Effect of deproteinizing agents on biochemical variability of total protein in *Cotugnia digonopora*

Waghmare S.B.*, Padwal N.D. and Jadhav B.V.
*Helminth Research Lab, Deptt of Zoology, Dr.B.A.M. University, Aurangabad-413004
Email- sachinash_red@yahoo.co.in, baba_v_jadhav@yahoo.co.in

Abstract- Biochemical profiles are subject of variation. Physiological state of the host exhibits profound influence on biochemical individuality of parasite. Methods for various biochemical assays which have been described in many journals are mainly pertaining to mammalian model. The same may not be good for other animal model particularly for helminth parasites. In view of above mentioned facts there is a need to evaluate various existing methods and also to modify the method suitably, to cope up with complexities of biochemical profiles of helminth tissue. The present investigation is planed to evaluate a suitable method related to estimate the total protein quantitatively in the cestode *Cotugnia digonopora*. Therefore in present investigation is aimed to know the biochemical variability of certain selected biochemical assay related to total protein of cestode *Cotugnia digonopora* occurred in domestic fowl *Gallus gallus domesticus* in Maharashtra, India. In the light of present findings it would seem to logical to recommend that a Biuret assay for the cestode parasites, when compared to Folin-Phenol method.
Key Words- Biochemical variability, *Cotugnia digonopora*, Deproteinizing agents

Introduction

Survey of literature on various aspect of biochemistry of parasites reveals that they exhibit intraspecific variation in biochemical variation. Observation recorded on the starvation of the host result in drastic decrease of polysaccharide in *Raillietina* Ried, 1942. Overall survey of literature reveals, the biochemistry of parasites would exhibit a remarkable intraspecific variability in biochemical composition. Critical assessment of factors, which responsible for biochemical variability of parasite is necessary, in order to understand physiological role of the biochemical components in the host parasite relationship. Halberg (1973) classified factors influencing individual biochemical variability as interindividual and intraindividual. William (1956) while studying biochemical individuality of organism has pointed out that most of data showing biochemical variability could be explained in terms of poor performance of method used in collection of data. Therefore, he suggest that before interpreting any individual variation in the biochemical compounds, the result of the repeated sample, has to be analyzed from the same individual. Precision and accuracy are the two aspects connected with reliability (Strabel, 1965). Precision is a measure of degree of reproducibility of biochemical measurement. This also depends on selecting suitable method for biochemical analysis. No attempts were made on to record such variation previously. Therefore it is very essential to see the reproducibility of the result on the various conditions of the same tissue, prior to interpretation of result. The various condition include fluctuations in functional efficiency of instrument and also time of reaction, quantitative and qualitative proportions of the reagent used in reaction mixture so on so forth.

Material and Methods

Cotugnia digonopora (Pasqual, 1880; Diamare 1893) is a poultry cestode, parasitizing in the country fowl, the *gallus domesticus* .For present study intestine of domestic fowl were collected from local commercial market at Aurangabad. These intestines were brought to laboratory and examined for parasitological study. The *Cotugnia digonopora* alone were separated for the study. *Cotugnia* were washed several times with chilled saline water to remove the adhering mucous The whole worm were transferred to Whatman filter paper No. 1 to remove an adhering moisture .To observe the biochemical variability of total protein the fresh and whole parasites were used with Folin-Ciocalteu method by Lowry's et, al 1951, Biuret method and ultraviolet absorption of protein method. The further analyses were made by using different precipitating agents, and time taken for the formation of optimal color of reaction was also observed.

Assaying Technique and Deproteinizing Agent

To carry out the biochemical estimation in biological extracts, it is necessary to remove the protein from extract, which interfere with many chemical reaction of analysis Separation of proteins from biological tissue is done on chemical basis by electing the chemicals which can disturb the normal relation of protein with other components of the tissue. Many chemical substances are used to precipitate the protein in biochemical analysis choice of selecting deprotenizing agent depends on several factors. An usually Precipitation of protein were done by three method 1)By heavy metals like $HgCl_2$, $AgNO_3$, $CuSO_4$ etc 2) Certain Acids and alkaloid reagents like TCA, picric acid, phosphotungstunic acid , tannic acid etc 3)By organic solvents like alcohol and acetones. Variability in biochemical composition quantitatively depends on the method employed for the estimation of biochemical components. Observation shows remarkable inter individuality occurring among the helminthes parasites (Fairbairn, 1958).

