CHANGES IN HUMAN SERUM PROTEIN FRACTIONS WITH AGE AND WEIGHT[1]

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Abstract

Serum protein, serum cholesterol, and inorganic phosphorus measurements were determined for 160 adult males. Height, weight, and age data were also collected. The serum proteins were separated into six components in terms of their electrophoretic mobility: albumin, α_1 globulin, α_2 globulin, β_1 globulin, β_2 globulin, and γ globulin. Analysis of the results showed that serum globulin fractions and total serum globulin increase linearly with age ($P<.01$), and decrease as weight increases ($P<.05$). Total serum cholesterol, cholesterol ester, and free cholesterol were not related to serum globulin fractions. Inorganic phosphorus was negatively related to the globulin fractions ($P<.05$). Serum albumin did not change with age, weight, height, cholesterol, or inorganic phosphorus. Albumin and globulin fractions were significantly related to each other ($P<.01$).

Introduction

Research on the human serum proteins has been directed toward problems of physiological and biochemical interest as well as toward those of medical and genetical interest. From the physiological and biochemical point of view, the serum proteins play important roles as biological buffer systems, in the maintenance of the osmotic pressure of the blood, control of blood pH, transport of lipids, dietary nitrogen, and other inorganic elements, and in immunological and other functions (1, 2). Medical interest in the serum proteins has arisen because of the marked changes which occur during disease and other disorders, and their absence in some congenital diseases. Reduction of serum albumin has been observed in nephritis, liver cirrhosis, malnutrition, injury reactions, and edema, while serum globulins have been observed to increase in many diseases (3). The discovery of inherited peculiarities in protein synthesis has aroused the interest of geneticists (4).

The nomenclature of Tiselius for the serum proteins has been widely adopted, due partly to the increasing use of the electrophoretic method in research and in clinical evaluation (2). The electrophoretic method distinguishes protein fractions in terms of their mobility in an electrical field. A major distinction is made between albumin (molecular weight 69,000) and the globulins (average molecular weight 170,000 to 175,000). The globulins in turn are separated into α_1, α_2, β_1, β_2, and γ fractions according to their electrophoretic mobility (5). Functions characteristic of specific fractions include the role of albumin in maintaining the fluid balance of the body and the antibody function of γ globulin (3).

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Although the serum proteins have been studied extensively in the past decade, little is known of their interaction with each other and of the factors influencing their variability in healthy individuals. Published research reports do not indicate whether the protein fractions change with such physical factors as height, weight, or age. Information on these aspects should be of relevance not only to physiologists and geneticists but also to physicians using serum electrophoresis records for diagnostic purposes. The present investigation was designed with these considerations in mind. The primary purpose was to determine whether the physical factors of height, weight, and age have any relationship with the serum proteins in healthy individuals. Interrelationships among the serum protein fractions were also examined for the same individuals, and serum cholesterol and inorganic phosphorus data were collected.

Methods

Data were collected for 160 adult male Bengalis (residents of the State of West Bengal, India) selected by random sampling within age groups of employees of the Indian Statistical Institute. The subjects ranged in age from 20 to 69 years, were all engaged in office work, and were all nonvegetarians whose main source of carbodydrate was rice. Subjects came to the laboratory in the morning in a fasting condition (no meal since the previous evening) and venous blood was removed for cholesterol, inorganic phosphorus, and serum protein determinations. The venous blood was always taken between 9.30 a.m. and 10.30 a.m., and was immediately processed for the different estimations. Height (ft) and weight (lb) were measured and age was recorded at the same time.

Bloor's method was used to estimate total serum cholesterol in milligrams per 100 milliliters (mg%), and Bloor and Knudson's method was used to determine cholesterol ester in mg % (6, 7). Free cholesterol was calculated by subtracting the ester from total cholesterol.

The method of Fiske and SubbaRow was used to determine inorganic phosphorus in mg % (8, 9).

