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# Variations in Levels of Blood Clotting Factors IX and X in a Population of Normal Men: Possible Genetic Polymorphisms

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The coagulation of blood may be regarded as the final step in a sequence of physiologico-chemical reactions, each prior step of which triggers a subsequent one [1, 2]. The components of the sequence, conventionally designated by Roman numerals but also possessing physiologic appellations, have been defined by combining biochemical analysis with the physiologic study of mutant phenotypes and members of their families. Mutant phenotypes have been described in humans for 10 of the 13 components or "factors" of the sequence (Factors I, II, V, VII, VIII, IX, X, XI, XII, and XIII) and in dogs for at least three (Factors VII, VIII, IX). As expected, most of the mutated loci reside on autosomal chromosomes (I, II, V, VII, X, XI, XII, XIII), but two are X linked in both species (VIII, IX). The mutations are rare, and the two most commonly encountered abnormal phenotypes are the X-linked hemophilias A (Factor VIII, AHF deficiency) and B (Factor IX, Christmas-factor deficiency). The frequency in humans of the abnormal gene producing hemophilia A has been estimated to be $1–1.7 \times 10^{-4}$ and for hemophilia B, $2–3 \times 10^{-5}$ [3]. The hemophilia-A locus is known to be linked to the colorblindness and glucose-6-phosphate dehydrogenase loci, but the hemophilia-B locus has not yet been linked to any other locus on the X chromosome [4].

The abnormal phenotypes are usually ascertained through the existence of a hemorrhagic diathesis, and confirmation is by use of an in vitro bioassay. Identification of the abnormal phenotypes has depended upon demonstration of retarded coagulation values under test-tube conditions. Specific identification of an abnormality has depended upon matching the abnormal plasma against a battery of previously defined abnormal plasmas in a type of complementation test. When the unknown abnormal plasma sample fails to improve coagulation of one member of the abnormal battery but is able to improve the retarded coagulation of all others,

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specific identification is regarded as complete. Improvement of retarded coagulation of all members of the battery may imply the discovery of a new abnormality, implying perhaps the existence of a previously unsuspected clotting factor.

Assay procedures have been developed by quantitative adaptation of the complementation principle. Under controlled conditions, using a specifically defective plasma for, say, Factor IX, the Factor IX content of an unknown sample can be assayed in comparison with a standard. The relative corrective effects of the two can be compared and the unknown expressed as a fraction of the standard. These assay procedures are highly specific qualitatively but are only moderately precise quantitatively, because the end point is a function of a very large number of variables.

Until recently, only the more severe mutations causing significant reductions in clotting-factor level have been definable, because of the relative imprecision of the assays and the fairly large variations within normal populations. The population variation is thought to have a physiologic as well as a genetic basis, since a number of the factors are affected by developmental and environmental influences such as age, hypermetabolism, pregnancy, drugs, and exercise. Additional genetic variability has been uncovered recently by application of immunologic techniques. For example, both $CRM^+$ and $CRM^-$ variants are recognizable for both hemophilia A [5–7] and hemophilia B [8]. An additional Factor IX variant, $B_m$ [9], has also been identified by coagulation procedures.

It occurred to us that study of a normal population of males for levels of Factor IX with a bioassay under careful control might reveal additional genetic variability. A polymorphism would be most easily detected for this factor, we reasoned, because Factor IX is related to a locus on the X chromosome, each male possessing only one such locus. The single sample collected on each subject was also assayed for Factors II and X (prothrombin and Stuart factor, respectively), because all three factors have many properties in common, that is, dependence upon vitamin K, susceptibility to coumarin compounds, adsorption and elution from alkaline earth particles, stability over time, and reasonably reliable assays. However, the results for Factor II are not reported in detail since (1) we were not able to control these assays as rigorously as the others, (2) the findings for this factor did not add significantly to the results, and (3) it is easier to understand the results of the statistical analyses in terms of two factors than in terms of three factors.

This paper will describe results which strongly suggest a relatively high frequency of polymorphism among normal males for Factor X (the Stuart factor) and a lower frequency of polymorphism for Factor IX.

## SUBJECTS AND METHODS

### *Study Population*

A group of 207 male students from the University of North Carolina volunteered for the experiment. All were Caucasians between the ages of 15 and 26 years. All professed to be in good health. Samples were obtained during a 3-week period in June 1965. The great majority of samples were obtained between 8 A.M. and 11 A.M. in the entry to the YMCA building from students en route to the snack bar. A few were obtained in

the dormitories at night. In no case were samples obtained from men recently engaged in strenuous exercise.

