BLOOD CHOLINESTERASE LEVELS AND RANGE OF PERSONAL VARIATION IN A HEALTHY ADULT POPULATION

BY

S. CALLAWAY, B.Sc.

D. R. DAVIES, M.Sc., F.R.I.C.

AND

J. P. RUTLAND

(From the Ministry of Supply, Porton)

The power to inhibit cholinesterase is shared by a number of chemical compounds now in use as insecticides, while others have been proposed for use in chemical warfare. When absorbed by the body all these compounds may produce a significant reduction in the level of the red-cell or plasma cholinesterase before symptoms of systemic poisoning become evident. For this reason information about the level of these enzymes in the blood of any individual will be of value in helping to decide whether or not he or she has absorbed any of these materials. Further, recovery from the effects of poisoning can be considered to be complete only when the blood cholinesterase has returned to normal levels.

Until normal limits and variations for blood cholinesterase levels have been determined, it is obviously impossible to decide whether or not an individual has or has not got a normal blood cholinesterase level at any given time.

The present work consists of a detailed analysis of the results of determining cholinesterase levels, both in plasma ("pseudo"-cholinesterase) and in red blood cells ("true" cholinesterase). In all, 247 healthy adults drawn from the armed Services and the civilian population have been examined. The limits of personal variation have been based on a series of eight successive determinations made at fixed intervals on ten healthy men.

Technique

Blood obtained by venepuncture was collected in ammonium and potassium oxalate (10 mg. per 5–10 ml. blood). The blood was centrifuged for 10 minutes at 500 g and the cells were washed twice with 0.85% NaCl, the final spinning being for 20 minutes at 500 g.

For the determination, 0.2 ml. of the plasma or packed cells was added to 11.8 ml. of distilled water, and 2 ml. of this dilution was used for analysis. The cells or plasma were added to 0.5 ml. of 0.12 M sodium bicarbonate in a Warburg vessel to which 0.5 ml. of a solution of acetylcholine had been added to the side arm. For analysis of the red cells 0.036 M solution of acetylcholine was used, and for the plasma a 0.36 M solution. Such preparations from normal blood produced an output of approximately 100 microlitres of CO₂ in 30 minutes at 38° C. The flask and contents were gassed for 10 minutes with a mixture of 95% nitrogen and 5% carbon dioxide in the Warburg bath at 38° C. The acetylcholine was then tipped into the well of the flask. After an interval of ten minutes for the temperature to reach equilibrium, manometer readings were taken every 15 minutes for one hour and the results calculated by the method of Aldridge, Berry, and Davies (1949). The

unit of enzyme activity was defined as the amount of enzyme contained in 1/30 ml. of cells or plasma which would produce one microlitre of carbon dioxide in 30 minutes.

Because of the inevitable delays between the taking of a blood sample and its arrival in the laboratory for examination, it was important to know what effect this would have on the enzyme levels in the cells and plasma. A large pooled sample of human blood was divided into two parts. One was retained as whole blood and maintained at room temperature. The other part was separated into plasma and red cells and kept at 0° C. Samples of each were examined daily for five days. The results are given in Table I.

TABLE I.—Effect of Storage of Whole Blood, Plasma, and Red Cells Upon Cholinesterase Activity

Time in Days	Cells and Plasma Stored at 0° C.		Whole Blood Stored at Room Temp.		Remarks
	Cells	Plasma	Cells	Plasma	
0	—	—	131	102	
1	136	102	132	104	
2	132	99	124	103	Haemolysis
3	134	100	122	106	"
4	132	100	118	110	"
5	137	99	90	124	Marked haemolysis

It will be seen that the activity of whole blood at room temperature is maintained for 48 hours, but with the onset of visible haemolysis the value of the red-cell enzyme decreases while that of the plasma increases. If the cells and plasma are separated and stored at 0° C. the cholinesterase content of each remains constant for at least five days.

Results

1. Variations in Plasma and Red-cell Cholinesterase in a Healthy Adult Population

The value of any estimate of the variation of blood constituents in health depends as much upon the accuracy with which the assessment of health is made as upon the precision with which the chemical analyses are carried out. The problem is as much statistical and demographic as analytical.

Data have therefore been obtained from three groups, each of which is further subdivided into a summer (August–September) group and a winter (November–March) group, and each of which is carefully defined with reference to its standard of health and sociological nature. The details are given in Table II.

