ELECTROPHORETIC PROTEIN PATTERN DISTRIBUTION AMONG THE NORMAL ASSAMESE PEOPLE

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The dietic habits and the socio-economic conditions of Assam are different from the other parts of India; and it is expected that the total serum protein and its fractionation may differ slightly (or grossly) from the values of the total serum protein and its fractionations found in people residing in the other parts of India. Moreover, the available literatures show that Banerjee (1958) only studied the total serum protein, albumin, and globulin amongst 82 residents of Assam following the Greenberg's method in a photoelectric colorimeter. That is the only study done extensively to determine the normal serum protein range in Assam. None did any work to find out the electrophoretic distribution of the serum protein pattern in Assam; in fact a very limited work has been carried out in India to find out the normal protein pattern distribution studied electrophoretically. Satoskar *et al.* (1954), Kumar and associates (1957), Chatterjee and Banerjee (957), Patel and co-workers (1957) Kulkarni and others (1958) studied the electrophoretic protein distribution in the different parts of this vast Republic. The electrophoretic method of protein fractionation is gaining its importance in the clinical practice and unless one knows the normal pattern it is not possible to interprete the results in the pathological conditions. With this view in mind, the present study has been carried out in 80 healthy medical students between the age group of 19 to 22 years and in 25 healthy individual belonging to the low economic status of the society. The first group in the present study is the Group A and the second group is the Group B. All the 80 medical students are the students of the 2nd year class of this college and they have been thoroughly examined medically by the specialists at the time of admission. The individual of the Group B either worked in the Department of Biochemistry and Chemistry of this college either as a Laboratory Boy or as a Laboratory Assistant or as a boy in the Assam Medical College Library for last 3—4 years, and none of them have taken leave from the duty during this period for their physical disability; even then they have been examined medically following the plan of the Indian Council of Medical Research by the author (P. G.) and none of them have been found to suffer from any physical or mental disorder. The stool and urine examination of this group of people revealed nothing much abnormality except occasional

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presence of round worm ova in the stool, which is regarded not as a gross pathological condition. The age of this group varies from 15 years to 48 years.

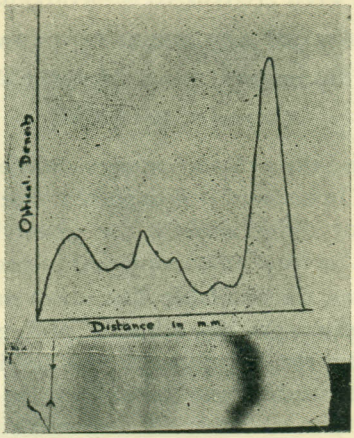

Fig. 1.

Method:—Electrophoresis of the sera was carried out according to Grassmann and Hannig (1952) at 110 volts for 12 hours in an open horizontal electrophoresis apparatus A sodium barbitone-sodium acetate buffer with pH 8·5 and ionic strength 0·1 was used. The filter paper (Schleocher and Schull No. 2043 a) strips after separation of protein were prepared, dried and stained with bromophenol blue (0·01%) following the conditions set up by Jencks, Jetton and Durrum (1955). The stained strips were oiled with mineral oil for 3 hours and then scanned in the Elphor scanner. Graphs were drawn and area found out by planimetry. The percentage of the different components are thus calculated out. The results tabulated in the Table No. 1 are the uncorrected results for the globulins as it has been observed that an arbitrary correction factor is not necessary (Goswami and Barua-1959). The effect of storage of the electrophoretic mobility of the different components of the serum protein has also been observed by us and an elaborate discussion of the same has been discussed elsewhere (Goswami and Barua-1959); but it is to be noted here that with a fresh serum it is possible to subfractionate the beta-globulin into $beta_1$ and $beta_2$-globulins. Similar subfractionation of the beta-globulin at pH 8·5 has also been observed by others with a fresh serum.

Results:—The results of the total serum protein determined by the Micro-Kjeldahl method and the electrophoretic fractionation of the serum protein have been tabulated in Table No. 1. The subfractionation of the beta-globulin into $beta_1$ and $beta_2$-globulin in the first 41 medical students has been possible because of the fresh sera.