Serum protein fractions were determined by the agar electrophoresis method of Giri (10). The serum was prepared by clotting at room temperature for 15 minutes and then incubated for 10 minutes at 28° C, after which it was centrifuged at 2000 r.p.m. for 10 minutes. The agar plate was made by applying 15μl of the clear, fresh, unhemolyzed serum to a Whatman No. 1 paper and placing it at the center of the agar gel (ionic strength 0.05) on a glass plate (5.1 by 30.3 cm); the agar plate was then covered with another glass plate. The agar electrophoresis unit which was used was made by the Vega Corporation, Bangalore, India. The electrophoresis was carried out (210 volts, 4.6 ma) in a veronal acetate buffer (pH 8.6, ionic strength 0.05) for 6½ hours, after which the agar plate was dried and stained with naphthalene black (Amido Schwarz 10B manufactured by E. Merck, W. Germany) for 30 minutes and washed twice with methanol containing 10% acetic acid. Densitometric curves were obtained by measuring the color density of the protein fractions on the agar plate with the photovolt densitometer (Photovolt Corporation, New York). Relative concentrations of the protein fractions were estimated by measuring the area under the densitometric curves with a planimeter. The resulting measurements were in square centimeters. To evaluate the reliability of the serum protein measurement, two agar plates were prepared from the same sample of serum and the densitometric curves and measurements were made for each plate separately for 11 of the subjects. The correlations between the two sets of measurements were .85 albumin; .89 α_1 globulin; .97 α_2 globulin; .90 β_1 globulin; .96 β_2 globulin; and .96 γ globulin.

Results

The basic descriptive data are presented by age groups in Table I. The means of the fractions expressed as percentages of relative total protein (estimated electrophoretically) are as follows for the total group: 60.10% albumin; 3.06% α_1 globulin; 9.26% α_2 globulin; 6.75% β_1 globulin; 2.47% β_2 globulin; and 18.35% γ globulin. These values can be calculated for the different age groups from the data given in Table I by adding albumin and total globulin (the sum of α_1, α_2, β_1, β_2, and γ fractions) and taking the total as relative total protein.

The correlations between height, weight, age, and the serum protein fractions are presented in Table II. They show that height and weight are significantly correlated, but that other correlations between height, weight, and age are insignificant. The serum globulin fractions are highly intercorrelated, with an average intercorrelation of +.78. Height is negatively correlated with α_1 globulin ($P<.05$); weight is significantly and negatively correlated with α_2, β_2, and γ globulins and total globulin ($P<.05$); and age is significantly and positively correlated with all of the globulin fractions and total globulin ($P<.01$). Serum albumin is not significantly correlated with height, weight, or age, but is significantly correlated with all of the globulin fractions ($P<.01$), with an average correlation of +.51. As age has the most significant relationship, the age groups have been compared for each of the globulins by analysis of variance (11). Albumin has been similarly analyzed. The analysis of variance summary tables are given in Table III. They show that the unpartitioned variation between groups is significant for all of the globulin fractions and total globulin but not for albumin. They also show that the component of variation between groups due to the linear regression of globulin on age is highly significant for all of the fractions and total globulin ($P<.01$) but that the remainder component of between-groups variation is uniformly insignificant. To compare the relative contribution of height, weight, and age to serum albumin and globulin, multiple regression equations (12) are presented in Table IV. The standard regression coefficients show that age and weight have a significant effect on all of the globulin fractions but not

TABLE I

Means and standard deviations for height, weight, and serum protein, cholesterol and inorganic phosphorus measurements

