verified

Journal of the Indian Institute of Science
V-16A. Part 6. 1933
PP. 69–83

Price, Re. 1.] [*Vol. 16A, Part VI, pp. 69 to 83.*

JOURNAL

OF THE

Indian Institute of Science

CONTENTS

JOURNAL

OF THE

INDIAN INSTITUTE OF SCIENCE

Volume 1 included 21 issues during 1914-18. Thereafter one volume was published annually until 1925, when Volumes 8 (A) and (B), followed by 9 (A) and (B) (1926) and 10 (A) and (B) (1927) appeared.

VOLUME 11 (A)—1928.

Parts I—XIX.

VOLUME 12 (A)—1929.

Parts I—XX.

VOLUME 13 (A)—1930.

Parts I—XV.

VOLUME 14 (A)—1931.

Part I.—Studies on the chemical composition and physical properties of plant tissue fluids. Part I. Effects of age and environment on the tissue fluids of French beans (*Phaseolus Vulgaris*). By C. Narasimha Acharya and B. N. Sastri. Price, As. 12.

Part II.—The Photochemical Oxidation of Organic Hydrocarbons. Part II. Toluene, and the Oxidation of Benzaldehyde. By C. J. Kothari and H. E. Watson. Price, Rs. 1-4

Part III.—I. Cantharidin from *Mylabris Pustulata* Fb., India. By B. H. Iyer and P. C. Guha. II. The Fatty acids from oil of Cantharis (*Mylabris Pustulata* Fb., India) By B. H. Iyer and P. Ramaswami Ayyar. Price, Re. 1.

Part IV.—I. Amylase from Rice. II. Dialysis of Some Cereal Amylases. By Vinayak Narayan Patwardhan and Dattatreya Vishnu Karmarkar. Price, As. 12.

Part V.—Contributions to the Study of Spike-Disease of Sandal (*Santalum Album.* Linn.), Part XII. Ecology of Sandal. By M. Sreenivasaya and S. Rangaswami. Price, As. 8.

Part VI.—Studies in Electro-Culture. Part I. Introduction. By K. Venkata Giri, T. J. Mirchandani and V. Subrahmanyan. Studies in Electro-Culture. Part II. Influence of Electrical Treatment on the Germination of Barley and the Diastatic Activity of Malt. By K. Venkata Giri and V. Subrahmanyan. Price, Rs. 2.

Part VII.—The Origin and Nature of the Peaty Soils of Travancore. By T. R. Narayana Pillai and V. Subrahmanyan. Price, Re. 1.

Part VIII.—The Relation between Seeds and Micro-Organisms. By T. R. Sathe and V. Subrahmanyan. Price, Rs. 1-4.

Part IX.—The Reactivity of Dimethyldihydroresorcin. Part I. Condensation with Aromatic Aldehydes. By Gopâl Chandra Chakravarti, Haripada Chattopadhyaya and Prafulla Chandra Ghosh. Price. Re. 1.

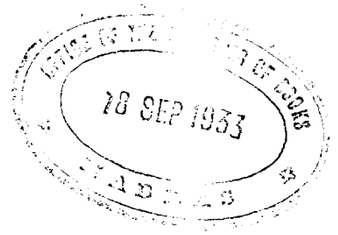
Part X.—The Reactivity of Dimethyldihydroresorcin. Part II. Behaviour towards Ortho-Nitro- and Ortho-Aminobenzaldehydes. By B. H. Iyer and G. C. Chakravarti. Price, Re. 1.

Price, Re. 1.] [*Vol. 16A, Part VI, pp. 69 to 83.*

JOURNAL

OF THE

Indian Institute of Science



CONTENTS

DILATOMETRIC STUDIES IN ENZYME ACTION. PART III.

Contraction constants of enzyme-substrate reactions.

By H. B. Sreerangachar and M. Sreenivasaya.

GLUCOSIDES.