TABLE No. 1

Group A

The total serum protein and the percent of the serum protein components studied by paper electrophoresis in 80 healthy medical students

Serial No.	Total serum protein in Gm. p. c.	Gobulin in percent of total protein					Albumin in Gm. p.c. of total protein
		Gamma	$Beta_2$	$Beta_1$	$Alpha_2$	$Alpha_1$	
1.	6·83	22·22	3·70	14·08	5·18	1·47	53·33
2.	7·53	19·77	3·49	11·62	6·98	2·33	55·69
3.	7·53	29·13	4·86	7·28	4·86	0·49	53·40
4.	7·31	23·69	3·95	10·53	7·90	1·32	52·64
5.	7·31	23·54	4·71	12·94	11·77	2·35	44·71
6.	7·31	28·81	5·09	15·25	8·47	1·69	49·18
7.	7·31	20·54	1·79	19·64	5·36	2·68	50·00
8.	7·07	21·33	2·70	6·75	4·05	1·35	63·85
9.	6·83	24·60	5·74	11·48	9·02	3·28	45·90
10.	6·37	25·40	3·80	10·13	4·97	1·27	54·43
11.	7·07	24·62	4·69	14·62	9·23	3·08	43·77
12.	7·53	18·52	3·70	13·89	7·41	0·93	55·55
13.	6·83	20·55	7·71	12·25	8·16	5·10	46·82
14.	7·53	21·95	7·20	10·20	9·98	3·66	47·24
15.	7·78	20·16	7·75	10·85	9·40	5·43	46·41
16.	7·78	14·81	7·41	10·81	9·63	5·93	51·41
17.	7·53	14·81	3·70	12·96	11·11	3·70	53·70
18.	7·53	20·31	5·18	10·76	7·69	3·85	52·22
19.	7·53	20·62	8·25	10·30	9·28	4·12	47·42
20.	6·83	16·98	8·51	10·64	6·38	4·26	53·19
21.	7·31	19·32	6·90	13·10	4·83	2·76	53·10
22.	7·53	15·79	3·95	6·58	2·63	2·63	68·42
23.	6·37	17·39	7·49	10·63	9·49	4·97	49·13
24.	7·31	13·48	4·49	12·96	10·48	2·25	51·94
25.	7·07	18·27	5·77	19·23	7·69	4·81	44·23
26.	6·83	27·46	3·92	6·86	4·90	1·96	54·91
27.	7·78	14·81	7·41	18·52	8·89	0·74	49·63
28.	7·78	29·70	6·25	14·06	3·13	1·56	45·32
29.	7·53	29·06	5·13	11·11	6·84	3·42	44·44
30.	7·31	19·54	2·87	16·68	9·20	2·30	49·40
31.	7·53	24·27	5·71	14·57	7·14	2·86	45·43
32.	7·53	22·41	3·85	15·38	4·81	1·92	51·89

Serial No.	Total serum protein in Gm. p.c.	Globulin in p.c. of total protein					albumin in p.c. of total protein.
		Gamma	Beta	$Beta_1$	$alpha_2$	$alpha_1$	
33.	7·31	18·68	5·69	15·78	9·89	4·40	45·57
34.	7·53	18·35	3·76	11·01	5·50	0·92	60·54
35.	7·07	21·15	4·81	12·50	9·60	1·92	49·98
36.	7·53	20·38	3·88	11·65	5·83	0·97	57·28
37.	7·31	27·83	5·69	15·65	5·22	1·74	43·87
38.	7·78	24·65	7·32	12·20	8·13	3·25	44·47
39.	7·31	15·54	6·90	10·94	9·19	4·60	47·96
40.	7·07	24·07	5·56	14·81	5·56	1·85	48·14
41.	7·53	22·24	2·47	12·35	7·41	1·24	54·37
42.	7·07	26·98	11·77	5·04	4·20		52.02
43.	7·78	24·78	10·47	6·14	3·19		55·40
44.	7·07	28·92	12·79	5·68	2·27		50·64
45.	8·01	19·22	16·55	8·91	3·00		52.45
46.	7·07	25·67	11·11	6·76	6·08		49·40
47.	6·83	24·68	15·19	10·76	6·33		43·03
48.	7·53	22·22	11·11	3·70	1·85		61·10
49.	7·07	26·37	14·29	8·79	4·40		46·15
50.	6·83	24·80	12·40	4·65	2·33		55·82
51.	7·07	28·30	13·61	7·08	2·04		49·99
52.	7·07	20·74	16·35	8·06	3·23		51·60
53.	7·31	27·93	11·93	6·57	1·79		51·80
54.	6·83	24·07	12·96	5·56	3·70		53·70
55.	7·07	25·48	16·13	10·29	1·61		46·48
56.	7·31	16·07	15·74	7·14	5·36		55·74
57.	7·07	23·08	14·23	8·54	3·85		50·32
58.	7·07	20·34	15·05	11·43	3·62		49·58
59.	6·83	25·30	10·21	8·16	5·44		50·90
60.	7·53	28·81	11·87	10·16	5·07		44·07
61.	7·53	23·67	12·93	9·98	5·92		47·51
62.	7·31	13·25	11·45	6·82	3·81		64·77
63.	7·07	14·55	10·91	9·09	3·64		61·67
64.	7·31	20·34	16·84	8·33	2·50		49·00
65.	7·53	19·62	18·62	8·11	3·60		49·84
66.	7·53	18·33	21·67	13·34	3·33		45·00
67.	7·31	24·63	7·75	1·55	0·78		65·12
68.	6·83	23·23	18·46	10·66	1·54		46·00
69.	7·78	26·67	15·55	6·67	2·22		48.90

