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An Examination of Variability of Blood Chemistry, Hematology, and Proteins, in Relation to Age

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Blood participates in and reflects the operation of homeostatic mechanisms controlling bodily functions. Constituents of blood reflecting homeostatic steady states include calcium, chlorides, serum pH, and serum albumin. Glucose and glycoproteins are among the constituents of blood participating in homeostasis. Levels of these and other blood constituents are evaluated clinically to determine whether or not the homeostatic mechanisms are functioning normally. Their use in medical diagnosis presupposes a normal range which indicates that they are under homeostatic control. Values of these constituents which exceed the normal range are interpreted as being out of control due to internal or external causes.

According to the hypothesis of biochemical individuality, a person perfectly 'normal' in all of his numerous blood constituents would be most unusual [Williams, 1956]. Furthermore, a person seemingly perfectly 'normal' in youth might have metabolic peculiarities which would reveal themselves only in postmaturational diseases, and hence, it follows from this hypothesis that 'normality' in all blood constituents would become increasingly unusual with advancing age [Williams, 1956]. It is the purpose of this study to test this hypothesis by defining 'normality' for a number of blood determinations considered simultaneously, and examining whether or not 'normality' so defined changes with age. The data being used are determinations of blood constituents obtained by chemical, hematological, and electrophoretic techniques.

Data

The first set of data analyzed comprises chemical and hematological determinations of twenty-one blood constituents measured on a single sample of ten ml of fasting venous blood taken from each of 478 adult males residing in Calcutta, India. The individuals tested may be regarded as a sample of the general, free living, employed male population. They were selected by stratified random sampling, with proportional allocation according to age group, of the employees of the Indian Statistical Institute. The routine adopted for collection of the data has been reported previously [DAS, 1964], and the laboratory methods employed have been specified elsewhere [DAS, 1967]. The following blood constituents were evaluated: amylase activity, calcium, chlorides, cholesterol (free), cholesterol ester, creatinine, glucose, nonprotein nitrogen, phosphatase (acid), phosphatase (alkaline), protein (total), serum pH, urea, uric acid, erythrocytes, leukocytes, neutrophils, lymphocytes, eosinophils, hemoglobin, and sedimentation rate. The subjects ranged in age from 20 to 74 years of age. Other results for these data have been reported previously [DAS, 1967].

The second set of data analyzed consists of electrophoretic determinations of fifteen protein components found in a single sample of ten ml of fasting venous blood taken from each of 284 adult males residing in Calcutta, India. These subjects were selected from the same population and by the same procedure adopted for the preceding study [DAS, 1968]. The following blood components were evaluated electrophoretically: serum albumin, serum globulins (alpha-1, alpha-2, beta-1, beta-2, gamma), alpha and beta lipoprotein, glycoproteins (alpha, beta, gamma), and amylase (alpha, beta-1, beta-2, gamma). Subjects 20 to 74 years of age were studied, and other results have already been reported [DAS, 1968].

Statistical Analysis

The first step was to transform the measurements to a common scale which would indicate the degree of deviation from the central tendency (arithmetic average or mean) of the individuals tested, taking into account the variability among the individuals. Taking all observations for a single blood constituent, the mean (M) and standard deviation (s) were computed. Then, each observation (X) was transformed to a normal deviate by computing $z = (X - M)/s$ [WALKER and LEV, 1953]. Observations on the twenty-one chemical and hematological determinations were transformed to normal deviates for 478 individuals, and observations on the fifteen electrophoretic determinations were similarly transformed for 284 individuals. For each individual examined, these measurements expressed as normal deviates, showed how his blood constituents deviated from the means of all individuals included in the study. The normal deviates were computed on the IBM 1401 Electronic Data Processing System of the Indian Statistical Institute.