*Collection and Storage of Blood*

A 5-ml sample of blood was drawn from each volunteer, using a single syringe technique. Then 4 ml of the blood was quickly mixed with 0.5 ml of 3.2% sodium citrate solution; the remainder was discarded. The samples were immediately packed in ice at 0° C and transported to the laboratory within several hours. After centrifugation under refrigeration, the sedimented cells were discarded and the pipetted plasma from each of the samples was stored at −20° C until assayed.

*Control Plasma*

A 50-ml sample of blood was drawn from each of 10 normal donors, anticoagulated with 3.2% sodium citrate in the ratio 1:8 v/v, and centrifuged. The 10 plasmas were then pooled and divided into lots of 0.2 ml each, a volume sufficient for one assay procedure, and stored at −20° C. A lot of the pooled control plasma was thawed prior to each procedure and used as the standard for comparison.

*Assay Procedure*

Factor IX was measured by a one-stage assay procedure developed by Barrow et al. [10] based upon the partial thromboplastin time. Factor X was assayed by a one-stage assay procedure developed by Hougie et al. [11], most recently described by Barrow and Graham [12]. Both assays were carried out on all samples during a period of 4 weeks by one of us (R. Lester).

## RESULTS

The results of the assays on the 207 volunteers are tabulated in the Appendix. Each assay was carried out twice, and each result shown in the table is the average value for each factor on each person; the standard error of such a result was calculated to be 7.5 for Factor IX and 5.9 for Factor X. Inspection of the tabulated data reveals nothing striking. We did not, for example, encounter by chance a single person with a sufficiently abnormal assay value to suggest that he be regarded as a "bleeder."

TABLE 1

BASIC DESCRIPTIVE STATISTICS OF THE SAMPLE, TESTING FOR DEPARTURE FROM A NORMAL DISTRIBUTION

| | AGE (Years) | FACTOR ASSAY | |
|---|---|---|---|
| | | IX | X |
| Mean | 20.72 | 123.98 | 106.91 |
| Variance | 3.41 | 745.79 | 623.85 |
| Skewness | 0.37* | 0.31* | 0.89** |
| Kurtosis | 3.72* | 3.65* | 3.26 |
| Range | 15–26 | 46–217 | 56–190 |

* $.01 \leq P \leq .05$.
** $.001 \leq P \leq .01$.

Table 1 shows the basic descriptive statistics computed from the sample studied. The distributions of both factors are positively skewed and leptokurtic; the skewness is especially significant for Factor X. It has been shown that platykurtosis is evidence of bimodality, and, conversely, that leptokurtosis indicates "least bimodality" [13, 14]; this would imply that neither of the factors can possibly have bimodal distributions. However, the arguments used hold only for symmetric distributions; since the distributions are skewed, the measure of kurtosis in this case tells us very little about bimodality. It should be noted that a log transformation of the data will reduce the skewness, but, for Factor X at least, cannot eliminate it entirely.

The pairwise correlations are as follows, the indicated significance levels being appropriate for pairs of variables that follow bivariate normal distributions: age–Factor IX, —0.05; age–Factor X, 0.18 ($P < .05$); Factor IX–Factor X, 0.30 ($P < .001$). Although the variables are not normally distributed (see table 1), this departure from normality is probably insufficient to seriously affect the significance levels [15].

Figures 1*a* and 1*b* compare the data with the best-fitting normal distributions, as determined from the means and variances given in table 1. Each solid curve is a cumulative normal distribution; the dots are an empirical plot of $[r(x) - \frac{1}{2}]/n$ (ordinate) against $x$ (abscissa), where $x$ is a factor measurement on one individual and $r(x)$ is the rank of $x$ among $n$ (207) measurements (the lowest measurement has rank 1, the highest has rank $n$). This method of plotting the data avoids the difficulty, inherent in drawing histograms, that the choice of class interval can greatly influence the picture obtained. It is immediately apparent from these plots that Factor IX is approximately normally distributed (even though it shows significant skewness and kurtosis), but that Factor X is a mixture of at least two normal distributions.

Using a very general computer program developed by Kaplan and Elston [16], maximum-likelihood estimates were obtained for a fit of a mixture of two normal distributions, with common variance, to the data for each factor. Thus, denoting the data by $x_i$, $i = 1, 2, \ldots, n$, the likelihood is proportional to

$$\sigma^{-n} \prod_{i=1}^{n} \left\{ p \exp\left[ -\tfrac{1}{2} \left( \frac{x_i - \mu_1}{\sigma} \right)^2 \right] + (1 - p) \exp\left[ -\tfrac{1}{2} \left( \frac{x_i - \mu_2}{\sigma} \right)^2 \right] \right\},$$

and this is maximized to obtain estimates of $\mu_1$ and $\mu_2$ (the two means), $\sigma^2$ (the common variance), and $p$ and $(1 - p)$ (the proportions of admixture). The standard errors of these estimates are calculated by inverting the observed information matrix, this latter being obtained by numerical double differentiation of the log-likelihood surface. The results are given in table 2, and a comparison of the data points and the corresponding theoretical curves are shown in figures 1*c* and 1*d*; these plots are similar to the plots shown in figures 1*a* and 1*b*, except that now the theoretical curve is, in each case, a mixture of the two corresponding distributions given in table 2. It is apparent that a mixture of two distributions fits the data