The present communication deals with the hydrolysis of four well-known glucosides, amygdalin, arbutin, aesculin and salicin by emulsin, the reactions being conducted in the dilatometer described in an earlier paper (*J. Indian Inst. Sci.*, 1932, **15A**, 17), which gives the experimental procedure in detail. In the present study, the fall in the dilatometric column was measured at intervals ; the degree of hydrolysis was also determined by an independent analysis of the reaction mixture, 5 c.c. aliquots of which, were drawn from a control flask maintained for the purpose. The amount of sugar thus determined gives the amount of glucoside hydrolysed. The contraction constant per gram molecule of the glucoside can therefore be calculated from the quantity hydrolysed and the corresponding dilatometric depression observed.

EXPERIMENTAL.

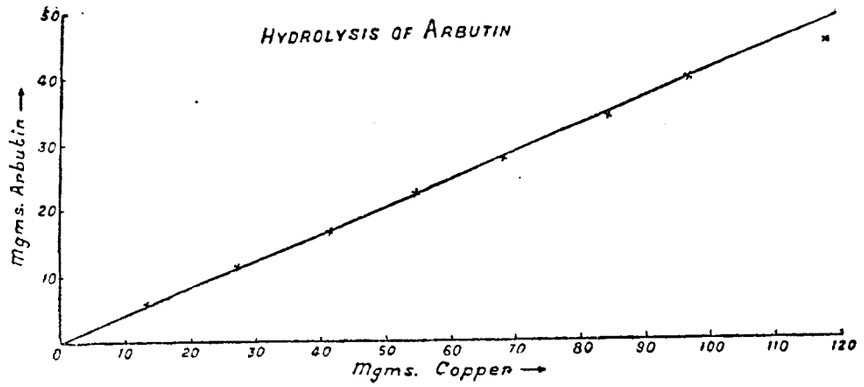
Mixtures containing 50 c.c. of about 1 per cent. solution of the glucoside dissolved in Sörensen's phosphate buffer (P 5.2) and 5 c.c. of 1 per cent. solution of almond emulsin (B.D.H.) were usually employed for the experiments. Aesculin and arbutin were preparations of Theodor Schuchardt, amygdalin, Kahlbaum's, and salicin, B.D.H.

Sugar formed in the hydrolysates was estimated by Bertrand's method. In the case of arbutin, however, the estimation had to be carried out immediately, since the hydroquinone accompanying the sugar in the hydrolysate became rapidly oxidised to quinone particularly in alkaline solutions. In calculating the amount of arbutin from the experimental copper value, the combined reducing action of sugar and hydroquinone have to be considered.

Copper values for mixtures of the two reducing constituents in the proportion in which they occur in arbutin were determined, and the values given in Table I are represented in the graph, from which, given the copper value of arbutin hydrolysate, the amount of glucoside hydrolysed can be directly read.

TABLE I

	1	2	3	4	5	6	7	8
Mixture, c.c. ...	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Copper reduction, mg. ...	13.8	27.1	41.4	54.7	68.5	84.8	97.1	118.3
Arbutin, mg. ...	5.6	11.2	16.8	22.4	27.5	33.5	39.1	44.7



Reduction values should be determined immediately after pipetting the arbutin hydrolysate or the artificially prepared sugar-hydroquinone mixture into the alkaline copper solution. Atmospheric oxygen quickly oxidises the hydroquinone in alkaline media as revealed by the following table, in which the results of back-titrating unused ferric iron according to the Bertrand method at different intervals, are given.