Results

I) Results of Folin-Phenol assay

The protein were estimated by Folin phenol reaction by Lowry's et.al; (1951) in *Cotugnia digonopora* The reaction was conducted with the protein extracted by various precipitating agents presented in Table 1 and Fig 1.The optical density was read at the intervals of 5 min. The precipitating agents include 5 % TCA, 10 % TCA, Ethanol, 5 PCA, 10 % PCA, 5 % TA, and 10 % TA. The protein values were found high in ethanol extracted samples (82.527 ± 2.33). The lowest values were obtained in 5% Tungstic acid (8.059 ± 1.10). The protein levels are in the order of Ethanol(82.527 ± 2.33), 10 % TCA 62.986 ± 1.66, 5 % TCA 28.5.9 ± 2.50, 10 % PCA 15.185 ± 2.48, 10% Tungstic acid 12.546 ± 1.51, 5 % PCA 10.300 ± 2.10 and 5 % Tungstic acid 8.059 ± 1.16 mg, Protein /gm fresh weight. The values are statistically significant. The completion of reaction was assessed by reading the optical density at regular intervals of 5 min. The optimal color formation was found at the end of first 5 min. Thereafter the color faded gradually. Influence of deproteinizing agents in Folin-Phenol reaction (Table 1) and time dependent optical densities are given in (Table 2).

II) Results of Biuret assay

The proteins were estimated by Biuret method (Gornall et, al; 1949) in *Cotugnia digonopora*. The reaction was conducted with the protein extracted by various precipitating agents and the results are given in table 3. The optical density was read at the intervals of 5 Min. The precipitating agents used include 5 % TCA, 10 % TCA, Ethanol, 5 % PCA, 10 % PCA, 5 % Tungstic acid, and 10% Tungstic acid. The protein values were found high in ethanol extracted samples. The lowest was obtained in the 5 % Tungstic acid. The protein values are: 5 % TCA 87.563 ± 2.08, 10 % TCA 133.66 ± 1.41, Ethanol 150.60 ± 1.08,5 % PCA 19.533 ± 1.13, 10 %PCA 35.980 ± 0.80, 5% TCA 16.323 ± 1.53,10 % TA 27.838 ±1.94 mg of protein/gm fresh weight (Table 3). The values are statistically significant. The completion of reaction was assessed by reading the optical density at regular intervals of 5 min. The optimum colour was found at 30 min; the color remained same. The intensity of the color gradually increases up to 30 min and maintained a uniform value from the 30 min onwards. Results were tabulated in (Table 4).

Discussion

The Folin-Phenol reaction described by Lowry's et, al: (1951) is generally used for the extraction of protein, when protein are found in micro quantities. The values obtained by this procedure in various parasitic helminthes were found to vary. The species specific difference was recorded in various helminth parasites. The quantities of protein *Cittotoina perplexa* 21 %, (Campbell 1960), *E.granulossus* 61 % (Agosin et, al; 1957), *M.expansa* 22 % (Campbell 1960), *R.cesticillus* 36 % (Ried, 1942), *T.taenaeformis* larva 27 to 29 % and adult 45 % (Von Brand and Bowman, 1961). Variation in the total protein content were found in different regions of fowl cestode *R.tetragona* .The highest amount was recorded in the immature proglotids, followed by mature and gravid proglottids (Madhava Reddy 1981). More or less similar observations were recorded in the parasite *Stilesia globipunctuata* (Patwari, 1982). It is clearly evident from the above mentioned results that the ethanol extracted proteins exhibiting significantly higher values when compared to the remaining deproteinizing agents, both in folin as well in Biuret assays. Further, it is also notices that the condition used in the

mammalian models particularly the incubation time of the reaction mixture is found to be very less in Helminth parasites. From this it appears that the observed specific variability may be due to variation in the incubation timings. When two methods are compared (Tab-5), Biuret assay did nor deviate much from mammalian system. But in terms of qualitative values, the Biuret method recorded high values than Folin Phenol method (Table 5: Fig 3).Often Folin-Phenol method is considered to be more sensitive and respond even to micro-quantity of proteins. Biuret reacts with nonprotein nitrogen, such as $CONH_2$ and may give higher values (Hawks 1954).When both the methods are compared with U.V. method which excludes non-protein nitrogen, protein values obtained by Biuret assay appears to be nearer to the U.V. method (table 5b). In the light of above findings it would seem to logical to recommend the Biuret assay for the cestode parasites, when compared to Folin-Phenol method.