To obtain the mean classification of the variables according to normal deviate class intervals for the different age groups, and to

classify individuals according to the distribution of their measurements expressed as normal deviates, frequencies were tallied for each individual according to the following nine normal deviate class intervals:

1. equal to or greater than +1.6000
2. equal to or greater than +1.1000 but less than +1.6000
3. equal to or greater than +0.6000 but less than +1.1000
4. equal to or greater than +0.0001 but less than +0.5999
5. equal to zero (0.0000)
6. less than 0.0000 but greater than −0.6000
7. equal to or less than −0.6000 but greater than −1.1000
8. equal to or less than −1.1000 but greater than −1.6000
9. equal to or less than −1.6000

Table I gives the expected distribution of normal deviates of 21 blood constituents for a single individual, according to his age group. This expected distribution has been computed by obtaining the mean number of deviates in each of the nine class intervals for all individuals within a given age group. The last row gives the classification of the 21 determinations into the nine normal deviate class intervals for all 478 individuals. This gives the empirical definition of 'normality' for a number of blood determinations considered simultaneously. Table IV gives a similar analysis of 15 electrophoretic determinations of serum protein, lipoprotein, glycoprotein, and amylase.

To classify individuals according to the distribution of the 21 normal deviates, a classificatory scheme was adopted consisting of 14 classes ranging from the minimum deviation to the maximum deviation. The scheme allocates the 21 normal deviates to the following sets:

1. The set $(-1.10, 0] \cup [0, 1.10)$, which is the union of the interval less than 0.0000 but greater than −1.1000, and the interval equal to or greater than 0.0000 but less than +1.1000.
2. The set $(-1.60, -1.10] \cup [1.10, 1.60)$ which is the union of the interval equal to or less than −1.1000 but greater than −1.6000, and the interval equal to or greater than +1.1000 but less than +1.6000.
3. The set $(-\infty, -1.60] \cup [1.60, \infty)$ which is the union of the interval equal to or less than −1.6000 but greater than $-\infty$, and the interval equal to or greater than 1.6000 but less than ∞.
4. The set $(-1.60, 0] \cup [0, 1.60)$ which is obtained by combining the first two sets.

Table I. Classification of 21 blood chemistry and hematology determinations in terms of normal deviate class interval: mean frequencies by age group

Age group (years)	N[1]	−1.6000 [2]	−1.1000 to −1.5999	−0.6000 to −1.0999	−0.0001 to −0.5999	0.0000	0.0001 to 0.5999	0.6000 to 1.0999	1.1000 to 1.5999	1.6000 [3]	Row Total
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
20–24	50	0.88	1.88	4.24	6.32	0.34	3.44	1.86	0.90	1.14	21.00
25–29	70	0.51	1.36	4.90	5.91	0.26	4.06	2.39	0.70	0.91	21.00
30–34	92	0.60	1.27	3.83	6.63	0.26	4.45	2.09	0.84	1.03	21.00
35–39	72	0.56	1.26	3.85	6.13	0.26	4.19	2.40	1.11	1.24	21.00
40–44	67	0.57	1.27	3.43	6.06	0.21	4.81	2.25	1.18	1.22	21.00
45–49	53	0.51	1.11	3.45	6.40	0.28	4.19	2.72	1.11	1.23	21.00
50–54	34	0.68	1.06	3.18	6.29	0.32	4.79	2.03	0.97	1.68	21.00
55–59	15	0.73	0.80	3.40	6.46	0.27	4.87	2.40	0.80	1.27	21.00
60–64	16	0.81	0.88	3.75	5.37	0.38	5.25	2.56	0.62	1.38	21.00
65–69	6	0.33	1.33	3.67	7.17	0.33	3.67	2.33	1.17	1.00	21.00
70–74	3	0.67	1.67	1.67	7.99	0.33	4.66	1.67	0.67	1.67	21.00
Pooled Total	478	0.61	1.29	3.86	6.26	0.27	4.32	2.27	0.95	1.17	21.00

[1] Number of individuals
[2] Including all values less than −1.6000.
[3] Including all values greater than +1.6000.

