

PHYSIOLOGICAL PRODUCTS OF THE LAC INSECT. PART I.

A preliminary investigation.

By N. K. Ranga Rao and M. Sreenivasaya.

Although modern trade and industry value lac primarily for its resinous incrustation, yet from the viewpoint of ancient Hindu medicine, the insect residues, which consist of nitrogenous and fatty bodies, have a therapeutic interest. Aqueous decoctions of the insect are administered in lung diseases. Germicidal, febrifugal and astringent properties are also attributed to lac which, finely powdered and mixed with honey, is administered as a specific for haemostosis. It forms the essential ingredient of a medicated oil *Lakshadi Thaila*, which is reputed to reduce chronic fevers, cure rheumatic pain and help the growth of foetus during pregnancy.

Ayurvedic literature or practice does not identify the component of stick lac to which the medicinal property is due. It is not known whether the brood lac with its living population of mother insects, or "phunky" lac containing the debris of insect skins, is to be employed for the above-mentioned preparations. Clinical experience points to the conclusion that the most efficacious and potent preparations are obtained from fresh brood lac before swarming; it is also suggested that wild lac, *Lakshadia communis*, which is usually found on *Ficus* trees, possesses a higher medicinal value. When aqueous decoctions are administered, the dissolved body fluids of the insect are involved, but the medicated oil should incorporate exclusively the wax and the insect fat, both of which probably constitute active ingredients.

A detailed study of the nitrogenous and fatty constituents of the insect has been undertaken in view of its importance in Hindu medicine. This investigation has an additional interest of economic value since in the washing of stick lac in North Indian factories, these valuable constituents run to waste. The wash-liquors should certainly yield products of economic value.

EXPERIMENTAL.

The brood lac employed in these studies was obtained from one of the plantations at Devarabetta, North Salem, three weeks before

the commencement of larval swarming. The insect mothers inhabiting the incrustation had therefore very nearly reached their maximum maturity, since this stage represented the peak on the growth curve. The incrustation was washed in a rapid stream of water to dissolve the honey-dew deposited on the surface. After wiping with clean cloth, the incrustation which was removed from the green twigs and carefully freed from plant-debris, provided the starting material for all subsequent investigations.

The incrustation (20 lbs.) was crushed in a porcelain end runner and extracted with 10 litres of 0.9 per cent. sodium chloride solution. The decanted liquid was passed through a cloth filter and the residue pressed in a hydraulic press at 2 tons to the square inch to recover as much of the extract as possible. On centrifuging at 3,000 R.P.M., the combined filtrates separated into three distinct layers:— (1) the uppermost layer consisting of scum which could be easily skimmed, (2) the middle layer, a thick, fairly clear scarlet fluid and (3) the residues comprising the chitinous debris of the insect and the finer particles of lac incrustation escaping the cloth filter.

The butter-like insect fat was easily separated, the middle layer carefully decanted and the residues kept separately. The fat was desiccated *in vacuo* over calcium chloride and subsequently purified by extraction with carbon tetrachloride forming a pale yellow solid.

The middle portion was filtered with considerable difficulty through fluted filters in the cold room at 0°. and the filtrate (about 10 litres) divided into three portions. The first portion was saturated with magnesium sulphate, and the second treated with ammonium sulphate to half saturation. In these cases small quantities of precipitate were obtained having nitrogen values of 7.6 and 7.5 per cent. respectively. On heating to 90°, the third portion did not yield a precipitate and was therefore treated with a few drops of acetic acid, the precipitate thus obtained containing 8.7 per cent. total nitrogen. The following is a schematic representation of the treatments.

Incrustation (20 lbs.)

Treated with 10 litres of sodium chloride (0.9 per cent.)

