

Price, As. 8]

[Vol 16A, Part IX, pp. 97 to 102.]

JOURNAL

OF THE

Indian Institute of Science

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PART II. INVESTIGATION OF THE WATER SOLUBLE NITROGENOUS
CONSTITUENTS

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PHYSIOLOGICAL PRODUCTS OF THE LAC INSECT

PART II. INVESTIGATION OF THE WATER SOLUBLE NITROGENOUS CONSTITUENTS

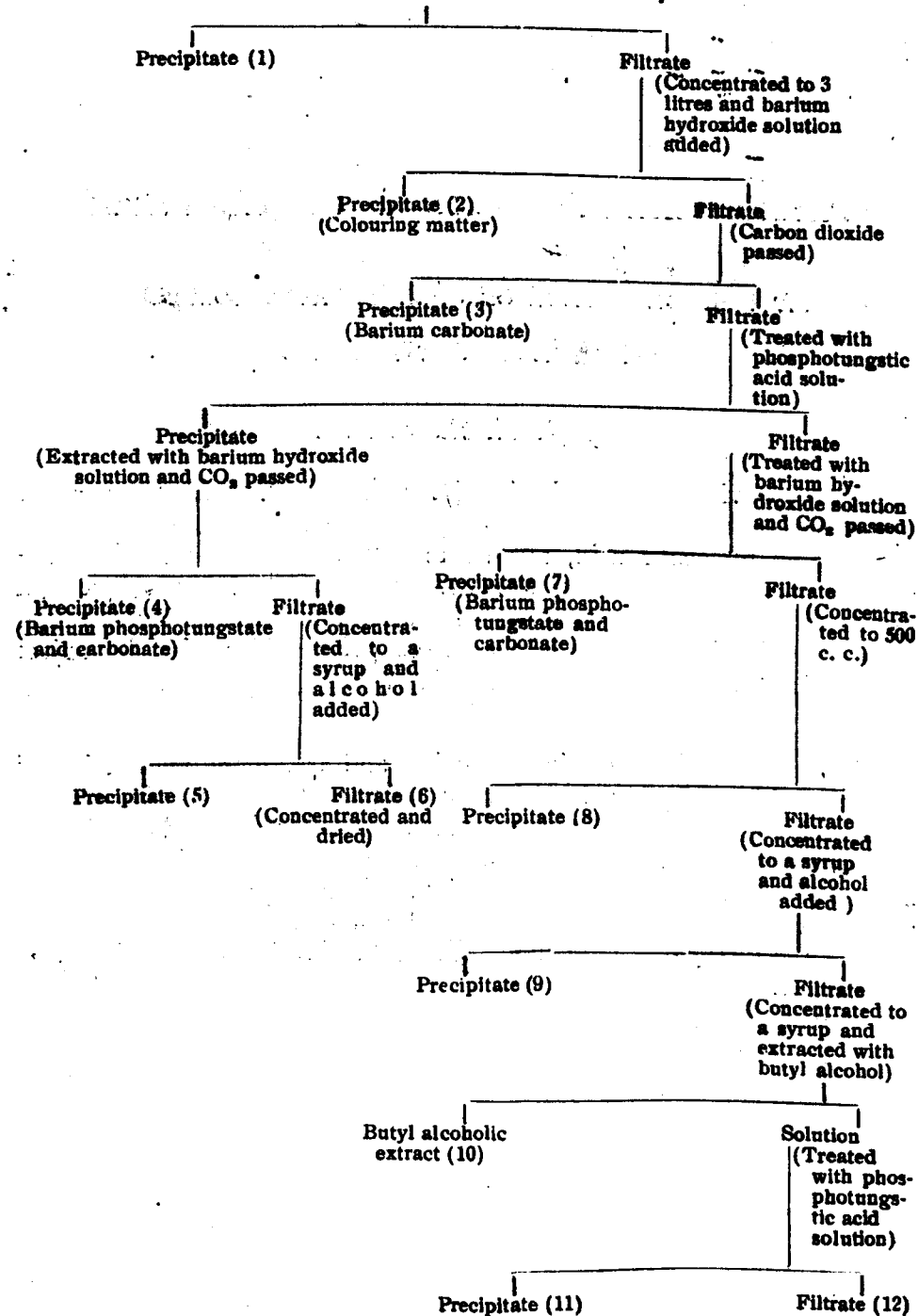
By N. K. Ranga Rao.

