 N sodium hydroxide for titrating 25 cc. of the peptic digest. Results were expressed in cc. per gm. of nitrogen in the material acted upon. For the birds' nests in a typical experiment this value was 9.60 cc. and for the egg white 15.47 cc. Similarly, the increase of 0.1 N hydrochloric acid to titrate the tryptic digest was 19.02 cc. for the birds' nests and 38.75 cc. for the egg white.

The percentage of carbohydrate in the hydrolyzed birds' nests could not be found with accuracy. Efforts using Benedict's (10) Method 2 failed to give concordant results. The material was prepared in the following manner: 1 gm. of the ground and sifted nests was dissolved in a small amount of concentrated hydrochloric acid by standing over night. It was then diluted with distilled water to make a 5 per cent acid solution and boiled with a reflux condenser for $1\frac{1}{2}$ hours. The hydrolyzed mixture was treated with phosphotungstic acid, filtered, and the filtrate was made up to a definite volume.

Concordant results were not obtained. Variations were therefore made in the strength of acid from 3 per cent to concentrated and the length of hydrolysis from ½ to 8½ hours. In some cases Levene's (11) method of introducing a little stannous chloride into the hydrolyzing mixture was followed. It was soon discovered that the reducing power of the birds' nest was gradually diminished by heating in an acid solution. A difference of 6.37

TABLE II.

Relation between Quantity of Reducing Sugar and Time of Hydrolysis.

Time.	Reducing sugar.
hrs.	*per cent*
½	17.36
1	15.99
1	16.03
2	13.63
3	13.11
8½	10.99

per cent of carbohydrate calculated as glucose between the samples hydrolyzed ½ and 8½ hours is shown in Table II. In this experiment 20 per cent hydrochloric acid was used, the highest figure, in the table, 17.36 per cent, is much lower than that reported by Müller (9) for the carbohydrate in salivary mucin. His is 37 per cent, estimated by making phenylosazone from the hydrolyzed mixture.

Distribution of Nitrogen.

For the distribution of nitrogen Van Slyke's (12) method was closely followed, except that the bases were precipitated from a volume of 250 cc. instead of 200 cc. and the correction for solubility of the basic phosphotungstates made accordingly. Approximately 2 gm. of the ground and sifted birds' nest were taken for each of the four series of experiments. Several months elapsed between each one of the series.

By a study of Table III it will be seen that it was possible to obtain a fairly complete result on the distribution of nitrogen, the results for the different nitrogen fractions in the three finished series totalling 99.84, 100.61, and 100.54 per cent, respectively.

Series II and III are in close agreement with each other and Series IV slightly different, probably due to the fact that the material used in Series IV was purchased at a different time from the others. In Series II and III with the exception of cystine nitrogen, humin nitrogen, and the non-amino nitrogen of mono-amino-acids, the differences between duplicate series are all

TABLE III.

Distribution of Nitrogen in the Edible Birds' Nests.

Series	I		II		III		IV		Average of II and III.	
Nitrogen.	Birds' nests.	Total nitrogen.	Birds' nests.	Total nitrogen.	Birds' nests.	Total nitrogen.	Birds' nests.	Total nitrogen.	Birds' nests.	Total nitrogen.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Amide	1.07	10.15	1.03	10.06	1.05	10.10	1.09	10.54	1.04	10.08
Humin	0.71	6.75	0.65	6.38	0.72	6.98	0.70	6.77	0.69	6.68
Arginine	1.45	13.69	1.43	13.96	1.44	13.93	1.25	12.12	1.44	13.95
Cystine	0.37	3.51	0.29	2.79	0.41	4.00	0.49	4.77	0.35	3.39
Histidine			0.60	5.90	0.67	6.53	0.67	6.47	0.64	6.22
Lysine			0.26	2.55	0.25	2.37	0.19	1.84	0.26	2.46
Amino nitrogen of mono-amino-acids			5.08	49.61	5.24	50.76	4.99	48.41	5.16	50.19
Non-amino nitrogen of monoamino-acids			0.87	8.54	0.61	5.90	1.00	9.62	0.74	7.22
Total nitrogen recovered			10.21	99.84	10.40	100.61	10.38	100.54	10.31	100.23
Total nitrogen determined	10.57		10.24		10.33		10.31		10.29	

within the maximun experimental error allowed by Van Slyke (12) for pure proteins. The presence of considerable fine feathers with their high sulfur content and the resulting difficulty in obtaining a pure sample may be the cause of the variation in the cystine nitrogen. The differences in the humin nitrogen are probably due to the carbohydrate in the nest proteins.