Measurement		Age groups 20–29	30–39	40–49	50–59	60–69	Total
		n†					
		59	34	34	19	14	
Height (ft)	M	5.4254	5.4850	5.3935	5.3626	5.4493	5.4259
	s	0.2287	0.1689	0.2305	0.2729	0.1702	0.2226
Weight (lb)	M	119.2881	122.5882	126.3529	131.2105	116.3571	122.6500
	s	21.5644	25.6906	27.1400	28.4728	25.8751	25.3740
Serum albumin*	M	3.2810	3.1721	3.2856	3.5558	3.3079	3.2938
	s	0.4681	0.4414	0.4990	0.4933	0.4800	0.4854
Serum globulin α_1*	M	0.1463	0.1494	0.1668	0.2205	0.2357	0.1679
	s	0.0670	0.0642	0.0790	0.0929	0.0755	0.0797
Serum globulin α_2*	M	0.4832	0.4682	0.4594	0.6753	0.5957	0.5076
	s	0.2638	0.2802	0.2890	0.3468	0.2210	0.2893
Serum globulin β_1*	M	0.3436	0.3303	0.3568	0.4874	0.4479	0.3698
	s	0.1725	0.1990	0.2356	0.2213	0.1573	0.2048
Serum globulin β_2*	M	0.1181	0.1235	0.1344	0.1721	0.1879	0.1353
	s	0.0506	0.0796	0.0952	0.0800	0.0715	0.0772
Serum globulin γ*	M	0.8647	0.9409	0.9788	1.3237	1.3929	1.0059
	s	0.4591	0.4744	0.7385	0.7102	0.5405	0.6003
Total serum globulin*	M	1.9558	2.0124	2.0962	3.0095	2.8600	2.2019
	s	0.9936	1.0373	1.3484	1.3225	0.9458	1.1623
Total cholesterol (mg%)	M	177.2744	182.6926	193.6612	206.4242	176.3207	185.2861
	s	41.7269	41.9548	37.9666	40.4376	37.2419	41.6887
Cholesterol ester (mg%)	M	115.7317	112.0550	117.8206	130.1742	117.3193	117.2483
	s	33.1551	24.9441	23.3702	26.4421	28.8035	28.9092
Free cholesterol (mg%)	M	61.5427	70.6376	74.9582	76.2500	59.0143	67.8503
	s	30.7805	36.5437	30.1551	26.8912	29.5016	32.1122
Inorganic phosphorus (mg%)	M	3.6003	3.4488	3.5362	3.5863	3.8050	3.5708
	s	0.5900	0.6422	0.7084	0.6259	0.2917	0.6206

*Relative area under densitometric curve in cm^2. † *n* = number; M = mean; s = standard deviation.

TABLE II

Correlations between height, weight, age, and serum protein measurements ($N = 160$)

	Weight	Age	Albumin	Globulin α_1	Globulin α_2	Globulin β_1	Globulin β_2	Globulin γ
Height	.3956†	−.0575	−.0727	−.1632*	−.1533	−.1357	−.1438	−.1113
Weight		.0734	.0212	−.1350	−.1815*	−.1446	−.1676*	−.1892*
Age			.1002	.3662†	.1566*	.2791†	.2845†	.2917†
Albumin				.4522†	.5540†	.5538†	.4588†	.4977†
Globulin α_1					.7626†	.7410†	.7559†	.7448†
Globulin α_2						.8778†	.7644†	.7523†
Globulin β_1							.7316†	.7962†
Globulin β_2								.7846†

* $P < .05$. † $P < .01$.