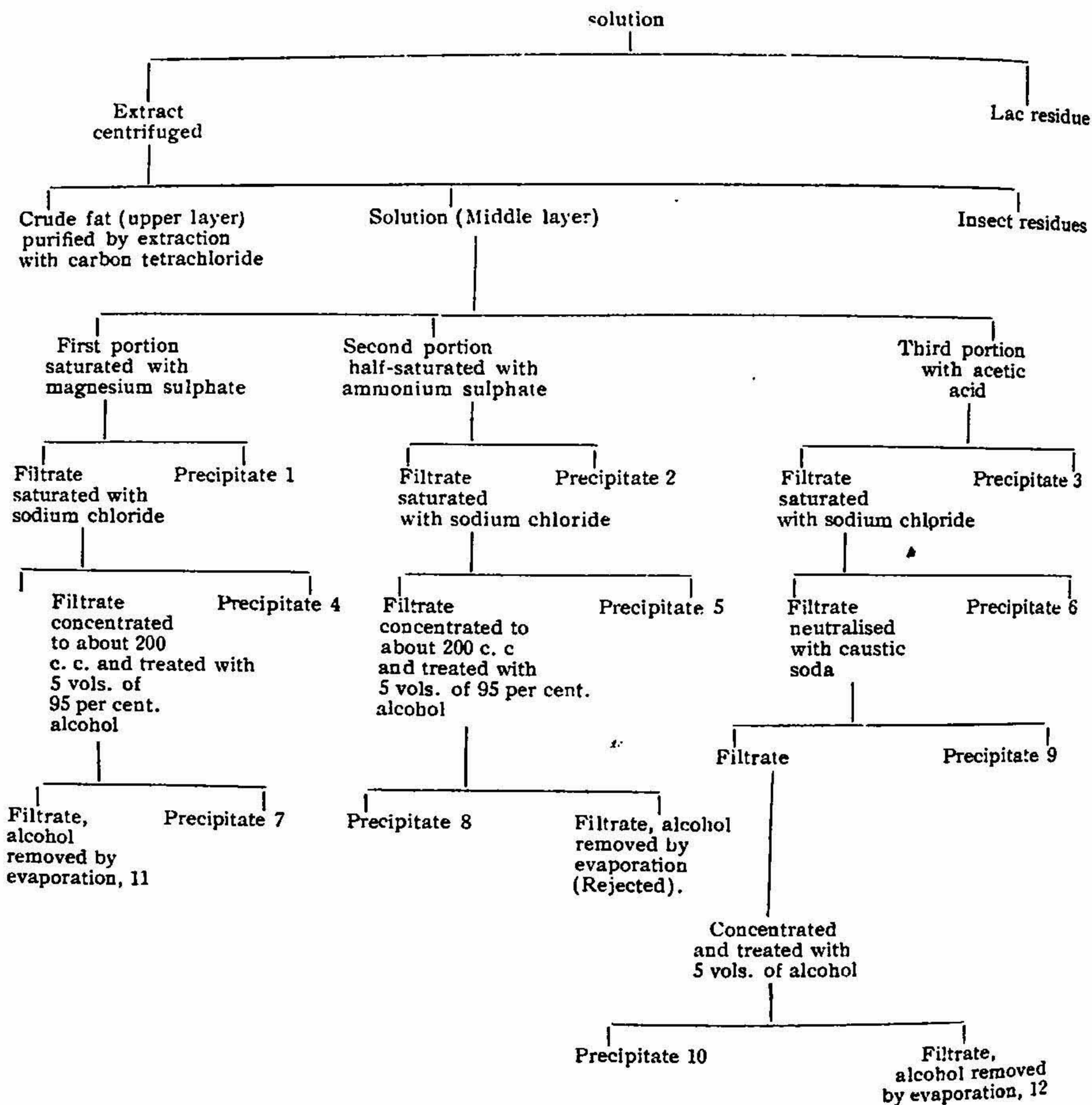


Table I gives the general appearance and characteristic properties of the above twelve fractions.

TABLE I

Fraction No.	Wt. of product gms.	Appearance	Nitrogen value per cent.	Other properties
1	1.0	Brick red powder	7.6	Amorphous
2	1.2	do.	7.5	do.
3	0.8	Dark red	8.7	do.
4	3.0	do.	2.72	Amorphous, answers biuret and α -naphthol tests
5	...	Light pink	...	Crystalline, ammonium sulphate
6	4.0	Dark red	3.34	Answers biuret test
7	6.0	Flesh colour	...	Highly hygroscopic, does not reduce Fehlings' solution answers α -naphthol test
8	...	Light pink	...	Crystalline, ammonium sulphate
9	5.0	Bright pink	0.00	Contains no nitrogen
10	7.0	Light brown	0.70	Soluble in water, answers α -naphthol test
11 & 12	250 c.c.	Scarlet liquid	11.0 (on the ash-free total solids)	

It will be seen from Table I that the three albuminous precipitates 1, 2, and 3 obtained by the three different methods contain about the same percentage of total nitrogen. The protein exists in combination with the strongly acidic laccaic acid which is responsible for the deep red colour of the preparations. The substance precipitated by acetic acid has a higher nitrogen value of 8.7. When purified by dissolving it in dilute caustic potash and reprecipitating with acetic acid the product

gave a nitrogen value of 12.1 per cent. Further purification did not increase the nitrogen content.

The Hausmann numbers of the first, second and third fractions as also the final solution (11 and 12) have been determined with a view to finding out the proximate proportions of the basic and non-basic forms of nitrogen.