TABLE II.—Source and Nature of the "Normal" Groups Examined

Group	Period of Observation	Source	No. in Group	Characteristics of Group
Ia Ib	Nov.–Dec., 1949 Aug.–Sept., 1950	R.N., Army, and R.A.F.	68 } 100 32 }	Males aged 18–30. All fit for combat duty everywhere
IIa IIb	Jan.–Mar., 1950 Aug.–Sept., 1950	Civil pop. of S.W. England, urban and industrial	52 } 81 29 }	Males aged 18–69. No history of serious illness for at least two years
IIIa IIIb	Jan.–Mar., 1950 Aug.–Sept., 1950	As IIa and IIb	53 } 66 13 }	Females aged 25–74. History as IIa and IIb

The first group consisted of 100 men aged 18–30 from the armed Services, each designated as "fit for full combat duty anywhere" (Service group). The second group

FIG. 1.—Distribution and activity diagrams showing levels of plasma and red-cell cholinesterase.

consisted of 81 men aged 18–60 from South-west England, and the third group consisted of 66 women aged 25–74 (civilian groups).

No member of the last two groups gave a history of any serious illness within the preceding two years, and as they were all blood donors they could be considered to be free from the common infectious diseases at the time the blood was taken.

Distribution-activity diagrams for the level of both the plasma and the red-cell enzyme in all three groups have been constructed. They show (Fig. 1) that the distributions do not depart significantly from normal, but the mode of the red-cell enzyme distribution occurs at a higher level of activity in Group I (Service group) than in either of the civilian groups.

In Table III the means with standard deviations for the level of each enzyme in all groups are given, and the figures show that there is no significant difference between the mean values of the plasma enzyme in any

group, while the mean value of the red-cell enzyme is significantly higher in the Service group. There is no difference between the mean values of the male and female groups of the civilian population.

The difference between the red-cell enzyme level in Group I and that in Group II cannot readily be explained except possibly on the higher level of physical fitness that might be expected in the Service group.

Age does not influence the level of either enzyme in the adult (Fig. 2), and a comparison of the Service group with a group of civilian males of the same age showed that the former still had a significantly higher level of red-cell enzyme.

No seasonal differences were observed. The mean value of the plasma enzyme during the summer was 87.1 (s.d. 20.6). During the winter it was 90.0 (s.d. 19.1). This difference is not significant ($P > 0.05$). The significant difference between Service and civilian red-cell values necessitates a separate comparison. The mean red-cell cholinesterase for the Service group was 116.4 (s.d. 11.0) during the summer and 118.2 (s.d. 13.9) during the winter; ($P > 0.05$). The comparable figures for the civilian group were 110.9 (s.d. 16.2) and 107.9 (s.d. 14.2); ($P > 0.05$).

These figures incidentally serve as a control of the method and indicate that the results obtained at different times are strictly comparable.

The limits of variation based on a 95% level of probability have been determined for the plasma enzyme level in the whole population examined, and for the

TABLE III.—Comparison of Plasma and Red-cell Cholinesterase in Healthy Persons

Group	No.	Mean	Stand. Dev.	Significance
Plasma:				
Service ..	87	86.7	17.0	$P > 0.05$
Civilian males ..	76	90.6	24.6	
Civilian females ..	65	88.3	20.6	
Red cell:				
Service ..	96	117.7	12.7	$P < 0.05$
Civilian males ..	79	109.6	16.4	
Civilian females ..	65	107.7	12.8

FIG. 2.—Diagram showing levels of plasma and red-cell cholinesterase according to age.

TABLE IV.—Limits of Variation in Cholinesterase (ChE) Levels in the Red Cells and Plasma of Adults

Enzyme	Group	No.	Mean	Stand. Dev.	Limits
			(ChE Units)		(ChE Units)
Plasma	All groups	228	89.3	19.4	51–128
Red cell	{ Service	98	117.7	12.7	92–143
	{ Civilian	145	108.2	16.7	75–142

red-cell enzyme for the Service and civilian groups. If the mean value of the enzyme activity is expressed as 100, and the standard deviation as a percentage standard deviation—i.e., the coefficient of variation—the limits can then be expressed in a more universally applicable form.

TABLE V.—The Coefficient of Variation of Plasma and Red Cell. Mean=100

Enzyme	Group	Coefficient of Variation	Limits
Plasma	Total	21.7	57–143
Red cell	{ Service	10.8	78–122
	{ Civilian	15.4	69–131

Using the coefficient of variation, it is possible to compare the results from experiments done by different techniques. The results of others have been calculated in this way and comparisons made. These are summarized in Tables VI and VII. With the exception of the continuous titration technique there is remarkably good agreement in the coefficient of variation of the results from the use of different methods.