Serial No.	Total serum protein in Gm. p.c.	Globulin in p c. of total protein				albumin in p. c. of total protein.
		Gamma	Beta	alpha$_2$	alpha$_1$	
70.	7·53	26·67	13·33	6·67	2·22	51·09
71.	7·53	23·26	14·64	6·98	2·33	55·82
72.	7·31	28·61	9·13	2·29	0·46	59·50
73.	7·07	25·58	13·33	10·00	4·44	46·67
74.	7·78	17·86	14·29	9·14	5·36	55·36
75.	6·83	25·62	12·16	5·41	2·70	54·05
76.	7·31	21·35	13·46	7 97	1·04	56·18
77.	7·53	17·91	10·45	8·97	2·99	59·70
78.	7.31	25·27	17·74	8·07	0·54	48·40
79.	7.07	27·59	7.94	3·97	0·79	59·52
80.	7.53	15·91	11·45	6·47	3·49	62·70
		Group B				
1.	6·49	36·98	11·77	5·04	4·20	42·02
2.	7·78	24·78	19·47	14·02	6·19	35·40
3.	7·66	36·92	12·79	5·68	2·27	42·34
4.	7·70	19·22	18·55	10·91	8·00	43·32
5.	7·10	25·67	14·19	6·76	6·08	49·40
6.	7·50	27·55	18·96	8·16	5·10	40·82
7.	7·30	24·68	15·19	10·76	6·33	43·03
8.	7·30	22·22	11·11	3·70	1·85	61·10
9.	7·60	26·37	14·29	8·79	4.40	46.15
10.	7·31	24·80	12·40	4·65	2·33	55 82
11.	7·78	31·30	13·61	4·0S	2·04	49·99
12.	6·84	17·74	13·95	8·06	3·23	51·60
13.	6·96	33·93	8·93	3·57	1·79	51·80
14.	6·84	24.07	12·96	5·56	3·70	53·70
15.	6·84	21·95	24·40	9 98	3·66	40·24
16.	7·54	20·16	18·60	12·40	5·43	43.41
17.	6·84	35·48	16·13	11·29	1·61	35·48
18.	7·07	14·81	22·22	9·63	5·93	47·41
19.	6·84	16·07	15·74	7·14	5·36	55·75
20.	6·84	14·81	16·66	11·11	3·70	53·70
21.	7·07	23·08	19·23	11·54	3.85	42·32
22.	7·78	33·34	19 05	11·43	7·62	28·58
23.	7·08	22·31	16·91	7·69	3·85	49·22
24.	7·07	31·30	10·21	8·16	5.44	44·90
25.	6·84	25·08	18·23	10·64	3·75	42·32

In the Table No. II the mean average of $beta_1$ and $beta_2$-globulin and also the "pooled mean value" of beta-globulin of 80 healthy medical students have been shown. In group B the subfractionation of the beta-components into two has already been pooled.

TABLE No. II

Mean average of the results in group A.

Total serum protein in Gm. p.c.	Globulin in per cent of total protein					albumin in p c. of total serum protein.
	γ	β_2	β_1	α_2	α_1	
Mean 7·27	22·25	5·28	12·52	7·46	2·93	51·72
S. D. 0·35	4·31	1·30	3·11	2·31	1·50	5·67
Variance 0·12	18·61	1·98	9·74	5·52	2·27	—
			Pooled beta globulin.			
Mean			11·14			
S. D.			3·33			
Variance			9·26			

Mean average of group B.