The classificatory scheme follows:

Class	Set (−1.10, 0] U [0, 1.10)	(−1.60, −1.10] U [1.10, 1.60)	(−∞, −1.60] U [1.60, ∞)
A	21	0	0
B	20	1	0
C	19	2	0
D	18	3	0
E	17	4	0
F_1	16	5	0
F_2	15	6	0
	(−1.60, 0] U [0, 1.60)		(−∞, −1.60] U [1.60, ∞)
G	20		1
H	19		2
I	18		3
J	17		4
K	16		5
L	15		6
M	14		7

For the blood chemistry and hematology determinations, table II presents the classification of individuals according to these 14 classes, separately by age group. To test the hypothesis that age group and classification according to distribution of normal deviates are independent, the chi square statistic has been computed [WALKER and LEV, 1953]. For table II, chi square = 129.1787 with 120 degrees of freedom, which is not statistically significant. (The value of chi square significant at the 5% point is 146.29.) Table V gives this classification for 284 individuals in terms of 15 protein determinations. The observed chi square for table V frequencies is 104.58 with 130 degrees of freedom, which is not statistically significant. (The 5% value of chi square is 157.32.)

If the observations made on an individual are equal to the corresponding mean observations for the population to which he belongs, he may be regarded as 'normal', with reference to that population. To test the hypothesis that the individual's observations on the 21 blood chemistry and hematology determinations, or on the 15 protein determinations, are 'normal', the statistic

$$S_0 = V_0' \mathfrak{Z}_0^{-1} V_0$$

Table II. Classification of 478 individuals according to distribution of 21 blood chemistry and hematology determinations expressed as normal deviates

Age group (years)	Class												
	A	B	C	D	E	F_1	G	H	I	J	K	L	M
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
20–24	0	1	4	2	1	1	18	7	8	1	2	4	1
25–29	4	2	7	2	2	0	20	25	5	1	1	1	0
30–34	2	10	10	0	1	1	28	17	8	10	5	0	0
35–39	3	0	3	1	3	1	24	19	11	3	2	2	0
40–44	0	4	4	2	2	1	20	14	11	6	3	0	0
45–49	1	3	5	2	1	0	14	13	7	5	1	1	0
50–54	1	0	2	0	1	2	10	5	3	4	3	1	2
55–59	2	0	0	0	0	0	5	3	2	2	1	0	0
60–64	1	0	2	1	0	0	3	3	2	2	1	0	1
65–69	0	0	0	0	0	0	4	2	0	0	0	0	0
70–74	0	0	0	0	0	0	0	2	1	0	0	0	0
Total	14	20	37	10	11	6	146	110	58	34	19	9	4

[RAO, 1965, p. 349, equation 6e. 2.4] has been computed for each of the 478 individuals in the first study and similarly for the 284 individuals in the second study. In this equation, V'_0 and V_0 give the individual observations, and S_0^{-1} is the inverse of the variance-covariance matrix of the observations for all individuals in the study, i.e., the population. Since S_0 is distributed approximately as chi square, it is possible to decide for each individual whether to accept or reject the hypothesis of 'normality' at a specified significance level. For these data, the 5% level of significance has been adopted for rejecting the hypothesis that an individual's observations equal the corresponding population mean observations. The computations were carried out on the IBM 1401 Electronic Data Processing System of the Indian Statistical Institute.

As all types of bodily functions might not behave similarly, the statistical analysis was carried out separately for seven sets of determinations.

(i) Blood chemistry related to regulatory processes, comprising calcium, chlorides, and serum pH.

(ii) Blood chemistry related to excretory processes, comprising creatinine, nonprotein nitrogen, urea and uric acid.