The humin nitrogen is shown much higher than that of any of the pure proteins. The highest Van Slyke (12) has reported is 3.6 per cent for ox hemoglobin, a difference of 3.08 per cent from that of the nest. This high humin nitrogen may be explained as the result of the presence of a carbohydrate radicle in the nest proteins. Gortner and Blish (13), Gortner (14), and Hart and Sure (15) reported that the presence of dextrose or any other carbohydrate caused an increase of humin nitrogen during the hydrolysis of zein, fibrin, and casein.

The influence of the presence of a carbohydrate radicle on the distribution of nitrogen is also shown by the comparatively low lysine nitrogen and the high histidine nitrogen of the nest proteins. Thus, in case of pure casein reported by Hart and Sure (15) the value given for lysine nitrogen is 9.41 per cent and that for histidine nitrogen is 5.95 per cent. On the addition of dextrose, sucrose, and starch, respectively, during the hydrolysis of casein the corresponding values for lysine nitrogen are 7.01, 6.38, and 5.54 per cent, and those for histidine nitrogen are 7.31, 7.65, and 7.30 per cent. In every case, therefore, there is a decrease in the lysine nitrogen and an increase in the histidine nitrogen. Although the nature of the carbohydrate radicle in the nest has not yet been completely determined, it seems to possess in common with other carbohydrates the power of causing a redistribution of amino-acids on hydrolysis of protein. The presence of feathers in the material is undoubtedly the cause of the high value, 3.39 per cent for the cystine nitrogen in the nest. The highest value Van Slyke gave for cystine nitrogen in pure proteins is 1.25 per cent in gliadin.

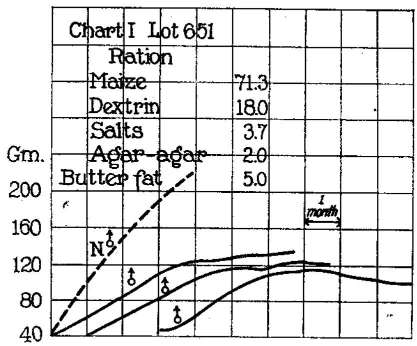
Biological Value of the Birds' Nests Proteins.

Feeding experiments were conducted on rats at Dr. McCollum's laboratory,[1] the School of Hygiene and Public Health, Johns Hopkins University, and the birds' nest was used to supplement a ration adequate in all respects but the character of the protein. Two unsatisfactory proteins, maize kernel and rolled oats, were chosen because they are of different character (16). Although

[1] For this part of the work I am indebted to Dr. McCollum and Miss Simmonds who kindly continued the experiments that I had started, and thus enabled me to secure the results.

when each is fed as the sole source of protein, they have approximately the same biological values for the support of growth, they are not at all similarly constituted. Oat proteins are supplemented well by the amino-acid mixture which comes from the digestion of gelatin, whereas the proteins of the maize kernel are not so supplemented in a degree which can be demonstrated by growth experiments with young animals.

The complexes which form the limiting factor in those two proteins are different, and, therefore, if the birds' nest protein has a high nutritive value it should at least supplement one of them. Since its addition failed to supplement either protein, it seems very probable that the birds' nest protein is of an inferior quality. It is of course possible that when taken together



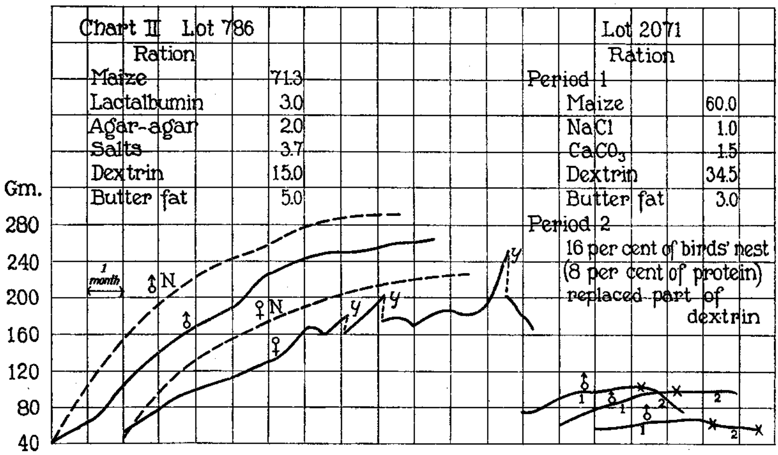
with certain other foods it might have value; but its value is problematical in any case, and it is certain that it is far from being a complete food protein.