TABLE III

Effects of age upon serum protein fractions: analysis of variance summary tables

Measurement	Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Albumin	Between groups	1.8826	4	0.4707	2.037
	Due to regression	0.4397	1	0.4397	1.904
	Remainder	1.4429	3	0.4810	2.082
	Within groups	35.8088	155	0.2310	
α_1 Globulin	Between groups	0.1563	4	0.0391	7.037†
	Due to regression	0.1340	1	0.1340	24.131†
	Remainder	0.0223	3	0.0074	1.339
	Within groups	0.8607	155	0.0056	
α_2 Globulin	Between groups	0.8095	4	0.2024	2.493*
	Due to regression	0.3529	1	0.3529	4.347†
	Remainder	0.4566	3	0.1522	1.875
	Within groups	12.5830	155	0.0812	
β_1 Globulin	Between groups	0.4474	4	0.1119	2.767*
	Due to regression	0.2839	1	0.2839	7.024†
	Remainder	0.1635	3	0.0545	1.348
	Within groups	6.2646	155	0.0404	
β_2 Globulin	Between groups	0.0866	4	0.0217	3.867†
	Due to regression	0.0763	1	0.0763	13.636†
	Remainder	0.0103	3	0.0034	0.611
	Within groups	0.8678	155	0.0056	
γ Globulin	Between groups	5.3592	4	1.3398	3.971†
	Due to regression	4.6257	1	4.6257	13.710†
	Remainder	0.7335	3	0.2445	0.725
	Within groups	52.3005	155	0.3374	
Total globulin	Between groups	23.6307	4	5.9077	4.309†
	Due to regression	17.3696	1	17.3696	12.668†
	Remainder	6.2611	3	2.0870	1.522
	Within groups	212.5171	155	1.3711	

* $P<.05$. † $P<.01$.

on albumin. The influence of age is relatively more than that of weight, and the two factors exert their influence in opposite directions. In order to estimate the relative amount of a protein fraction (Y'), given height, weight, and age, multiple regression equations are given. The multiple correlations are significant for all of the globulin fractions. The same analysis is given for albumin for comparison with the globulins.

Figure 1 presents photographs of the agar plates of three individuals representing three age groups, the twenties, the forties, and the sixties. The proteins appear in order from left to right, starting with albumin. Densitometric curves for these plates are given in Fig. 2.

Correlations between cholesterol measurements, inorganic phosphorus, and serum protein fractions are given in Table V. The correlations between the cholesterol measurements are +.63 between total and ester, +.62 between free and total, and −.0007 between free cholesterol and the ester. Weight is correlated .3373 with total cholesterol, .3881 with cholesterol ester, and .1850 with free cholesterol. These results are in agreement with those reported earlier (13, 14). No cholesterol measurements are significantly related to

TABLE IV

Standard regression coefficients, multiple regression equations, and multiple correlations of height, weight, and age on serum proteins ($N = 160$)

Serum protein	Multiple regression equations‡	Standard regression coefficients			Multiple correlation
		Height	Weight	Age	
Albumin	$Y' = 4.0734 - .1888h + .0009w + .0035a$	−.0866	.0486	.0916	.1285
α_1 Globulin	$Y' = 0.3076 - .0329h - .0004w + .0023a$	−.0919	−.1263	.3729†	.4106†
α_2 Globulin	$Y' = 1.1572 - .1037h - .0018w + .0037a$	−.0798	−.1622*	.1639*	.2594*
β_1 Globulin	$Y' = 0.6541 - .0585h - .0011w + .0046a$	−.0636	−.1408*	.2858†	.3298†
β_2 Globulin	$Y' = 0.2462 - .0214h - .0005w + .0018a$	−.0616	−.1652*	.2931†	.3463†
γ Globulin	$Y' = 1.2366 - .0314h - .0049w + .0143a$	−.0116	−.2076†	.3062†	.3604†
Total globulin	$Y' = 3.0940 - .1506h - .0089w + .0267a$	−.0288	−.1934*	.2953†	.3498†

* $P < .05$.
† $P < .01$.
‡ h = height in ft; w = weight in lb; a = age in years.

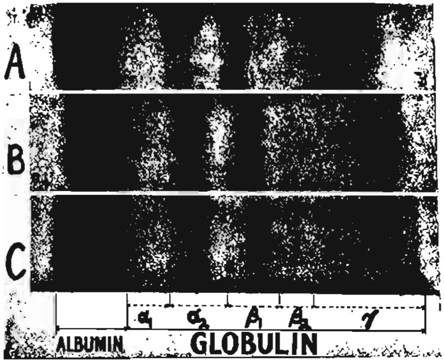

FIG. 1. Photographs of agar electrophoresis plates showing the electrophoretic serum protein patterns for three subjects: A, age 22 (top); B, age 46 (middle); and C, age 63 (bottom).