(iii) Blood chemistry related to metabolic processes, comprising amyl-

Table III. Classification of 478 individuals according to the hypothesis that the individual observations equal the corresponding population mean observations: blood chemistry and hematology

Age group (years)	Hypothesis for blood chemistry: regulatory		Hypothesis for blood chemistry: excretory		Hypothesis for blood chemistry: metabolic		Hypothesis for hematology	
	accept	reject	accept	reject	accept	reject	accept	reject
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
20–24	48	2	47	3	44	6	30	20
25–29	66	4	69	1	68	2	43	27
30–34	85	7	86	6	87	5	61	32
35–39	66	6	67	5	66	6	50	21
40–44	61	6	62	5	57	10	48	20
45–49	49	4	51	2	43	10	38	15
50–54	31	3	30	4	30	4	23	10
55–59	12	3	13	2	13	2	12	3
60–64	16	0	13	3	15	1	12	4
65–69	5	1	6	0	6	0	5	1
70–74	3	0	3	0	3	0	2	1
Total	442	36	447	31	432	46	324	154

ase activity, free cholesterol, cholesterol ester, glucose, acid phosphatase, alkaline phosphatase, and total protein.

(iv) Hematology, comprising erythrocytes, leukocytes, neutrophils, lymphocytes, eosinophils, hemoglobin and sedimentation rate.

(v) Serum protein, consisting of albumin, and alpha-1, alpha-2, beta-1, beta-2 and gamma globulins.

(vi) Lipoprotein, alpha and beta, and glycoprotein, alpha, beta and gamma components.

(vii) Amylase, having alpha, beta-1, beta-2, and gamma components.

For blood chemistry and hematology, table III reports the number of individuals for which the hypothesis of 'normality' is accepted and the number for which it is rejected, separately by age group and type of bodily function. Table VI reports the same analysis for the proteins. To test the independence of 'normality' so defined and age, chi square has been computed for each class of blood determinations. The computed values of chi square are:

(i) blood chemistry related to regulatory processes, chi square = 7.2088.

(ii) blood chemistry related to excretory processes, chi square = 10.9955.
(iii) blood chemistry related to metabolic processes, chi square = 14.9706.
(iv) hematology, chi square = 5.8495.
(v) serum protein, chi square = 10.8448.
(vi) lipoprotein and glycoprotein, chi square = 13.9841.
(vii) amylase, chi square = 13.9193.

Each of the chi square values is based on 10 degrees of freedom, for which the 5% level is 18.3070. As each of the computed chi square values is less than 18.3070, the hypothesis of independence is accepted for each of the above classes of blood determinations.

Discussion

Two possible measures of 'normality' for a number of blood determinations, considered simultaneously, have been proposed in this paper. The first is the expected number of observations falling into several normal deviate class intervals (see tables I and IV), and the second is a statistical test of the hypothesis that the individual observations equal the corresponding population mean observations (see tables III and VI). Each of these measures will be considered separately, after which their relationship to age will be examined.

If the values of an individual's observations on a number of blood determinations are distributed according to the normal probability function, the number of determinations predicted for each of the class intervals in table I is as follows:

Class interval	Number		Table I
	Predicted	Observed	Column Number
≥ 1.60	1.15	0.61	(3)
$\geq 1.10, < 1.60$	1.70	1.29	(4)
$\geq 0.60, < 1.10$	2.91	3.86	(5)
$\geq 0, < 0.60$	4.74	6.26	(6)
$\leq 0, > -0.60$	4.74	4.59	(7), (8)
$\leq -0.60, > -1.10$	2.91	2.27	(9)
$\leq -1.10, > -1.60$	1.70	0.95	(10)
≤ -1.60	1.15	1.17	(11)

The similarity of the predicted and observed numbers will be noted, and a chi square test of the goodness of fit permits acceptance of the hypothesis that the values of an individual's observations are normally distributed. The numbers in table I may be interpreted as those which would be expected for members of the general, free living population. They provide an empirical definition of 'normality' on a number of blood determinations considered simultaneously, that is, the distribution of values which would be expected on the basis of chance alone. 'Abnormality' would then be regarded as a distribution of individual values deviating significantly from that predicted under the 'normal' condition. Thus for a 'normal' individual, at least two values would be expected by chance alone to fall within the set $(-\infty, -1.60] \cup [1.60, \infty)$, obtained by combining columns (3) and (11) of table I. Using this criterion of 'normality', all individuals falling within Classes A to H of table II would be regarded as 'normal'. Applying a chi square test to determine significant deviation from 'normality' shows that only individuals of Classes K to M can be regarded as 'abnormal'. These results confirm the basic premise of the hypothesis of biochemical individuality, namely, that values for a series of determinations are not identical, but rather exhibit variability. However, when the variability expected is defined in terms of the normal probability distribution, it is seen that 'normal' individuals constitute the majority, and are not rare or unusual. For clinical diagnosis, it is pertinent to add that positive normal deviates equal to or greater than 2.58, or negative normal deviates equal to or less than –2.58, considered together would have an expected probability of occurrence of 0.01, and would indicate 'abnormality'. These conclusions also apply to the data in tables IV and V.