TABLE 2

ESTIMATED PARAMETERS, AND STANDARD ERRORS, WHEN A MIXTURE OF TWO NORMAL DISTRIBUTIONS WITH COMMON VARIANCE IS FITTED TO SAMPLE

| | FACTOR IX | | FACTOR X | |
|---|---|---|---|---|
| | Parameter | SE | Parameter | SE |
| Mean of first distribution | 122.98 | 2.06 | 96.03 | 1.23 |
| Mean of second distribution | 195.68 | 34.13 | 146.10 | 2.52 |
| Common variance | 654.15 | 75.50 | 194.31 | 22.75 |
| Proportion in first distribution | 0.98 | 0.02 | 0.78 | 0.03 |
| Proportion in second distribution | 0.02 | 0.02 | 0.22 | 0.03 |

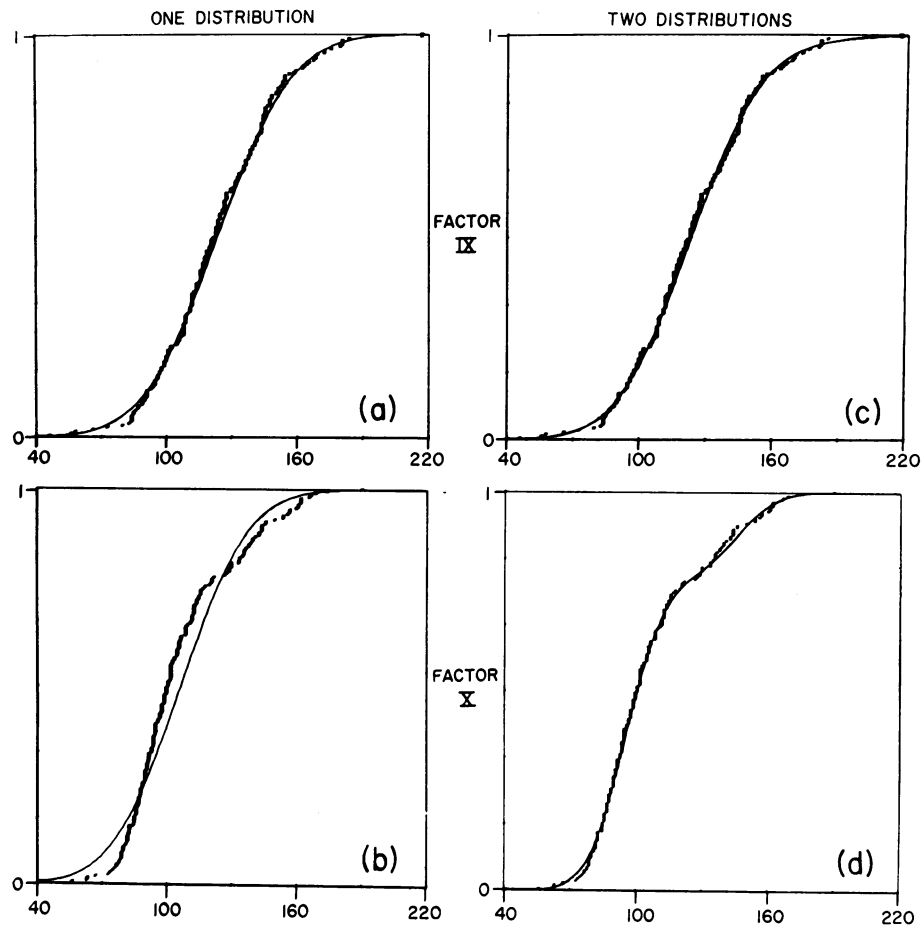


FIG. 1.—Empirical and theoretical cumulative plots when one normal distribution (*a*,*b*) or a mixture of two normal distributions (*c*,*d*) are fitted to the data for Factors IX and X.

better, as is to be expected, and that the poor fit for Factor X is greatly ameliorated. That the fit for Factor X is significantly better when a mixture of two distributions is postulated is seen by noting that the estimated proportion in the second distribution, 0.22, differs from zero by more than seven times its standard error. In the case of Factor IX, however, the corresponding proportion is not significantly different from zero.