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***Table 1**:-Influence of deproteinizing agents in Folin-Phenol reaction*

Sr.No	5% TCA	10% TCA	Ethanol	5 % PCA	10% PCA	5 % TA	10 % TA
1	24.125	61.258	77.213	8.124	11.526	7.118	13.256
2	27.165	63.125	84.123	8.752	14.985	9.523	11.263
3	28.952	64.856	81.956	9.215	12.452	5.963	12.564
4	27.256	62.984	84.568	10.856	14.254	9.123	8.564
5	29.452	60.589	82.3758	10.245	14.784	7.524	10.521
6	30.456	61.895	83.456	8.456	18.124	7.985	11.564
7	28.124	61.254	83.984	13.213	17.489	8.587	12.548
8	32.546	64.986	82.546	13.546	17.871	8.654	12.546
Mean	28.5095	62.61838	82.52773	10.30088	15.18563	8.059625	11.60325
S.D ±	2.504787	1.662904	2.338708	2.105879	2.480952	1.163652	1.510041

Values are expressed in mg of protein /gm fresh weight

***Table 2:-**Time variation in Folin-Phenol reaction*

Sr.No	Deproteinizing agents	1 min	5 min	10 min	15 min	20 min	25 min	30 min
1	5 % TCA	18.254	28.325	26.215	21.584	21.564	19.265	17.458
2	10% TCA	44.854	60.215	55.426	51.265	51.256	47.236	48.265
3	Ethanol	49.546	81.956	68.245	58.254	54.215	51.265	49.457
4	5 % PCA	7.652	11.235	11.265	8.265	8.265	8.457	6.254
5	10 % PCA	10.562	13.856	12.562	11.512	11.265	9.251	7.548
6	5 % TA	6.854	8.256	8.265	7.265	6.985	5.985	6.547
7	10 % TA	9.562	12.542	10.265	9.985	9.125	7.867	7.245

Values are expressed in mg of protein /gm fresh weight.

***Table 3:-** Influence of deproteinizing agents on Biuret reaction*

Sr.No	5 % TCA	10% TCA	Ethanol	5% PCA	10% PCA	5 % TA	10 % TA
1	90.102	135.258	152.541	21.548	36.548	16.548	28.656
2	85.265	135.541	150.254	18.564	38.547	14.658	30.564
3	86.245	133.547	151.654	18.652	36.541	15.654	26.548
4	89.235	132.541	150.254	20.541	34.587	15.624	24.658
5	84.254	134.235	149.548	20.23	35.548	14.658	27.154
6	87.874	134.215	149.457	19.321	34.658	16.325	30.254
7	88.548	131.541	150.245	19.325	35.865	18.652	27.215
8	88.985	132.451	151.548	18.245	35.547	18.468	27.658
Mean	87.5635	133.6661	150.6876	19.55325	35.98013	16.32338	27.83838
S.D. ±	2.080472	1.410394	1.087583	1.135221	0.804878	1.537926	1.949437

Values are expressed in mg of protein /gm fresh weight.

Table 4 *Time variation in Biuret assay reaction*

Sr.No	Deproteinizing agents	1 min	5 min	10 min	15 min	20 min	25 min	30 min
1	5 % TCA	20.754	22.235	25.698	27.598	30.254	50.654	88.654
2	10% TCA	24.985	28.658	30.265	31.584	41.254	112.654	130.587
3	Ethanol	23.265	58.632	81.548	84.564	90.325	130.265	151.548
4	5 % PCA	1.654	2.654	4.658	6.754	9.658	15.625	19.658
5	10 % PCA	2.658	6.985	10.265	13.785	14.985	25.654	34.658
6	5 % TA	1.895	3.658	5.625	8.125	9.788	12.547	15.898
7	10 % TA	4.589	6.325	7.985	10.235	15.485	20.215	27.584

Values are expressed in mg of protein /gm fresh weight.

Table 5 *Comparison between Folin-Phenol and Biuret reactions*

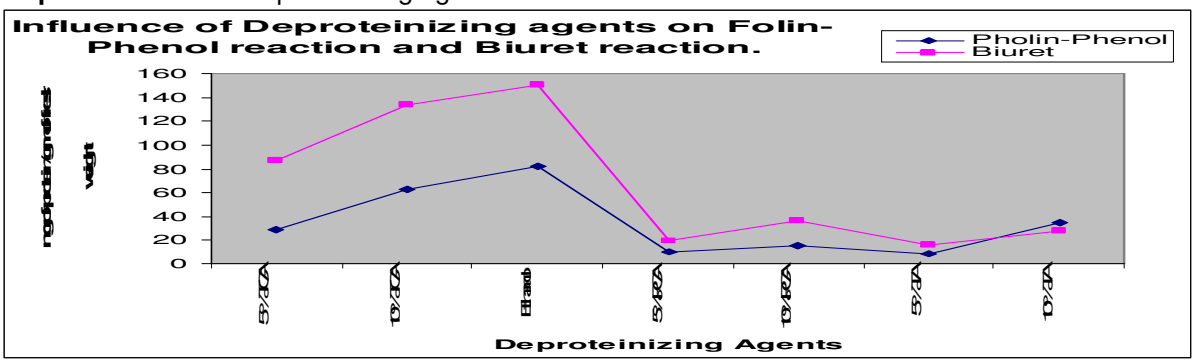
Sr. NO	Deproteinizing agent	Folin	Biuret
1	5 % TCA	28.509	87.5635
2	10% TCA	62.986	133.6661
3	Ethanol	82.527	150.6876
4	5% PCA	10.3	19.55325
5	10% PCA	15.185	35.98013
6	5 % TA	8.059	16.32338
7	10 % TA	12.546	27.83838

Values are expressed in mg of protein /gm fresh weight.