Another measure of 'normality' is provided by the test of the hypothesis that an individual's observations equal the corresponding population mean observations. This test is most simply carried out when the individual observations are transformed to normal deviates, for then the corresponding population mean observations are all zero. On the basis of this statistical test, individuals for whom the hypothesis of equality is rejected can be regarded as 'abnormal'. The last rows of tables III and VI show that the percentage of such individuals is 10% for blood chemistry determinations reflecting metabolic processes, 8% for blood chemistry determinations of regulatory processes and for serum proteins, and 6% for blood chemistry determinations reflecting

Table IV. Classification of 15 protein determinations in terms of normal deviate class interval: mean frequencies by age group

Age group (years)	N[1]	Normal deviate class interval									Row Total
		−1.6000 [2]	−1.1000 to −1.5999	−0.6000 to −1.0999	−0.0001 to −0.5999	0.0000	0.0001 to 0.5999	0.6000 to 1.0999	1.1000 to 1.5999	1.6000 [3]	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
20–24	30	0.48	1.45	2.35	3.66	0.16	2.68	2.16	0.74	1.32	15.00
25–29	37	0.59	1.62	2.11	4.38	0.08	2.95	1.43	0.89	0.95	15.00
30–34	49	0.57	1.51	2.37	3.88	0.12	2.82	2.06	0.67	1.00	15.00
35–39	36	0.26	1.06	2.63	4.90	0.09	2.69	1.54	0.80	1.03	15.00
40–44	39	0.54	1.05	2.67	4.06	0.15	3.00	2.02	0.69	0.82	15.00
45–49	29	0.24	1.10	2.48	3.83	0.14	3.52	2.00	0.83	0.86	15.00
50–54	24	0.52	1.04	2.56	3.72	0.00	3.04	2.20	0.84	1.08	15.00
55–59	15	0.53	0.80	1.87	4.80	0.13	4.00	1.47	0.53	0.87	15.00
60–64	17	0.35	0.82	2.53	4.54	0.12	2.82	2.35	0.76	0.71	15.00
65–69	6	0	0.83	1.00	5.50	0	3.16	2.17	0.67	1.67	15.00
70–74	2	0	0	2.00	2.00	0.50	4.00	2.50	1.50	2.50	15.00
Pooled Total	284	0.45	1.22	2.39	4.16	0.11	3.00	1.92	0.76	1.00	15.00

[1] Number of individuals

[2] Including all values less than −1.6000.

[3] Including all values greater than +1.6000.

Table V. Classification of 284 individuals according to distribution of 15 protein determinations expressed as normal deviates

Age group (years)	Class													
	A	B	C	D	E	F_1	F_2	G	H	I	J	K	L	M
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
20–24	0	2	1	1	2	0	0	8	7	6	2	1	0	0
25–29	2	2	2	1	1	1	1	12	9	2	1	1	2	0
30–34	2	5	2	2	1	1	0	15	11	6	2	1	1	0
35–39	1	4	5	3	1	0	0	11	5	4	0	0	1	1
40–44	2	2	6	1	0	0	0	16	6	3	1	1	0	1
45–49	2	3	3	1	1	0	0	10	6	2	1	0	0	0
50–54	1	3	2	0	0	0	0	8	6	2	1	0	0	1
55–59	2	4	2	0	0	0	0	2	1	2	1	0	0	1
60–64	0	3	1	0	0	0	0	9	3	1	0	0	0	0
65–69	0	0	1	0	0	0	0	3	1	0	0	1	0	0
70–74	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Total	12	28	25	9	6	2	1	94	56	29	9	5	4	4

excretory processes as well as for the lipoprotein and glycoprotein class, but 32% for hematology, which reflects temporary infectious conditions. Using this statistical criterion yields results consistent with those obtained for the first measure of 'normality', namely, that individuals do vary in their values, but that when the acceptable degree of variation is established, a relatively small number of individuals are 'abnormal'.