Examination of the empirical plot for Factor X suggests the possibility of its being a mixture of three normal distributions, and so the best-fitting three-distribution mixture was also obtained, with the results given in table 3. The likeli-

TABLE 3

ESTIMATED PARAMETERS, AND STANDARD ERRORS, WHEN A MIXTURE OF THREE NORMAL DISTRIBUTIONS WITH COMMON VARIANCE IS FITTED TO FACTOR X DATA

| | Parameter | SE |
|---|---|---|
| Mean of first distribution | 95.30 | 1.26 |
| Mean of second distribution | 138.00 | 6.98 |
| Mean of third distribution | 160.71 | 8.36 |
| Common variance | 166.42 | 24.10 |
| Proportion in first distribution | 0.76 | 0.04 |
| Proportion in second distribution | 0.18 | 0.05 |
| Proportion in third distribution | 0.06 | 0.06 |

hood of the data under this model is 4.3 times larger than the likelihood for a mixture of two distributions; but, as is seen in table 3, the proportion of the third distribution is not significantly different from zero. If we assume the three proportions in table 3 are the population frequencies of three genotypes *AA*, *Aa*, and *aa*, then assuming Hardy-Weinberg equilibrium, the gene frequencies are 0.85 and 0.15. These result in the expected genotypic frequencies of 0.723, 0.255, and 0.022, which are not significantly different from the observed proportions. Furthermore, on the assumption that the rarer gene is dominant, the gene frequencies calculated from the two-distribution proportions in table 2 are similarly 0.88 and 0.12. The data are thus consistent with the hypothesis of polymorphism at an autosomal locus. Figure 2 shows the theoretical density functions for the best-fitting mixture of two distributions; the overall density for a mixture of three distributions, which may be theoretically more realistic, is indistinguishable from figure 2 when similarly graphed.

In view of the highly significant correlation between the two factors, it is of interest to determine whether this polymorphism for Factor X is in any way related to the distribution of Factor IX. With this in mind, maximum-likelihood estimates were obtained for a fit of a mixture of two bivariate normal distributions, with common variance matrix, to the data. Quite unexpectedly, two local maxima were found on the likelihood surface. Upon reflection, however, this finding is not surprising if the factors are parts of biologically independent systems. This appears

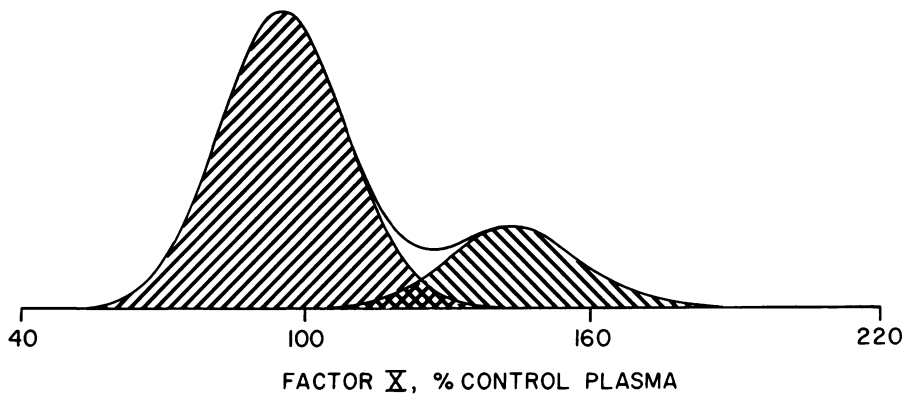


FIG. 2.—Theoretical component and overall density functions for a mixture of two normal distributions fitted to the data for Factor X.

to be confirmed by the estimates obtained, shown in table 4; the higher maximum is about $10^{12}$ times as large as the lower maximum.

Looking at the results for the higher maximum first, it is seen that the means and variance for Factor X, and also the proportions of admixture, are not significantly different from those obtained for Factor X in table 2. This maximum thus corresponds to the polymorphism for Factor X. It should be noted that the estimated means for Factor IX at this maximum are not significantly different, and, further-