TABLE II

Fractions	Percentage of total nitrogen				
	1	2	3	4	11 and 12
Humin (insoluble) } ,, (soluble) }	2.08	2.02	1.94	5.24	5.65
			0.23	18.32	3.38
Amide ...	13.8	13.29	14.36	10.37	4.52
Basic ...	25.0	24.15	25.89	20.46	45.81
Non-basic ...	58.5	58.83	58.25	42.54	40.67
Total ...	99.38	98.29	100.64	96.93	100.03

Table II shows that the first three fractions have approximately the same composition, the fourth fraction on acid hydrolysis yields a high proportion of humin nitrogen due to the presence of carbohydrates as indicated by the α -naphthol test. The solution is characterised by a high percentage of the basic nitrogen.

The most interesting among the nitrogenous bodies which merited detailed investigation appeared to be the first three fractions representing the serum albumins of the lac insect and the residual serum (11 and 12) consisting of the simpler polypeptides. The third fraction, after purification as previously indicated, and the residual serum were subjected to Van Slyke analysis. In the solution the basic fraction was further investigated by Kossel's silver baryta method.

TABLE III

Forms of Nitrogen	Percentage of Total Nitrogen			
	fractions			Portamine-like substance from <i>Sardinia coerulea</i> (1)
	3	11 and 12		
Humin	2.83
Acid-insoluble	...	1.94	5.65	...
Acid-soluble	...	0.23	3.38	...
Amide	...	14.36	4.52	0.86
Basic	...	25.89	46.41	...
Arginine	...	8.09	21.02 20.31*	27.83
Histidine	...	5.99	23.70 23.28*	23.02
Lysine	...	11.81	1.69	5.48
Cystine	...	Nil	Nil	0.60
Non-basic	...	58.25	40.67	40.22
Total	...	100.67	100.63	100.84

* Kossel's method.

DISCUSSION.

Table III gives the results of analysis. The serum albumin has high proportions of basic and amide-nitrogen and in general corresponds in composition to the vegetable globulins. The serum-nitrogen not precipitated by saturation with any of the salts, consists of a series of lower peptones and polypeptides. The fact that this fraction contains 11.0 per cent. of nitrogen on the total solids, indicates that the nitrogenous bodies in solution are moderately pure. About 9 per cent. of the total nitrogen exists in the amino-form, which increases to 43 per cent. on acid hydrolysis. This small (less than five times) increase in amino-nitrogen shows that the nitrogenous materials in solution are not complex in structure and consist of a series of the simpler polypeptides. The free amino-nitrogen of gelatin is only 0.1 per cent. of

its total nitrogen ; on hydrolysis, however, the amino-nitrogen increases to about 60 per cent., i.e., 600 times.

The high arginine- and histidine-content of the serum is noteworthy, and ranks this complex with the protamine-like substance isolated by Dunn (*J. Biol. Chem.*, 1926, **70**, 697) from the testicles of *Sardinia coerulea*. Analytical data for this protamine are presented for comparison in Table III, showing close agreement between Van Slyke and Kossel methods with regard to the arginine and histidine-content. Fractionation of the serum with a view to isolating individual polypeptides is now in progress.

The fat.—The fat isolated during the process of centrifuging and purified by extraction with carbon tetrachloride yields a pale yellow solid, having the following constants :—Iodine value, 30.4 ; saponification value, 182 ; acid value, 5. It is soluble partially in 95 per cent. alcohol and completely in petrol ether or chloroform. It was separated into an alcohol-soluble and an alcohol-insoluble fraction, the latter being very similar to the lac wax of the incrustation. A detailed investigation of the fat is contemplated and meanwhile the product, which can be easily isolated from the washings of stick lac, has already been used in the preparation of shoe polishes, and finishes for furniture and automobile bodies.

SUMMARY.

1. A preliminary investigation of the physiological products of the lac insect, now running to waste, is presented in this paper. The importance of some of these products in Hindu medicine and their economic value are indicated.
2. A method for isolating the various fractions from brood lac is described in detail.
3. The nitrogenous constituents have been fractionated into an albumin, precipitable by acetic acid or by salt saturation, and a serum mainly consisting of the simpler polypeptides.
4. A Van Slyke analysis of the above two fractions has shown that the albumin is allied in composition to the vegetable globulins while the serum polypeptides have revealed the existence of protamine-like substances closely related to that isolated by Dunn from the

¹ Dunn, M. S., *J. Biol. Chem.*, 1926, **70**, 697.

testicles of *Sardinia*. Attempts to isolate individual polypeptides are in progress.

5. The isolation of the insect-fat by centrifuging, and its subsequent purification are described; the constants point to its being a mixture of a true fat and wax. A detailed analysis of this fat is contemplated.

*Department of Biochemistry,
Indian Institute of Science,*

Bangalore.

[Accepted, 29-3-33.]

PRINTED AND PUBLISHED
BY V. M. PHILIP AT THE DIOCESAN PRESS
POST BOX 455, MADRAS—1933. C8017