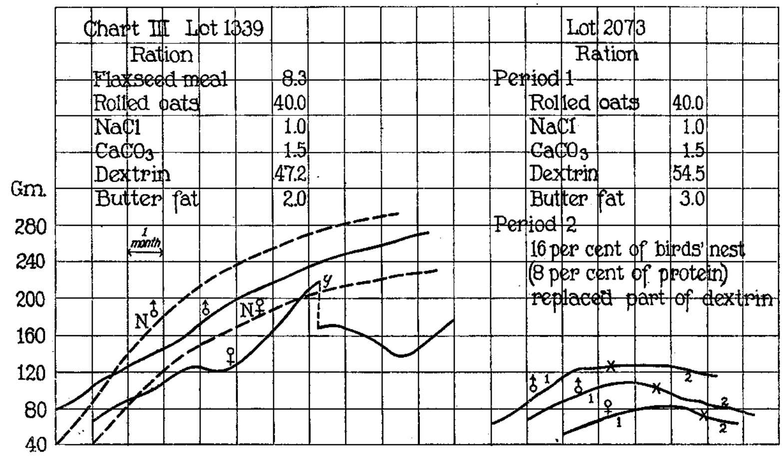
Lot 651 (Chart I) represents the growth curves for a group of rats which were restricted to a diet containing about 7 per cent of corn protein. The diet was otherwise fairly satisfactorily constituted. It failed to induce growth at a rate corresponding to about half the normal rate, and the animals became stunted when they were about half the normal adult size.

This diet is greatly improved by the addition of even so small an amount of purified protein as 3 per cent of lactalbumin. This is shown in the growth curves of Lot 786 (Chart II).

Lot 2071 (Chart II) shows the very slow rate of growth of a group of rats which were restricted to a diet comparable in all respects to those just mentioned except that the corn protein was fed at the plane of 6 per cent of the food mixture. This ration, when supplemented with a protein having an appreciable biological value, would become capable of inducing growth, as illustrated in the case of Lot 786.

When a group of young rats were fed this diet for a period of 13 weeks and had been able to increase in weight but very little, and had finally completely failed to grow, 16 per cent of the Chinese edible birds' nest,





equivalent to 6 per cent of N × 6.25, was added in the place of a portion of the carbohydrate of the diet. If the birds' nests protein had any appreciable nutritive value, it should have enabled the young animals to respond with growth. A wide experience has demonstrated that such response will be observed when the protein added is of such a nature as to furnish certain essential amino-acids which form the limiting factor in the proteins of the corn (maize). In this experiment there was no growth following this addition of birds' nests. The only conclusion which can be drawn is that the birds' nests do not supplement the protein and the corn kernel.

Lot 2073 (Chart III) shows that young rats cannot grow much when the diet contains only the amount of protein which will be furnished by a content of rolled oats equivalent to 40 per cent of the food mixture. This amounts to approximately 6 per cent of protein in the diet. Lot 1339 (Chart III) shows that the same food mixture, supplemented with the proteins of flaxseed oil meal sufficient to furnish an additional 3 per cent of proteins, is sufficient for the maintenance of a rate of growth somewhat more rapid than half the rate at which the young rat is capable of growing. Animals on this diet have reached approximately the full adult size, and young have been produced.

The fact that Lot 2073 in Period 2 failed to respond with growth when 16 per cent of the edible birds' nest was added to the diet, leaves no room for doubt that this substance, although a protein of a peculiar character, does not supplement the proteins of the oat kernel in any appreciable degree.

SUMMARY.

1. The Chinese edible birds' nest has the properties of a protein as well as those of a carbohydrate. It belongs, therefore, to the class of glycoprotein.

2. Its percentage composition resembles that of salivary mucin. Its ash is high, but there is no sandy material present. It contains 10.29 per cent nitrogen and at least 17.36 per cent carbohydrate.

3. Artificial digestion experiments indicated that the birds' nest was digested by both pepsin hydrochloric acid and trypsin at a slower speed than boiled egg.

4. The distribution of nitrogen showed a higher value for both humin nitrogen and cystine nitrogen than for pure proteins. The former is probably due to the carbohydrate radicle in the nest while the latter is due to the presence of fine feathers. Other fractions were similar to those of pure proteins.

5. Feeding experiments indicate that the nest protein is probably of an inferior quality. It failed to supplement a ration adequate in all respects, except that the source of protein was derived from either maize kernel or rolled oats. Although both of them were unsatisfactory proteins they were different in character.

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