TABLE 4

ESTIMATED PARAMETERS, AND STANDARD ERRORS, WHEN A MIXTURE OF TWO BIVARIATE NORMAL DISTRIBUTIONS WITH COMMON VARIANCE MATRIX IS FITTED TO SAMPLE

| | Higher Local Maximum | | | | Lower Local Maximum | | | |
|---|---|---|---|---|---|---|---|---|
| | Factor IX | | Factor X | | Factor IX | | Factor X | |
| | Parameter | SE | Parameter | SE | Parameter | SE | Parameter | SE |
| Means of first distribution ..... | 122.26 | 2.16 | 96.61 | 1.26 | 122.69 | 2.20 | 106.34 | 1.86 |
| Means of second distribution ..... | 130.73 | 4.45 | 147.36 | 2.65 | 193.93 | 40.46 | 137.93 | 20.48 |
| Common variances ..... | 730.57 | 72.02 | 204.01 | 24.51 | 652.05 | 77.13 | 603.07 | 61.74 |
| | Parameter | | SE | | Parameter | | SE | |
| Common correlation .... | 0.34 | | 0.10 | | 0.26 | | 0.11 | |
| Proportion in first distribution ..... | 0.80 | | 0.03 | | 0.98 | | 0.03 | |
| Proportion in second distribution ..... | 0.20 | | 0.03 | | 0.02 | | 0.03 | |

more, that the estimated common correlation between the two factors is not significantly different from that obtained (0.30) when a single bivariate distribution is assumed. Figure 3 is derived from the estimates obtained at the higher maximum and clearly shows that they correspond to a polymorphism for Factor X, virtually independent of Factor IX levels.

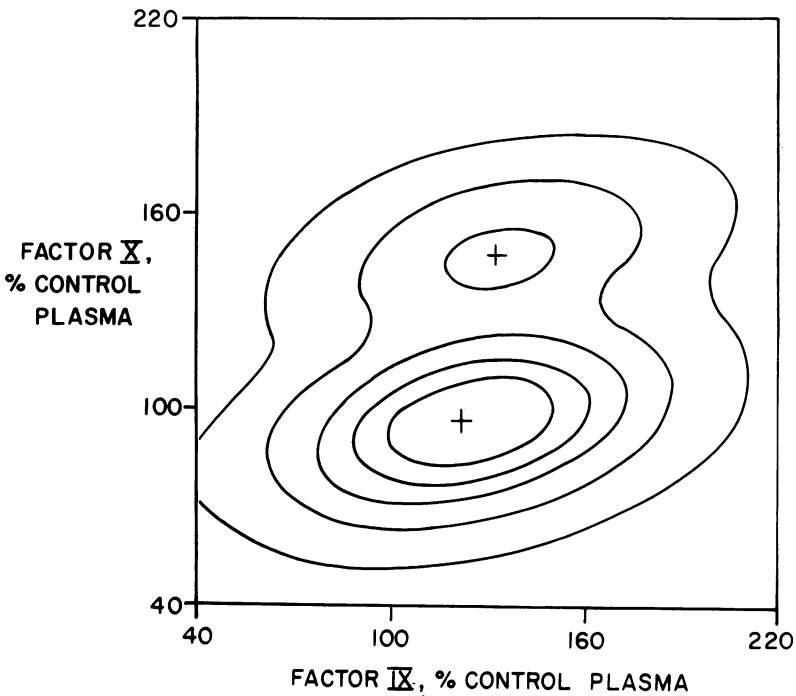


FIG. 3.—Equiprobability contours and means (+) for a mixture of two bivariate normal distributions fitted to the data, using maximum-likelihood estimates corresponding to the larger local maximum on the likelihood surface.

Conversely, if we look at the results given in table 4 for the lower maximum, we see that the estimates for Factor IX are virtually the same as the corresponding estimates for Factor IX in table 2 and that now the two means for Factor X are not significantly different; and, again, the correlation is not significantly changed. This can be seen pictorially in figure 4, indicating there may be a real polymorphism for Factor IX that is not significantly dependent upon levels of Factor X. However, a comparison of figures 3 and 4 shows that the polymorphism for Factor IX is much less clear. The significant correlation that is consistently found between the factors is most likely due to the common treatment undergone by each blood sample prior to the factor assays or the effects of environmental agents which affect both factors, for example, aspirin, etc.

Finally, in view of the significant correlation between age and Factor X, trivariate analyses were performed to investigate the possibility that the above results are