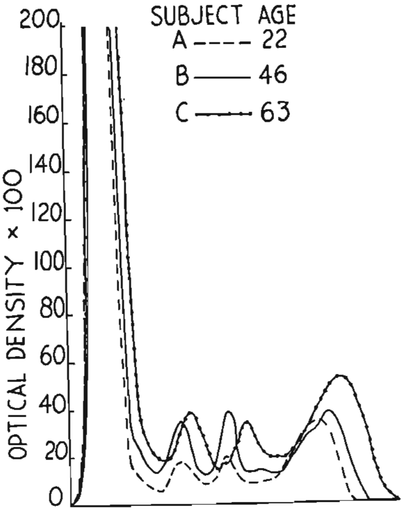

FIG. 2. Densitometric curves plotted from the agar electrophoresis plates given in Fig. 1 illustrating the relative concentration of serum protein fractions at different ages. From left to right the fractions are albumin, α_1 globulin, α_2 globulin, β_1 globulin, β_2 globulin, and γ globulin.

serum protein fractions, but inorganic phosphorus is negatively and significantly related to α_2, β_1, β_2, and γ globulins and total globulin.

TABLE V

Correlations between serum protein, serum cholesterol, and inorganic phosphorus ($N = 160$)

Serum protein	Serum cholesterol			Inorganic phosphorus
	Total	Ester	Free	
Albumin	.0973	−.0400	.1069	.0457
α_1 Globulin	.0207	.0574	.0254	−.1157
α_2 Globulin	.1008	−.0181	.1239	−.1766*
β_1 Globulin	.0978	.0160	.1183	−.2196†
β_2 Globulin	.0640	−.0790	.0939	−.1794*
γ Globulin	−.0315	−.0436	−.0003	−.2134†
Total globulin	.0459	.0950	.0725	−.1459

* $P < .05$.
† $P < .01$.

Discussion

Comparison of the serum protein values obtained for the Bengali sample with those published for European samples may be of interest. Lederer (15) summarizes the results obtained by a number of investigators using paper electrophoresis. Albumin fractions, expressed as percentage of the relative total protein, ranged from 55.4% to 72.9% in the eight studies cited from Germany, Italy, Scandinavia, and Spain. The number of subjects per study ranged from 10 to 39. The present value of 60.10% for the sample of 160 Bengali males, obtained by agar electrophoresis, falls well within their range of albumin values. They report values from 1.4% to 4.4% for α_1 globulin, 3.5% to 8.1% for α_2 globulin, 8.6% to 11.1% for β globulin, and 13.6% to 22.2% for γ globulin. The corresponding Bengali values are 3.06% α_1, 9.26% α_2, 9.22% β (combining β_1 and β_2), and 18.35% γ globulin. With the exception of α_2 globulin, the Bengali percentage values fall within the ranges reported by Lederer. The mean cholesterol values for the present sample are 185.29 mg % for total cholesterol, 117.25 mg % for the ester, and 67.85% for free cholesterol. These values conform to those reported earlier for Bengalis (13, 14). Walker, Boyd, and Asimov (5) report a mean total cholesterol of 180 mg %, with a standard deviation of 28 mg %, for North American males, which is in agreement with this value. Other workers, however, report values of 197 mg % for males between the ages of 18 and 35, and 239 mg % for males between the ages of 45 and 65 for total cholesterol (16). In the absence of weight data for their samples, direct comparison with the Bengali data may not be possible. Previous work in this laboratory (13, 14) has shown that cholesterol ester, free cholesterol, and total cholesterol increase significantly with weight but not with age. For every 10-lb increase in weight, cholesterol ester may be expected to increase 3.13 mg %, free cholesterol 4.16 mg %, and total cholesterol 7.31 mg % (14). Mean inorganic phosphorus values of

3.5 mg %, 3.7 mg %, and 3.8 mg % have been reported by various investigators in North America. The level of inorganic phosphorus in the serum is apparently the same in Bengalis, with a mean of 3.57 mg % for the total group.