TABLE VI.—A Comparison of "Normal" Limits of Plasma Enzyme as Determined by Various Authors

Author	Technique	No. of Observations	Coefficient of Variation
Present paper		141	22.8
Levine and Hoyt (1949)	Warburg: substrate acetylcholine	66	20.4
		67	18.9
Michel (1948)		80	22.5
Callaway and Davies (unpublished)		42	21.5
		11	18.1
Wilson (1949)	Warburg: substrate benzoylcholine	100	28.5
Davies and Rutland (1950)		49	25.8
Davies and Rutland (1950)	Electrometric: substrate acetylcholine	47	24.5
Michel (1949)		42	21.4
Piccoli and Longo (1947)	Continuous titration: substrate acetylcholine	30	12.5
Meyer <i>et al.</i> (1948)		15	30.2

2. Variation in the Red-cell and Plasma Cholinesterase in a Healthy Individual

Blood samples were taken on eight occasions over a period of four weeks from ten soldiers aged 18–30, each engaged on normal service duty. The mean plasma cholinesterase, with its variation, is given for each individual in Table VIII. The overall mean is 89.0 and the average variance is 58.6. It is necessary to consider

TABLE VII.—A Comparison of "Normal" Limits of Red-cell Enzyme as Determined by Various Authors

Author	Technique	No. of Observations	Coefficient of Variation
Present paper		98	10.7
Callaway and Davies (unpublished)	Warburg: substrate acetylcholine	27	12.3
Present paper		145	15.4
Wilson (1949)	Warburg: substrate acetylcholine	100	12.5
Davies and Rutland (1950)		50	15.4
Davies and Rutland (1950)	Electrometric: substrate acetylcholine	48	15.9
Meyer <i>et al.</i> (1948)	Continuous titration: substrate acetylcholine	15	10.2

whether this variance is homogeneous or whether it is an average of variances which differ widely from subject to subject.

TABLE VIII.—The Homogeneity of the Personal Variation of Plasma Cholinesterase

Subject	Mean ChE	Variance	Stand. Dev.
A	86.1	49.3	7.0
B	95.0	19.7	4.4
C	89.4	37.1	6.1
D	101.5	69.2	8.3
E	84.1	97.0	9.9
F	73.2	63.4	8.0
G	100.0	83.7	9.1
H	90.2	66.7	8.2
J	84.4	23.4	4.8
K	86.2	76.8	8.8

Bartlett's (1937) test for the homogeneity of these variances provides a value for χ^2 of 8.0 with $P=0.54$. Although the variances range from 19.7 to 97.0, with corresponding standard deviations of 4.4 to 9.9, their average values of 58.6 and 7.6 respectively may be considered to be the best estimate of the personal variation of the plasma enzyme.

The essential purpose of this paper is to facilitate the recognition of abnormal values of cholinesterase with the minimum of observations on the normal. This problem may be stated in more precise terms as follows:

1. If a single estimate of the enzyme is available, what must be the difference between this and a second estimate for the latter to differ significantly? Such differences between observations which have been derived from a single population with variance σ_1^2 are distributed normally about zero mean with variance $\sigma^2 = 2\sigma_1^2$. An estimate of σ_1^2 is given above—viz., 58.6—and thus σ^2 becomes 117.2 and the standard deviation 10.8.

Since it is intended to use the information resulting from this inquiry to investigate processes involving the inhibition of cholinesterases, only negative values of such differences are of interest. In consequence, the appropriate multiplier of the standard deviation for 5% significance is 1.64. Thus the second result must be 17.7 units below the first.

2. Similarly, if two such estimates, x_1 and x_2 , are available, what must be the difference between the mean of these two and a third, x_3 ? Here again the distribution of such differences must be considered. If the mean of the first two estimates be regarded as the equivalent of the initial estimate of the previous example the same treatment is applicable, but here the variance of this mean is $\sigma_1^2/2$. Thus, the difference will be distributed normally around zero mean with variance $2\sigma_1^2/2$.

3. Similarly, with three initial estimates, the difference between their mean and a fourth estimate will be distributed normally about zero mean with variance $4\sigma_1^2/3$.

On this basis an instructive table (Table IX) has been drawn up in which the value of such significant differences has been calculated for a varying number of initial estimations.

TABLE IX.—Minimum Differences for the Recognition of Abnormal Personal Red-cell and Plasma Cholinesterase Values

No. of Initial Estimations	Standard Deviation of Difference of Suspected Observation from Mean	Significant Difference 1.64
1	$\sigma\sqrt{2} = 10.8$	17.7
2	$\sigma\sqrt{3/2} = 9.4$	15.4
3	$\sigma\sqrt{4/3} = 8.8$	14.5
4	$\sigma\sqrt{5/4} = 8.5$	14.0
5	$\sigma\sqrt{6/5} = 8.4$	13.8
10	$\sigma\sqrt{11/10} = 8.0$ $\sigma = 7.6$	13.1 12.6

The red-cell data have been accorded a similar treatment, and the results are recorded in Table X.