TABLE II

Time of estimation.	$KMnO_4$ ($N/10$) required in c.c.
Immediate.	5·95
After 1 hour.	4·70
After aeration for 1 hour.	2.85

TABLE III

Emulsin—amygdalin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Amygdalin hydrolysed in mg.	*Dilatometer readings* in cms.		Dilatometric depression
			Exptl.	Control	
23	28.6	165.8	2.8	16.7	0.0
46	47.0	330.3	2.8	16.7	0.0
78	51.6	363.8	2.8	16.7	0.0
135	59.8	424.5	2.8	16.8	0.0

TABLE IV

Emulsin—arbutin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Arbutin hydrolysed in mg.	*Dilatometer readings* in cms.		Dilatometric depression
			Exptl.	Control	
100	15·3	70.4	4·3	12.2	0.0
194	25.2	116.1	4·3	12.2	0.0
342	39.2	176.0	4·3	12.2	0.0
610	62.3	279.4	4·3	12.2	0.0
1230	83.8	375.1	4·4	12.3	0.0

TABLE V

Emulsin—Salicin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Salicin hydrolysed in mg.	*Dilatometer readings* in cms.		Dilatometric depression cms.	Contraction constants
			Exptl.	Control		
...	...	...	4.2	5·4	...	...
28	9.8	89.8	5.2	5·4	1.29	4.12
80	17.8	172.4	7.1	5·4	2.46	4.08
236	14.4	139.2	8.6	5·4	2.00	4.11

Since a preliminary study of the emulsin-aesculin system revealed no volume-change while the hydrolysis proceeded to the equilibrium point, no contraction constant can be obtained for this system.

DISCUSSION.

Of the four glucosides, the hydrolyses of which have been dilatometrically investigated, salicin is the only one which gives a depression and has a contraction constant. The absence of any volume-change during the enzymatic splitting of the other three glucosides is noteworthy.

To eliminate the possibility of counter volume-changes which might accompany the solution of the reaction products in petroleum, experiments were conducted with the old type of instrument which eliminates the use of the solvent. Neither this gave any depression, showing that the factor of solubility in petroleum does not affect the result. Attention should be drawn to the fact that the volume-change measured in the dilatometer is the net result of a series of physical and chemical changes accompanying the hydrolysis, such as the solubility of the hydrolytic products in the reaction media, the disappearance of water during hydrolysis, and the configurational readjustment of the new molecules resulting from hydrolysis. It is difficult at this stage to apportion the change to each of the above factors, but an attempt will be made on a future occasion when a greater variety and number of substances would have been investigated.

SUMMARY.

The hydrolysis of four glucosides, aesculin, amygdalin, arbutin and salicin by emulsin has been investigated in the dilatometer.

Emulsin-salicin gives a contraction constant of 4.1 while other systems do not reveal any volume-change during hydrolysis.

POLYSACCHARIDES.

A dilatometric study of the enzymatic hydrolysis of the colloidal polysaccharides starch and glycogen is described in the present communication. As previously mentioned (*J. Indian Inst. Sci.*, 1932, **15A**, 17) the contraction constant is expressed in cubic centimetres per 100 grams of the colloidal substrate. The preparation of solutions of these collodial substrates under standard and reproducible conditions is of great importance in these experiments. For example, the hydration of a given starch is regulated by hydrogen ion, the presence or absence of salts, and temperature. Since this hydration itself is accompanied by certain volume changes, the starch solutions were kept in the thermostat for 19 hours, so that the hydration equilibrium was attained before mixing the substrate and the enzyme.

EXPERIMENTAL.

Lintner's soluble starch (B.D.H.) was used throughout the experiments : glycogen was a Pfansteil's c.p. sample. They were subjected to the action of four different diastases, widely varying in origin. Pancreatin and malt diastase were Merck's preparations ; ptyalin was prepared in the laboratory, and Taka-diastase was a product of Parke Davis.

Except in the case of glycogen, all hydrolyses were conducted at four different concentrations of the substrate. Completion of the reaction was determined by the dilatometric column reaching a steady

value, usually in the course of 4 to 5 hours. The degree of hydrolysis was independently determined by estimating the reducing value of the hydrolysate by Bertrand's method. For both series of studies, the respective optimal reactions were maintained with Sörensen's phosphate buffer :—Pancreatin, 7.0 ; ptyalin, 6.6 ; malt and Taka-diastase, 5.3.