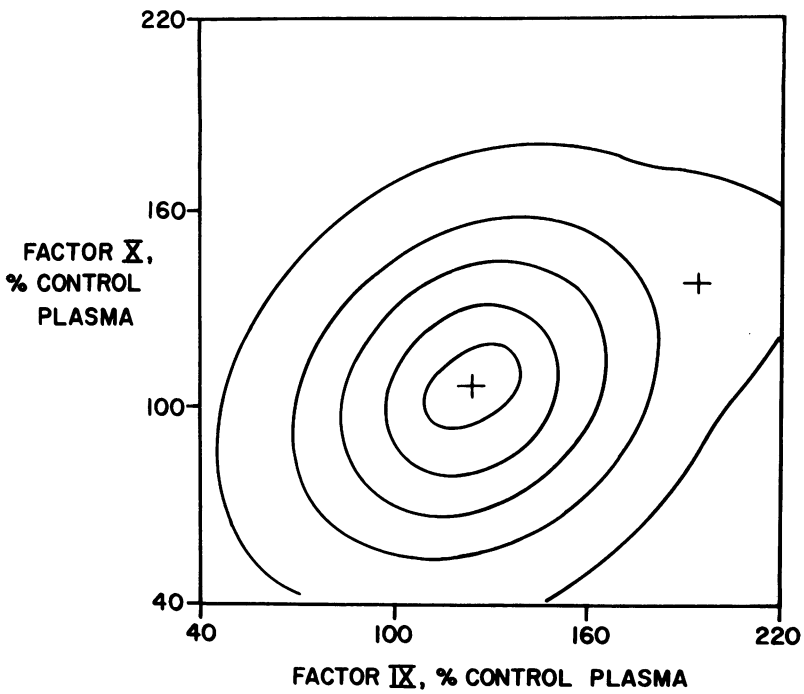


FIG. 4.—Equiprobability contours and means (+) for a mixture of two bivariate normal distributions fitted to the data, using maximum-likelihood estimates corresponding to the smaller local maximum on the likelihood surface.

an artifact, due perhaps to heterogeneity in the age distribution; this was found not to be the case. Similarly, in view of significant correlations with Factor II, appropriate quadrivariate analyses were performed; as indicated earlier, nothing was found that could invalidate our conclusions.

## DISCUSSION

There are questions, both physiologic and genetic, which can be examined by a study of these members of the so-called prothrombin complex. Most opinion favors the view that their synthesis and physiologic action are independent, although the factors possess a number of common characteristics and are very difficult to separate by chemical means. An alternative theory to complete independence is that Factor II (prothrombin) is a "parent" molecule and Factors IX and X are derived from it by cleavage during coagulation [17]. Thus, in a study such as ours, *no correlation* between the levels of these factors would be consistent with the usual hypothesis, and *very strong correlation* would support the Seegers hypothesis. The observed correlation coefficients are of the order of .30, values which are significant in a sample of this size, but which cannot be regarded as very strong correlations. Furthermore, it is significant that our analyses suggest a polymorphism for Factor X that is independent of Factor II and IX levels, and also the possibility of a poly-

morphism for Factor IX that is independent of Factor II and X levels. The results of this study are thus more compatible with the theory that the factors are synthesized independently, although they cannot completely rule out the alternative hypothesis.

Although highly suggestive, this study does not provide direct evidence for the existence of any genetic polymorphisms determining variations in the levels of the factors considered. Such evidence must come from the study of related individuals, using such methods of analysis as those proposed by Elston and Stewart [18]. There can be little doubt that the level of Factor X is a mixture of two (or more) distributions. Although the results may be considered ambiguous with regard to Factor IX, it should be noted that the second local maximum in the bivariate analysis confirms the univariate analysis. Day [19] has indicated that it is very easy, when performing such multivariate analyses, to estimate and find a mixture of two distributions when in fact sampling is from a single multinormal distribution. Using the estimates in table 4, it is possible to obtain estimates of the generalized distance between the pairs of populations; these are 3.68 at the higher local maximum and 2.85 at the lower one. The corresponding distances based on the univariate analyses (table 2) are 3.59 and 2.84 for Factors X and IX, respectively. Day considers the particular case of samples of size 200 for bivariate normal distributions, and it would seem probable from the simulation results he presents that all of these estimated generalized distances are significantly different from zero at the 5% level and, hence, unlikely to be due to sampling variation. We must therefore conclude that there may well be genetic polymorphisms for variation in the levels of both Factors IX and X. This suggests that carefully planned and executed family studies may clearly establish genetic polymorphisms; the task will, of course, be easier for Factor X.

SUMMARY

A study has been made of the plasma levels of physiologically closely related blood-clotting factors in a population of normal men. Analysis of the data strongly suggests a relatively high frequency of polymorphism for the Stuart factor (Factor X), with gene frequencies of about 0.85 and 0.15. There is also evidence of polymorphism for the Christmas factor (Factor IX), but at a much lower gene frequency. Study of the segregation of the factor levels in families ascertained through members with a high level should provide crucial evidence bearing on the question of polymorphism for these clotting factors.