For the data presented by age group in the tables, the results of chi square tests of the independence of age and 'normality' have been reported in the preceding section. The results consistently show that 'normality' as defined is independent of age, i.e., it does not change with age in a general, free-living employed male population. This finding does not rule out the possibility that older individuals tend to be 'abnormal', as such individuals would have to leave this population due to their condition. For the population which has been sampled, the variability of blood constituents does not increase with age in the manner which would be predicted on the basis of the hypothesis of biochemical individuality.

Homeostatic steady states are maintained within certain tolerance limits. Within these limits, they can vary in a single individual, or

Table VI. Classification of 284 individuals according to the hypothesis that the individual observations equal the corresponding population mean observations: protein determinations

Age group (years)	Hypothesis for serum protein		Hypothesis for lipoprotein and glycoprotein		Hypothesis for amylase	
	accept	reject	accept	reject	accept	reject
(1)	(2)	(3)	(4)	(5)	(6)	(7)
20–24	26	4	30	0	29	1
25–29	36	1	35	2	34	3
30–34	47	2	43	6	40	9
35–39	31	5	36	0	35	1
40–44	37	2	37	2	36	3
45–49	28	1	28	1	27	2
50–54	23	1	22	2	21	3
55–59	13	2	12	3	14	1
60–64	14	3	16	1	16	1
65–69	5	1	6	0	6	0
70–74	2	0	2	0	1	1
Total	262	22	267	17	259	25

show different levels or values when a number of individuals are compared. To equate values of different steady states, it is necessary to transform them to a common metric. One such metric is the expression of values as normal deviates. The data analyzed in this paper have been transformed to normal deviates so that deviation from the average, and variability among a number of blood constituents reflecting and participating in homeostasis, could be compared. The tolerable limits for the operation of homeostatic mechanisms are indicated by the empirical definitions of 'normality' which have been arrived at. If an individual is classified as 'normal' by these criteria, then his homeostatic mechanisms may be assumed to be under control. On the contrary, if he is classified as 'abnormal', it may be concluded that his homeostatic mechanisms are not functioning normally, due to internal or external causes. The acceptable degree of variability defines the normal range within which the homeostatic mechanisms are under control. It is seen that, for individuals from the free-living, employed male population, age is not related to variability. Hence, individuals staying within this population are able to maintain homeostatic control over their bodily functions throughout the first forty years of post-

maturational life. These results, which have been obtained by examining the data in a new way, indicate that the hypothesis of an age-related increase in variability cannot be accepted without empirical demonstration.

Summary

According to R. J. WILLIAM's hypothesis of biochemical individuality, a person perfectly 'normal' in all of his numerous blood constituents would be most unusual, and variability among these blood constituents would increase with age, because a person seemingly 'normal' in youth might have metabolic peculiarities which would reveal themselves only in postmaturational diseases. To examine the suitability of this hypothesis, variability of blood constituents was examined for employed adult males ranging in age from 20 to 74 years. Twenty-one blood chemistry and hematology determinations obtained for 478 subjects, and fifteen protein determinations made on 284 subjects, were analyzed separately. The mean frequency of determinations, classified according to normal deviates, for individuals grouped according to age, supports the hypothesis that variability characterizes the blood constituents. Classification of individuals in terms of age and the variability of their blood constituents, shows that variability so measured is independent of age. This conclusion holds good for blood chemistry and hematology determinations considered together or in terms of function tested, i.e. regulatory, excretory, metabolic, or defense. It also holds good for protein determinations considered together or separately for the serum proteins, lipoproteins and glycoproteins, and amylase.

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