Total serum protein in Gm. per cent	Globulin in per cent of total protein				albumin in p.c. of total serum protein.
	γ	β	α_2	α_1	
Mean 7·20	25·38	16·03	8·43	4·30	45·99
S.D 0·35	6·42	3·78	2·95	1·40	7·26
Variance 0·11	42·45	14·29	8·69	2·02	52·64
	2·75	6·11	1·67	3·97	4·07

It is useless to compare the results of the distribution of the protein patterns observed by the different workers, unless one mentions the particular method of electrophoresis, the nature of the buffer solution, the staining procedure, even then, a comprehensive comparison of the results of the protein components is tabulated in Table No. III.

TABLE No. III.

Comparison of the results of the electrophoretic fractionation of the protein components in health.

Workers	Per cent of total protein					No. of cases studied.
	alb.	α_1	α_2	β	γ	
*Pluckthun and Gotting (1951)	59·0	4·2	8·0	10·6	18·0	15
Grassmann and Hannig (1952)	61·3	4·1	8·1	11·0	15·5	25
*Koiw and Others (1952)	72·9	1·4	3·5	8·6	13·6	10
*Gras	66·6	10·1	10·1	8·7	14·6	26
*Caspani & Bernasconi (1952)	62·7	3·9	7·5	10·7	14·9	11
*Goa (1952)	59·2	3·8	6·9	11·1	19·6	17
*Sommerfelt (1952)	55·4	4·4	8·1	10·0	22·2	13
*Brante (1952)	64·9	7·6	7·6	11·1	16·4	39
*Esser, Heinzler & Wild (1953)	59·0	4·3	7·7	12·5	16·5	44
*Antonini and Piva (1953)	55·7	4·0	7·9	12·6	19·5	15
*Knapp and Sieler (1953)	60·5	4·0	9·5	11·0	15·0	8
*Sonnet and Rodhain (1953)	61·7	4·3	7.5	10·2	16.3	13
*Bogdanowies, Osinski and Stein (1953)	61·9	4·2	8·0	11·3	14·6	25
*Tenks (1956)	68·9	10·2	10·2	9·0	12·0	—
*Levin (1953)	62·4	10·6	10·6	14·3	12·7	—
*Kaplan et al. (1957)	51·6	5·8	10·6	14·0	17·9	49
*Klatskin et al. (1956)	58·6	3.4	8·0	12·5	17·8	10
*Knights et al. (1957)	50·0	5·3	9·6	14·5	20·6	20
*Taylor (1949)	64·22	4·10	7·77	11-29	12·61	11
*Sterling (1949)	60·34	3·99	9.70	12·69	13·26	10
*Baptista (Plasma-1956)	60·64	3·92	7·09	11·61	11·61	—
*Keys (1950)	62·74	4·01	8·02	12·03	12·89	30
*Reiner et al. (1950)	56·0±2·8	7·2±1·3	8·8±1·9	13·1±2·5	14·7±2·6	80
*Cooper & Associates (1946)	67-0	3·3	9·1	12.5	14·9	13
*Portillo (1953)	62·3	6·5	4·5	10·5	15·2	18
*Gonzalez (1953)	57·6	4·40	9·16	11·44	17·44	15
*Lobo-Parga and co-workers (Chile- 1949)	57·6±0·6	7·4±0·4	7·4±0·4	14·3±0·4	20·7±0·6	25

TABLE No. III (continued)

Workers	Per cent of total protein					No. of cases studied
	alb.	α_1	α_2	β	γ	
*Edozein (Nigeria-1957)	49·26	2·94	7·50	9·41	30·88	50
Vera & Roche (Caracas-1956)	55·16	3·70	7·49	11·35	22·30	80
Kumar et al. (1957)	62·95	7·59	7·59	9·58	19·88	72
Satoskar & Lewis (1954)	58·24	2·46	4·15	10·44	24.75	100
Verghese et al. (1957)	54·0	5·3	8·3	12·0	20·3	20
Chatterjee & Banerjee (Plasma-1957)	56·44	13·50	13·50	9·36	16·91	22
Kulkarni, Sheth & Gokale (Plasma-1958)	61·31	2·05	5·89	8·91	15·91	187
*Syuni (Africa-1951)	49·09	11 61	11·61	14·17	25.13	—
This paper.						
Group A	51·72	2·93	7·46	11·14	22·15	80
	±5·67	±1·50	±2·31	±3·33	±4·31	
Group B	45·99	4·30	8·43	16·03	25 38	25
	±7·26	±1·40	±2·95	±3·78	±6·42	

*Not cited in References.