TABLE I

Starch

Substrate concentration per cent.		0.25	0.50	1.0	2.0	Average constant
Pancreatin	Observed depression in cm.	0.65	1.45	2.7	5.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	8.9	17.2	34.3	73.8	
	Contraction constant	0.64	0.71	0.66	0.71	0.68
Ptyalin	Observed depression in cm.	0.7	1.6	2.65	5.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	...	...	41.34	80.14	
	Contraction constant	0.69	0.65	0.65	0.71	0.67
Diastase	Observed depression in cm.	0.8	1.60	3.2	6.5	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	10.8	...	38.2	85.2	
	Contraction constant	0.79	0.79	0.79	0.79	0.79
Taka-diastase	Observed depression in cm.	1.6	3.15	6.25	13.0	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	16.5	40.55	67.4	136.1	
	Contraction constant	1.57	1.55	1.55	1.59	1.57

TABLE II

Glycogen

Substrate concentration per cent.		0.25	0.5	Average constant
Pancreatin	Observed depression in cm.	0.45	0.95	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	6.1	11.8	
	Contraction constant	0.44	0.47	0.45
Ptyalin	Observed depression in cm.	0.85	1.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	6.6	13.8	
	Contraction constant	0.83	0.88	0.85
Diastase	Observed depression in cm.	0.55	1.1	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	7.7	15.3	
	Contraction constant	0.54	0.54	0.54
Taka-diastase	Observed depression in cm.	1.5	2.9	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	23.0	42.4	
	Contraction constant	1.47	1.43	1.45

DISCUSSION.

Tables I and II reveal that highest depressions are obtained with Taka-diastase at all concentrations of the substrates tested. The highest degree of hydrolysis is brought about by Taka-diastase as indicated by the corresponding copper values also. A strict proportionality (within reasonable limits of experimental error) is maintained between the various concentrations not only with respect to dilatometric depressions but also with regard to the copper values of the hydrolysate. The contraction constants of each system obtained at various concentrations of the substrate show good agreement and therefore these constants can be used for the estimation of starch or glycogen. Taka-diastase, giving the highest constant, should prove most useful in the analytical dilatometry of starch or glycogen.

The results show that the degree of hydrolysis of polysaccharides can be followed in the dilatometer, although it may be difficult to follow the disruption of the molecule; there is no doubt that each of these diastases act differently on starch. We are dealing here with a complex substrate consisting of amylopectin and amylose, the former according to Pringsheim being closely related to glycogen. The enzyme preparations represent a mixture of several saccharifiers and one liquefying component. Therefore a detailed study of this system by effecting a separation of the components of the substrate and enzyme systems is being made.

SUMMARY.

1. Starch and glycogen in concentrations of 0.25, 0.5, 1.0 and 2 per cent. have been separately subjected to the action of four diastases differing widely in origin, pancreatin, ptyalin, malt and Taka-diastase, in the dilatometer.

2. Contraction constants for each of these eight systems have been determined and the possibility of their being employed for the dilatometric estimation of starch and glycogen with particular reference to Taka-diastase, has been indicated.

Department of Biochemistry,

Indian Institute of Science,

Bangalore.

[Accepted, 29-3-33.]

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 powered 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.

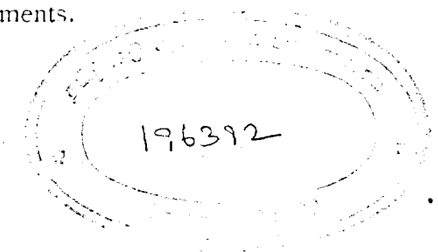
The brood lac employed in these studies was obtained from one of the plantations at Devarabetta, North Salem, three weeks before

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.)