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## APPENDIX

CLOTTING FACTOR ASSAYS ON 207 MALE VOLUNTEERS

| Subject No. | Age | Factor IX* (%) | Factor X* (%) | Subject No. | Age | Factor IX* (%) | Factor X* (%) |
|---|---|---|---|---|---|---|---|
| 1 | 20 | 85 | 78 | 56 | 26 | 143 | 162 |
| 2 | 20 | 80 | 136 | 57 | 19 | 142 | 130 |
| 3 | 21 | 141 | 134 | 58 | 19 | 136 | 174 |
| 4 | 18 | 109 | 92 | 59 | 20 | 180 | 161 |
| 5 | 18 | 84 | 87 | 60 | 18 | 148 | 157 |
| 6 | 20 | 117 | 112 | 61 | 21 | 145 | 111 |
| 7 | 22 | 100 | 102 | 62 | 20 | 142 | 109 |
| 8 | 21 | 108 | 91 | 63 | 20 | 217 | 160 |
| 9 | 21 | 84 | 83 | 64 | 19 | 124 | 136 |
| 10 | 19 | 87 | 134 | 65 | 23 | 153 | 162 |
| 11 | 19 | 56 | 83 | 66 | 20 | 145 | 162 |
| 12 | 22 | 66 | 95 | 67 | 20 | 126 | 113 |
| 13 | 23 | 99 | 101 | 68 | 19 | 148 | 112 |
| 14 | 21 | 90 | 92 | 69 | 19 | 144 | 93 |
| 15 | 18 | 91 | 94 | 70 | 20 | 110 | 91 |
| 16 | 18 | 86 | 88 | 71 | 18 | 142 | 89 |
| 17 | 23 | 109 | 113 | 72 | 20 | 120 | 96 |
| 18 | 21 | 89 | 105 | 73 | 20 | 90 | 81 |
| 19 | 19 | 112 | 90 | 74 | 26 | 121 | 90 |
| 20 | ... | 96 | 91 | 75 | 21 | 100 | 63 |
| 21 | 21 | 176 | 89 | 76 | 21 | 144 | 86 |
| 22 | 21 | 155 | 114 | 77 | 21 | 119 | 95 |
| 23 | 21 | 217 | 129 | 78 | 20 | 131 | 95 |
| 24 | 20 | 184 | 116 | 79 | 20 | 137 | 82 |
| 25 | 22 | 155 | 95 | 80 | 19 | 154 | 100 |
| 26 | 21 | 127 | 94 | 81 | 20 | 152 | 100 |
| 27 | 16 | 121 | 63 | 82 | 22 | 173 | 106 |
| 28 | 17 | 85 | 83 | 83 | 22 | 114 | 87 |
| 29 | 22 | 165 | 103 | 84 | 17 | 130 | 56 |
| 30 | 20 | 132 | 102 | 85 | 19 | 118 | 73 |
| 31 | 18 | 137 | 92 | 86 | 20 | 127 | 81 |
| 32 | 20 | 164 | 99 | 87 | 19 | 133 | 86 |
| 33 | 22 | 132 | 99 | 88 | 19 | 116 | 76 |
| 34 | 19 | 98 | 87 | 89 | 19 | 111 | 96 |
| 35 | 22 | 160 | 157 | 90 | 24 | 111 | 80 |
| 36 | 22 | 137 | 97 | 91 | 20 | 128 | 105 |
| 37 | 20 | 173 | 107 | 92 | 20 | 136 | 130 |
| 38 | 20 | 122 | 79 | 93 | 22 | 96 | 114 |
| 39 | 20 | 139 | 92 | 94 | 19 | 97 | 86 |
| 40 | 21 | 123 | 113 | 95 | 26 | 58 | 82 |
| 41 | 21 | 181 | 141 | 96 | 20 | 89 | 88 |
| 42 | 15 | 123 | 99 | 97 | 20 | 119 | 102 |
| 43 | 22 | 126 | 94 | 98 | 18 | 127 | 90 |
| 44 | 19 | 106 | 154 | 99 | 22 | 121 | 88 |
| 45 | 22 | 116 | 82 | 100 | 21 | 123 | 102 |
| 46 | 26 | 46 | 144 | 101 | 18 | 102 | 86 |
| 47 | 22 | 112 | 140 | 102 | 20 | 115 | 129 |
| 48 | 21 | 113 | 161 | 103 | 18 | 111 | 105 |
| 49 | 18 | 95 | 167 | 104 | 22 | 97 | 95 |
| 50 | 22 | 96 | 154 | 105 | 19 | 125 | 94 |
| 51 | 18 | 117 | 121 | 106 | 20 | 138 | 143 |
| 52 | 20 | 108 | 169 | 107 | 20 | 112 | 114 |
| 53 | 18 | 114 | 150 | 108 | 22 | 151 | 121 |
| 54 | 23 | 91 | 146 | 109 | 20 | 152 | 166 |
| 55 | 19 | 107 | 156 | 110 | 21 | 157 | 144 |