Previous work on serum proteins has been primarily concerned with biochemical, medical, and physiological aspects. The literature does not indicate whether serum protein fractions are affected by physical characteristics of individuals. In the present data, the relative amount of the serum globulin fractions (as estimated by electrophoresis) was found to increase linearly with age. For these data, a 10-year advance in age would be expected to increase the relative amounts of the globulin fractions as follows: .022 α_1; .036 α_2; .032 β_1; .017 β_2; .129 γ globulin; and total globulin, .251. The way the electrophoretic pattern is altered with increasing age is illustrated by Figs. 1 and 2. While the total amount of globulin is increasing, the albumin fraction remains constant. But if the data are expressed as percentage of total protein (determined electrophoretically), these changes would not be apparent. In fact, the percentage of albumin would appear to be decreasing with age, when it is actually not changing. Weight also influences the globulin fractions, but the relationship is inverse and again, albumin is not affected. It may be possible that, if weight increases with age, these two influences may counteract each other. As age and weight are uncorrelated in the present data, the age influence is more obvious. Height apparently has little effect on either globulin or albumin. These findings suggest that at least part of the variability in serum globulin fractions in the healthy human male population may be attributed to differences in age and weight. Serum albumin is apparently uninfluenced by these factors. The multiple regression equations permit estimation of globulin fractions, obtained by the agar electrophoretic method, knowing only height, weight, and age.

It has been mentioned in the literature that the existence of separate serum proteins in the living organism has not been satisfactorily demonstrated (16). A further suggestion has been made that the fractions are actually components of one or more protein complexes which have been broken off by the method of separation (2). While it is generally agreed that the proteins are not separated into entirely homogeneous compounds by the majority of the existing methods, attempts have been made to study the functional relationships and processes entered into by specific fractions. The correlational data presented in Table II suggest that albumins and globulins do, in fact, form two separate but related entities, as the average correlation between albumin and the five globulin fractions is +.51. These results are in agreement with conclusions drawn by other workers in terms of molecular weight, structure, and physiological and biochemical functions. From the correlations given in Table II it can be seen that the globulin fractions as measured are highly interrelated, with an average correlation of +.78. The fractions also behave in a similar manner toward albumin (Table II), height and weight (Tables II and IV), age (Tables II, III, and IV), and cholesterol and inorganic phosphorus (Table V). These results could be interpreted as consistent with the view that the fractions represent components of a protein complex. The site of synthesis of some globulin fractions, excluding γ globulin, has been attributed to the liver (1), but the evidence has not been accepted by all workers (see, for example, ref. 3). The high degree of intercorrelation among the globulin fractions observed in these data suggests that the hypothesis of a common site or mode of synthesis is tenable.

One of the functions of the serum proteins is the transport of lipids, and β globulin is supposed to contain cholesterol, according to Tiselius (as reported in ref. 2). The present data were examined to determine whether the serum protein fractions are related to serum cholesterol components. The correlations which are reported in Table V suggest that for the serum proteins, as measured, the relationships are not significant for β globulin or any other fraction. Interest was also directed toward possible relationships between inorganic phosphorus and serum protein fractions. The data show that inorganic phosphorus is significantly and inversely related with each of the globulin fractions, but not with albumin. Phosphorus in the blood is utilized in many ways: it is incorporated into phospholipids, it must be available for phosphorylations in the cells, it is concerned with acid–base regulation, and it forms one of the main inorganic buffer systems of the body. Serum proteins also bear lipids, have a buffering function, and serve to transport inorganic ions, but whether any of these functions accounts for the observed relationship with inorganic phosphorus would have to be determined by further studies.

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