TABLE X.—The Homogeneity of the Personal Variation of Red-cell Cholinesterase

Subject	Mean ChE	Variance	Stand. Dev.
A	122.5	100.3	10.2
B	105.8	52.2	7.2
C	108.9	17.9	4.2
D	120.3	53.6	7.3
E	113.0	74.3	8.6
F	107.5	69.7	8.3
G	129.4	25.7	5.1
H	118.8	74.2	8.6
J	112.1	53.6	7.3
K	117.4	62.3	7.9

Bartlett's test for the homogeneity of variances yields from the above data a value for χ^2 of 7.2 with 9 degrees of freedom ($P=0.62$). Thus, although the variances range from 17.9 to 100.3, with corresponding standard deviations of 4.2 to 10.2, the average values of 58.4 and 7.6 respectively may be accepted as the best estimates of personal variation.

The rather unexpected result has emerged that the average personal variance is numerically identical in both plasma and red-cell enzyme; thus the significant differences given in the third column of Table IX are applicable to the plasma enzyme.

Using the present data, an average value for the coefficient of variation can be calculated, and on the basis of a mean value of 89 for the plasma enzyme and 115.6 for the red-cell enzyme the respective coefficients are 8.5 and 6.6. These values may be used for the construction of a table similar to Table IX wherein column 3 will give percentage differences only. Such a table (Table XI) would be applicable to any other technique of analysis provided that sufficient data are available to permit the calculation of a mean to which these coefficients may be related.

TABLE XI.—Percentage Minimum Differences for the Recognition of Abnormal Red-cell and Plasma Cholinesterase Values

No. of Initial Estimations	Percentage Differences	
	Plasma	Cells
1	19.9	15.3
2	17.3	13.3
3	16.3	12.5
4	15.7	12.1
5	15.5	11.9
10	14.7 14.1	11.3 10.9

Discussion

On the basis of a large number of results from a healthy adult population it has been possible to lay down limits between which the normal levels of red-cell and plasma cholinesterase will lie. Sex, age, occupation, and season are without effect upon the level of the plasma enzyme. But in the case of the red-cell enzyme a group of Service men were found to have a significantly higher mean level than a male civilian population of comparable age. It is conceivable that this may be due to the active outdoor life led by all of the first group together with a high general level of physical fitness.

The level of cholinesterase has a normal distribution among the adult population. If the variations are expressed as coefficients of variation rather than as standard deviations for the results of a particular method, it is possible to compare the results obtained by workers using different techniques. But, it need hardly be added, each worker must establish a satisfactory means for his own results.

It is thus possible to take a sample of blood from an individual and say whether or not the plasma or red-cell enzyme level lies within normal limits. However, the range of these limits is rather great, and further work was done to establish the limits for an individual's variation in the level of either enzyme when estimates were made at different times.

It was found that the range of these limits was considerably smaller than that of the whole population. This means that, where an individual is exposed to an inhibitor of cholinesterase and absorbs some of it, the level of cholinesterase in his cells or plasma may be significantly depressed below his own normal level and yet this new low level may be within the limits of normality for the population as a whole.

If it is possible to make only a single estimation from individuals exposed to these compounds, then it can be

said whether or not their level falls outside the normal range, and if it is below normal it is good evidence that they have absorbed some of the inhibitor. Fortunately, the lower normal level of the population is well above the highest level at which symptoms of systemic poisoning are likely to become apparent.

If, however, two successive samples of blood can be taken over a period during which the individual was exposed to these compounds, or over a period immediately following such an exposure, it might be possible to show that the variations of his individual levels varied beyond the range of his personal normal level and indicated absorption of a cholinesterase inhibitor.

The limits have been accurately defined and the degree of significance of the variations has been described.

A more universal application of the technique will await the development of methods less complicated than the one used in this work and the adoption of these newer techniques as a routine method in chemical pathological laboratories.

Summary

The variations in plasma and red-cell cholinesterase have been determined in a large group of 247 healthy adults drawn from the armed Services and civilian population.

Limits of variation have been defined for the plasma enzyme for the whole sample, but separate limits were necessary for the red-cell enzyme from the civilian and Service groups. These limits lie between 57 and 143% of the mean value for the plasma enzyme and 78 and 122% of the mean red-cell enzyme value for Service personnel. The corresponding limits for the red-cell cholinesterase for civilians are 69 and 131% of the mean.