## APPENDIX (*Continued*)

| Subject No. | Age | Factor IX* (%) | Factor X* (%) |
|---|---|---|---|
| 111 | 22 | 144 | 134 |
| 112 | 23 | 140 | 137 |
| 113 | 19 | 166 | 144 |
| 114 | 20 | 169 | 103 |
| 115 | 18 | 118 | 83 |
| 116 | 22 | 95 | 85 |
| 117 | 22 | 84 | 109 |
| 118 | 19 | 127 | 117 |
| 119 | 22 | 100 | 109 |
| 120 | 19 | 112 | 120 |
| 121 | 21 | 87 | 139 |
| 122 | 19 | 116 | 118 |
| 123 | 19 | 98 | 88 |
| 124 | 18 | 127 | 87 |
| 125 | 21 | 147 | 143 |
| 126 | 20 | 84 | 85 |
| 127 | 19 | 159 | 102 |
| 128 | 21 | 141 | 115 |
| 129 | 19 | 168 | 102 |
| 130 | 19 | 170 | 122 |
| 131 | 24 | 149 | 98 |
| 132 | 21 | 116 | 85 |
| 133 | 22 | 101 | 89 |
| 134 | 20 | 128 | 107 |
| 135 | 21 | 99 | 98 |
| 136 | 21 | 134 | 135 |
| 137 | 19 | 138 | 104 |
| 138 | 23 | 109 | 80 |
| 139 | 20 | 124 | 82 |
| 140 | 20 | 139 | 101 |
| 141 | 19 | 130 | 98 |
| 142 | 18 | 181 | 128 |
| 143 | 17 | 140 | 90 |
| 144 | 18 | 123 | 74 |
| 145 | 21 | 146 | 92 |
| 146 | 23 | 181 | 97 |
| 147 | 19 | 106 | 85 |
| 148 | 24 | 123 | 116 |
| 149 | 20 | 120 | 102 |
| 150 | 22 | 125 | 110 |
| 151 | 23 | 112 | 136 |
| 152 | 17 | 136 | 78 |
| 153 | 19 | 139 | 106 |
| 154 | 21 | 133 | 190 |
| 155 | 20 | 105 | 67 |
| 156 | 21 | 116 | 109 |
| 157 | 22 | 149 | 106 |
| 158 | 23 | 101 | 93 |
| 159 | 19 | 91 | 98 |
| 160 | 23 | 83 | 80 |
| 161 | 20 | 144 | 127 |
| 162 | 22 | 119 | 121 |
| 163 | 22 | 144 | 107 |
| 164 | 20 | 117 | 113 |
| 165 | 21 | 144 | 109 |
| 166 | 18 | 145 | 105 |
| 167 | 20 | 163 | 140 |
| 168 | 19 | 117 | 99 |
| 169 | 20 | 108 | 104 |
| 170 | 23 | 134 | 90 |
| 171 | 20 | 128 | 100 |
| 172 | 19 | 93 | 79 |
| 173 | 21 | 124 | 93 |
| 174 | 21 | 121 | 95 |
| 175 | 21 | 154 | 88 |
| 176 | 20 | 148 | 98 |
| 177 | 22 | 132 | 102 |
| 178 | 18 | 146 | 94 |
| 179 | 19 | 125 | 144 |
| 180 | 22 | 145 | 102 |
| 181 | 18 | 146 | 112 |
| 182 | 18 | 108 | 115 |
| 183 | 21 | 108 | 95 |
| 184 | 21 | 155 | 146 |
| 185 | 17 | 118 | 94 |
| 186 | 16 | 101 | 79 |
| 187 | 21 | 99 | 100 |
| 188 | 21 | 146 | 138 |
| 189 | 21 | 124 | 113 |
| 190 | 18 | 73 | 79 |
| 191 | 21 | 116 | 112 |
| 192 | 19 | 169 | 130 |
| 193 | 22 | 109 | 116 |
| 194 | 20 | 108 | 97 |
| 195 | 17 | 105 | 77 |
| 196 | 19 | 91 | 75 |
| 197 | 19 | 135 | 90 |
| 198 | 19 | 95 | 91 |
| 199 | 18 | 114 | 99 |
| 200 | 18 | 102 | 101 |
| 201 | 18 | 151 | 87 |
| 202 | 21 | 109 | 86 |
| 203 | 22 | 112 | 106 |
| 204 | 19 | 114 | 77 |
| 205 | 21 | 112 | 85 |
| 206 | 21 | 128 | 138 |
| 207 | 17 | 94 | 83 |

* See Methods section for standard.