In a previous communication (*J. Indian Inst. Sci.*, 1933, **16A**, 77) it has been shown that the water soluble nitrogenous bodies not precipitated by acetic acid, alcohol or salt solution, consisted of the simpler polypeptides, among which a protamine-like body with a high percentage of basic nitrogen was found. The present investigation deals with the fractionation of the serum polypeptides with the object of isolating simpler mixtures if not individual compounds.

Several modifications in extraction and treatment were adopted with the object of obtaining the polypeptides, as far as possible, in their native state and without any admixture of foreign bodies. Thus, the extraction of the material was carried out with distilled water instead of with 0.9 per cent. salt solution. Concentration of the aqueous extract was effected by freezing out the water. The proteins were precipitated directly with alcohol. The following is a schematic representation of the treatment.

INCRUSTATION (35 lbs.)

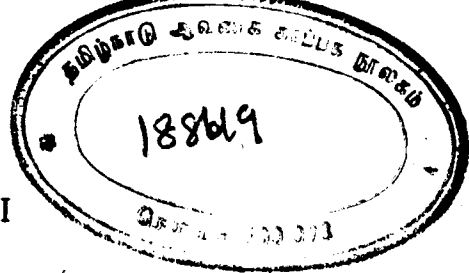
(Extracted with 10 litres of water, concentrated to 3 litres and treated with 5 vols. of alcohol)



Fractions 1, 2, 3, 4 and 7 were dried at 97° and bottled. Numbers 3, 4 and 7 were white powders: 1 and 2 were dark red in colour. Fraction 8 crystallised in the form of fine, white needles. Fractions 5 and 9 were obtained as brownish powders. They were washed thoroughly with absolute alcohol and preserved in a vacuum desiccator. On removing alcohol under reduced pressure, fractions 6 and 10 yielded brown powders which were also preserved in the vacuum desiccator.

Results.—The nitrogen contents of the different fractions were estimated.

TABLE I



Fraction No.	Total Nitrogen content in gms.	Percentage on total water soluble nitrogen	Weight of substance gms.	Percentage of nitrogen in the preparation
1	1.50	16.48	...	...
2	0.26	2.86	63.0	0.41
3	0.06	0.66	8.0	0.75
4	0.21	2.31	40.0	0.05
5	0.20	2.20	2.5	11.41*
6	3.40	37.37	23.2	14.67
7	0.25	2.75	140.0	0.18
8	0.23	2.53	3.1	7.60
9	0.87	9.56	28.0	4.30*
10	1.42	15.61	15.0	9.47
11	0.30	3.30	...	...
12	0.31	3.41	...	...
	9.01	99.04		

* On ash-free sample.

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TABLE II
Nitrogen distribution.

	Fraction 5	Fraction 6	Fraction 9	Fraction 10
Humin N	8.44	5.08	5.43	4.52
Acid amide N	2.62	13.02	2.62	2.82
Basic N	47.89	41.67	16.55	25.80
Arginine N	18.73	18.05	12.29	19.03
Histidine N	30.35	19.43	4.31	8.87
Lysine N	Nil	4.19	Nil	Nil
Cystine N	...	...	...	...
Non-basic N	39.20	39.93	75.43	66.94
Amino N in basic form	13.60	15.20	4.47	5.61
Amino N in nonbasic form	40.12	33.57	40.63	28.07

TABLE III
Properties of the nitrogenous compounds.