The highest values in Group A for albumin, alpha$_1$, alpha$_2$, beta (pooled) and gamma-globulin are 68·42, 6·33, 13·34, 18·62, 29·70, respectively, and the lowest values for the same components of the same group are 43·03, 0·49, 1·55, 4·72, 13·21. In group B, the highest values for albumin, alpha$_1$, alpha$_2$, beta, and gamma-globulins are respectively 61·10, 7·62, 14·16, 24·40, 36·98 and the lowest values for these components in order are 28·32, 1·61, 3·57, 8·93, 14·81. Like Vera and Roche (1956), in many instances particularly in group B the highest levels of the different components are "well above those generally reported", and even, the normal albumin and globulin ratio has been reversed; but there is no clinical manifestation of it.

Banerjee by Greenbergs technique obtained the mean values for total serum protein as 7·12±0·43 in summer season and 6·77±0·55 in winter season; and the serum albumin and globulin were found to be 4·4±0·38, 2·74±0·44 in summer and 4·19±034 & 2·62±0·48 in winter. There is a slight reduction of the total serum protein, serum albumin during the winter season.

DISCUSSION

The total serum protein in this investigation is more or less same as found by Banerjee (1958); the slight variation of the results of the present investigation from his results is not outside the experimental error and is due to the different techniques. The total protein determined by Chatterjee and Banerjee (1957) is slightly higher than the present investigation and most likely due to the fact that they determined total protein in plasma and not in serum. Our findings are identical also with the findings of Kumar *et al.* who have also determined the serum protein by the Micro-Kjeldahl method. The difference of the total serum protein in the medical college students and the doctors on one hand and the subjects belonging to the low economic status on the other is not significant in our investigation and also in the findings of Kumar *et al.* suggesting that the normal serum protein level cannot be taken as a measure of the nutritional status of any individual and any society. In the present investigation statistically significant difference has been observed between the two groups in case of beta-globulin, alpha$_1$-globulin and albumin, the calculated values in those instances are higher than the tabulated values of "t".

From the Table No. III, it has been seen that, in general, the albumin value is lower in country like India, Africa, Nigeria, Venezuala, Chile; though, Kulkarni and others observed a value equal to that of European countries. It has been suggested that the dietary habit of Indians might be a factor for the high gamma-globulin level among the Indians, in general, and this view has been supported by the Whipple's (1942) observation that the amino-acids from proteins of vegetable source mainly synthesise the gamma-globulin. The high gamma-globulin is not only observed in India alone but in other tropical countries like Africa, Nigeria, Caracas, Chile, and is explained therefore, as a greater immunity acquired consequent to repeated exposure to infection. This is only a supposition and is yet to be proved. The general health examination of both Groups A and B does not reveal any abnormality physically and mentally, but the level of the gamma-globulin often reflects the picture of an "old battle" of disease in the body; or it might be that there are some undetected abnormalities in the body and in that case it might be an acceptable explanation of this high gamma-globulin level in the tropics. Vera and Roche (1956) also studied the gamma-globulin level in 21 white and observed a lowered value than 52 meztizos and much below than the values of the gamma-globulin in Negroes. This led them to suggest that the per cent distribution of the gamma-globulin might have also some racial influence, and comparing the levels of gamma-globulin in the tropical countries with the other countries one cannot exclude the possibility.

SUMMARY

1. Total serum protein by microkjeldahl method and paper electrophoretic fractionation on 80 healthy medical students and 25 individual of lower

ncome group have been studied. There is not much difference of the values of the total serum protein between the two groups, but a statistically significant difference is observed in the values of beta-globulin, alpha$_1$-globulin and albumin.

2. The value of gamma-globulin found in the present investigation is higher than the levels or the gamma-globulin in the European countries, but a higher gamma-globulin level is observed in all the tropical countries, the explanation of which might be either (a) repeated exposure to infection or (b) probable racial difference. The levels of alpha$_1$, alpha$_2$-globulins also correspond to the findings of other tropical countries.

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