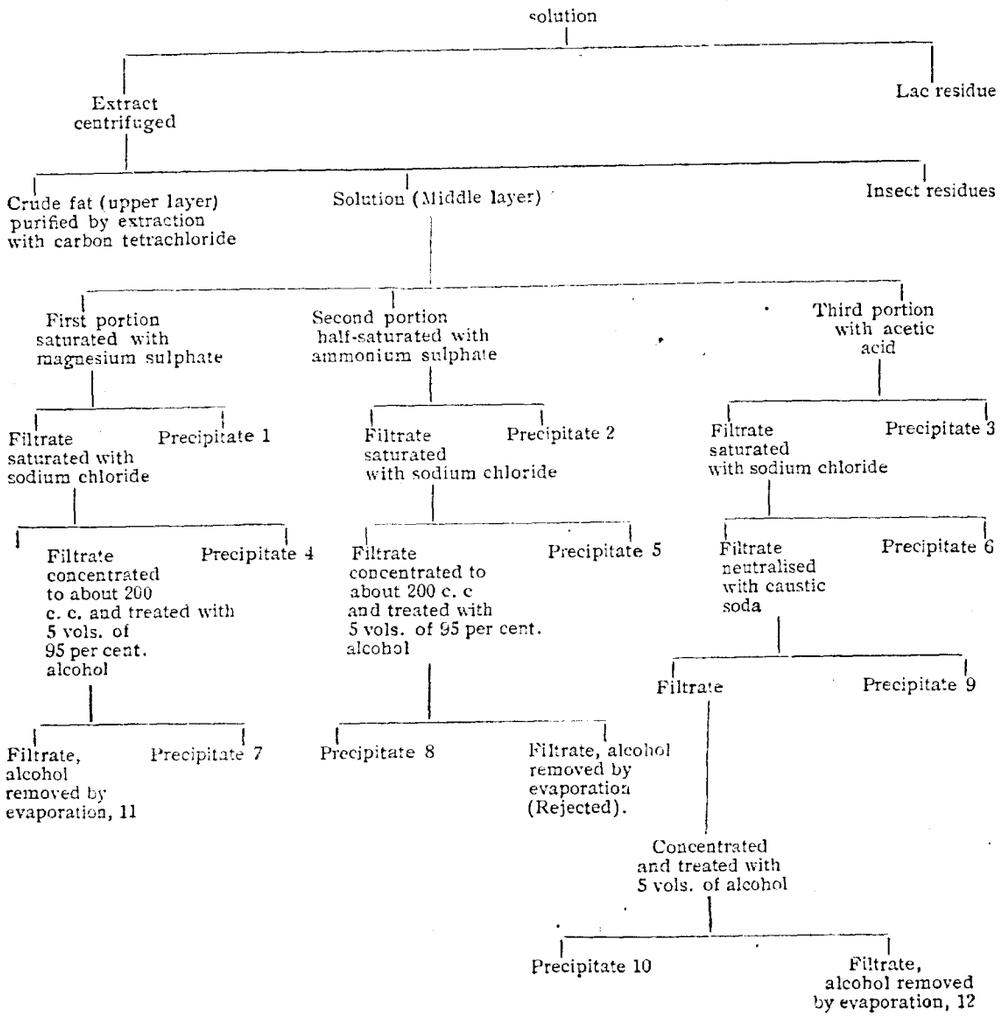


Table I gives the general appearance and characteristic properties of the above twelve fractions.

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) }	2.08	2.02	1.94	5.24	5.65
,, (soluble) }			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 *Sardinia coerulea* (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 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.

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

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

VOLUME 15 (A)—1932.

Part I.—Salt Stains on South Indian Hides and Skins. By V. N. Patwardhan and M. Subramania Sastry. Price, As. 12.

Part II.—Chemical Examination of the Root-Bark of *Plumbago Rosea*, Linn. By M. C. Tummin Katti and V. N. Patwardhan. Price, As. 8.