There were no sex, age, or seasonal differences in either enzyme.

It has been shown that the coefficient of variation is a valuable index for defining the limits of variation in both enzymes.

The personal variation has been studied in 10 soldiers by examining blood samples upon eight separate occasions.

It has been possible to make estimates of the fall in enzyme level necessary to indicate departure from the normal. This value varies with the number of initial observations, but is numerically the same (in enzyme units) for both plasma and red-cell enzymes.

A knowledge of the results here presented permits the early recognition of abnormal levels of red-cell and plasma cholinesterase.

We are greatly indebted to Surgeon Commander W. H. McKee, R.N., and to Dr. R. S. Tovey, Director of the South-western Region Blood Transfusion Service, for supplying us with blood samples. We wish also to thank the Service and civilian personnel who co-operated in this inquiry. Finally, we wish to record our appreciation of the careful and accurate assistance we have received from Miss A. M. Eyres and Corporal E. Kerr, R.A.M.C. Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish this paper. Crown copyright reserved. Reproduced with permission of the Controller of H.M. Stationery Office.

REFERENCES

- Aldridge, W. N., Berry, W. K., and Davies, D. R. (1949). *Nature, Lond.*, **164**, 925.
 Bartlett, M. S. (1937). *J. roy. Statist. Soc.*, Suppl. **4**, 137.
 Davies, D. R., and Rutland, J. P. (1950). *Biochem. J.*, **47**, xxi.
 Levine, M. G., and Hoyt, R. E. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 50.
 Meyer, L. M., Sawtisky, A., Ritz, N. D., and Fitch, H. M. (1948). *J. Lab. clin. Med.*, **33**, 189.
 Michel, H. O. (1948). *Med. Div. Inf. Prog. Rep.*, July, p. 38.
 — (1949). *J. Lab. clin. Med.*, **34**, 1564.
 Piccoli, R., and Longo, G. (1947). *Boll. Soc. ital. Biol. sper.*, **23**, 356.
 Wilson, A. (1949). Unpublished Prog. Rep. to Ministry of Supply.

BLOOD CHOLINESTERASE LEVELS IN WORKERS EXPOSED TO ORGANO-PHOSPHORUS INSECTICIDES

BY

J. M. BARNES, M.B., B.Ch.

Medical Research Council Toxicology Research
Unit, Carshalton

AND

D. R. DAVIES, M.Sc., F.R.I.C.

Ministry of Supply, Porton

During the last twelve months several reports have appeared which emphasize the toxic properties of a group of substances commonly referred to as the organo-phosphorus insecticides (Report to Pesticides Committee, 1950; Goldblatt, 1950; Bidstrup, 1950; Grob *et al.*, 1950). At least 30 fatalities have occurred among workers handling these compounds in America (Abrams *et al.*, 1950). No fatal cases of poisoning have occurred in Great Britain, and only a single non-fatal case has been reported (Milles and Salt, 1950). A working party has been set up by the Ministry of Agriculture to consider the various problems that arise in connexion with the use of toxic chemicals in agriculture. Its report and recommendations are awaited.

These organo-phosphorus insecticides are valuable adjuvants to modern agricultural practice. It is obvious that, like any other poisonous compounds, they can be handled only if suitable safe techniques can be devised. One very important safety procedure would be the development of a clinical test that would make it possible to detect the absorption of the poisons before enough had been taken in to produce a dangerous or fatal intoxication.

All the organo-phosphorus compounds used as insecticides have one common property. When they are introduced into the body they inhibit the enzymes that hydrolyse acetylcholine. In man both the "pseudo" and the "true" cholinesterase circulate in the blood; the former is found in the plasma and the latter in the red blood corpuscles. Furthermore, observations by one of us (D. R. D.) on animals have shown that the amount of these enzymes circulating in the blood may be lowered by at least 80% without symptoms of poisoning (due to the accumulation of acetylcholine) appearing.

Since techniques for measuring the level of cholinesterase in the blood are available, a determination of the cholinesterase level at any given time could be used to decide whether the poison was being absorbed even in quantities too small to produce symptoms of poisoning. Garlick (1950) has already emphasized the value of blood cholinesterase determinations to those responsible for the medical care of workers exposed to these insecticides during their manufacture.

Ideally, a series of determinations of the blood cholinesterase should be done on any individual before, during, and after exposure to the insecticides, whether in the factory or in the field. Callaway *et al.* (1951) have determined the range about which the level of a normal individual may vary, and so it would not be possible to say in any individual case whether or not a reduction