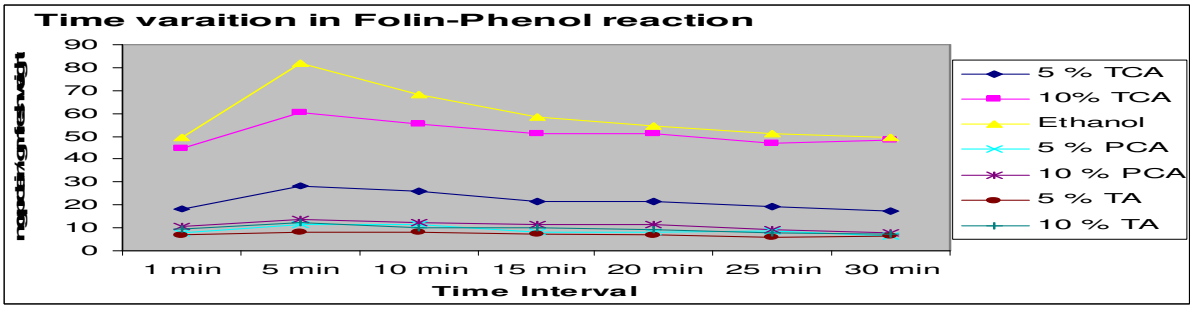
Table 5b*: Protein assay by U.V. method*

Sr.No	5 % TCA	10% TCA	Ethanol	5% PCA	10% PCA	5 % TA	10 % TA
1	90.548	132.265	156.254	27.235	41.325	20.325	33.325
2	90.658	132.256	154.236	26.325	42.325	21.321	32.362
3	89.362	131.254	155.236	25.325	40.235	23.021	33.021
4	89.265	133.265	153.583	26.325	42.215	18.362	31.251
5	90.251	132.265	154.235	25.326	40.325	19.325	32.265
6	91.236	131.652	153.265	26.254	40.321	18.325	31.251
7	91.235	133.265	154.251	25.213	42.325	20.325	31.254
8	90.215	131.214	155.669	26.265	42.321	20.321	35.214
Mean	90.34625	132.1795	154.5911	26.0335	41.424	20.16563	32.49288
S.D. ±	0.744846	0.780484	1.034158	0.696061	0.979138	1.554285	1.366133

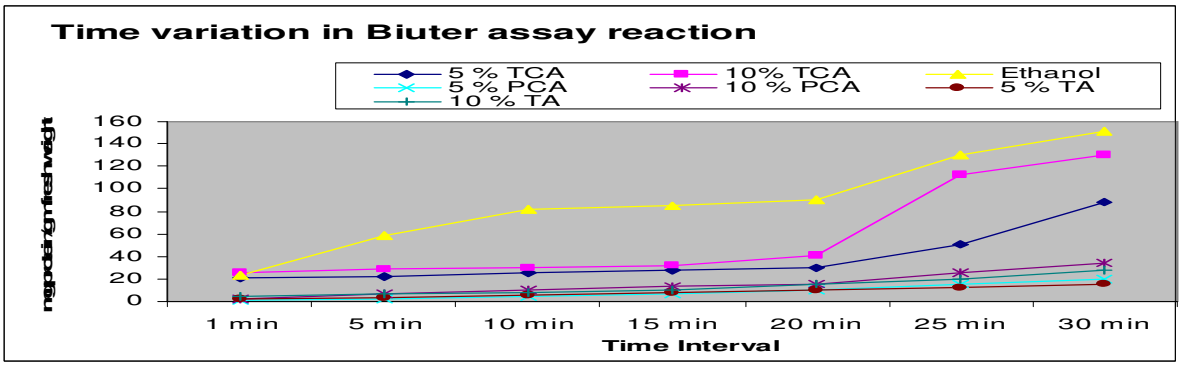
Graph-1 Influence of deproteinizing agents in Folin-Phenol reaction & Biuret Reaction



Graph- 2 Time variation in Folin-Phenol reaction



Graph-3 Time variation in Biuret assay reactions



Graph-4 Comparison between Folin-Phenol and Biuret reactions