Part III.—Dilatometric Studies in Enzyme Action. Part II. Contraction Constants of Enzyme-Substrate Reactions. By M. Sreenivasaya and H. B. Sreerangachar. Price, As. 12.

Part IV.—Aryl Di- and Poly-Stibinic Acids, Distibinous Oxides and Distibino-Compounds. By Sohrab M. Mistry and Praphulla Chandra Guha. Price, Re. 1.

Part V.—Bhadravati Wood-Tar and its Utilization. By Y. K. Raghunatha Rao, B. Sanjiva Rao and H. E. Watson. Price, Re. 1.

Part VI.—The Equilibrium between Dimethyl Ether, Methyl Alcohol and Water. By N. G. Gajendragad, S. K. Kulkarni Jatkar and H. E. Watson. Price, Re. 1.

Part VII.—Studies in Indian Essential Oils. I. Essential Oil from Leaves of *Litsæ Zeylanica* Linn. By B. Sanjiva Rao. II. Essential Oil from Flower-Heads and Stalks of *Andropogon Kuntzeanus*, Hack, *Var. Foveolata*, Hack. By B. Sanjiva Rao. III. Essential Oil from Leaves of *Thymus Serpyllum*, Linn. By Jagjit Singh and B. Sanjiva Rao. IV. Essential Oil from Leaves of *Cinnamomum Zeylanicum*, Breyn. By (the late) V. P. Shintre and B. Sanjiva Rao. Price, Re. 1.

Part VIII.—Organic Manure from Sewage, Town Refuse and Waste Vegetation. By J. Jagannatha Rao and V. Subrahmanyan. Price, Rs. 1-8.

Part IX.—I. Studies in Enzyme Action. Part V. Ageing of Amylases in Aqueous Solution. By K. Venkata Giri and V. Subrahmanyan. II. Studies in Enzyme Action. Part VI. Heat Inactivation of Pancreatic Amylase. By K. Venkata Giri. III. Studies in the Proteins of Indian Foodstuffs. Part IV. The Proteins of Fenugreek (*Trigonella Foenum Graecum*). By Y. V. Srinivasa Rau and M. Sreenivasaya. Price Rs. 1-8.

Part X.—Studies in Bridge Formation. Part I. Attempts to Synthesise Bicyclic Terpene Derivatives by a New Method. By P. P. Patel and P. C. Guha. Part II. Formation and Stability of a Bridged *cyclo*Hexenone, 1—Dimethylethane—$^{1:2:2}$ III $^{1:4:4}$ *cyclo*butane-2-one. By P. S. Mayuranathan and P. C. Guha. Price, Re. 1.

VOLUME 15 (B)—1932.

Part I.—A New Permeameter. By K. V. Karantha. Price, Rs. 1-8.

Part. II.—Measurement of Power Factor and Loss in Dielectrics. By T. J. Mirchandani, G. Yoganandam, S. K. Roy and N. V. Narayanaswami. Price, Rs. 1-4.

Part III.—Transients in Negative Constant Series Circuits. By L. C. Verman. Price, Rs. 1-4.

Part IV.—Telephony by Carrier and One Side Band. By S. P. Chakravarti. Price, Re. 1.

VOLUME 16 (A)—1933.

Part I.—Rhaponticin, and Anthraquinone Derivatives from *Rheum Emodi*, Wall. (Indian, or Himalayan Rhubarb). By M. Ghouse Mohiuddin and M. C. Tummin Katti. Price, As. 12.

Part II.—Constitution of the So-Called Dithiourazole of Martin Freund. Part V. Isomerism of Hydrazodithiodicarbonamides, Iminothiolthiobiazoles and Di-R-Iminothiobiazoles. By Shaha L. Janniah and P. C. Guha. Part VI. Isomerism of Hydrazomonothiodicarbonamides, Iminothiobiazolones and Monothiourazoles. By S. L. Janniah and P. C. Guha. Price, Re. 1-4.

Volume 16 (B)—1933.