	FRACTION				
	5*	6	8	9*	10
Solubility in water.	Soluble	Readily soluble	Soluble in hot water	Readily soluble	Readily soluble
Solubility in alcohol.	Soluble	Soluble	Insoluble	Soluble	Soluble
Solubility in butyl alcohol.	Soluble	Insoluble	Insoluble	Soluble	Soluble
Phosphotungstic acid.	Precipitate	Precipitate	No precipitate	Precipitate in acid medium	Precipitate in acid medium
Mercuric acetate.	Precipitate	Precipitate	No precipitate	Precipitate	Precipitate
α -naphthol test (Molisch test).	—	+	—	—	+

* Freed from metallic radical.

Discussion of Results.—Fraction 1 is a mixture of proteins and peptones which are precipitated with 5 volumes of alcohol. Since fraction 2 is made up chiefly of the lac dye, which is known to be free from nitrogen, it is to be inferred that 0.26 gm. of nitrogen found in that fraction (63 gms.) may be regarded as having been occluded. Fractions 3, 4 and 7 are barium salts of phosphotungstic and carbonic acids and they have in all 0.52 gm. of nitrogen precipitated with them. Fractions 5 and 6 are nitrogenous bodies precipitated by phosphotungstic acid, but fraction 5 differs from fraction 6 in that it forms a compound with barium hydroxide and is soluble in butyl alcohol. Fractions 9 and 10 are nitrogenous bodies not precipitated by phosphotungstic acid, but fraction 9 differs from fraction 10 since it forms a hygroscopic compound with barium hydroxide. The nitrogenous bodies not extracted by butyl alcohol are represented by fraction 12.

A detailed investigation of the fractions which had either a high percentage of nitrogen or contained comparatively large quantities of nitrogen was carried out.

Fraction 8.—This is a white needle-shaped crystalline substance soluble in hot water. It has been identified as tyrosine by its combustion values, nitrogen content, amino nitrogen content and Millon's reaction. (Found: C, 59.0; H, 6.10; N, 7.68. $C_9H_{11}O_3N$ requires C, 59.64; H, 6.12; N, 7.74 per cent.)

Fractions 5, 6, 9 and 10.—The distribution of nitrogen in these compounds has been determined by the Van Slyke method. Fraction 5 contains 47.9 per cent. of basic nitrogen of which 30.4 per cent. is histidine and 18.7 per cent. arginine. The ash content of this fraction is 29.8 per cent. About 32 per cent. of the total water soluble nitrogen separated as fraction 6 which contains 42 per cent. of basic nitrogen of which 18 per cent. is arginine and 19, histidine. The non-basic nitrogen of compounds of fractions 5 and 6 occur almost entirely in the amino form. Fractions 9 and 10 are poor in the basic fraction and are not therefore readily precipitated by phosphotungstic acid. The former forms a stable compound with barium hydroxide from which the free polypeptide cannot be obtained by passage of carbon dioxide as is usually the case but has to be treated with sulphuric acid to remove barium. The non-basic nitrogen content of this compound is very high, being 75.4 per cent. Its ash content is 21.8 per cent. Further work on this compound is in progress. Fraction 10 is neither precipitated by phosphotungstic acid, nor forms a compound with barium hydroxide. The nitrogen distribution of this compound is given in Table II.

SUMMARY

1. The nitrogenous bodies of the lac wash liquor have been separated into four fractions, two of which are precipitated by phosphotungstic acid. These fractions are rich in basic nitrogen and constitute about 35 per cent. of the total water soluble nitrogen. The other two fractions not precipitated by phosphotungstic acid are rich in the non-basic fraction (75 per cent.) and constitute about 25 per cent. of the total nitrogen.

2. Free tyrosine has been obtained from the body fluids of the lac insect. It has been found to be present to the extent of 2.5 per cent. on the total water soluble nitrogen.

My grateful thanks are due to Mr. M. Sreenivasaya and Professor V. Subrahmanyam for their many helpful suggestions in the course of this investigation.

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[Accepted, 21-7-33.].



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