Genetic Factors Influencing Human Serum Trehalase Activity

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Trehalase (EC Number 3.2.1.28; trehalose 1-glucohydrolase) is an enzyme which splits the disaccharide trehalose (1- α -D-glucopyranosyl- α -D-glucopyranoside) to glucose; and it is widely distributed in both plants and animals. It was first discovered by Bourquelot (1893) in *Aspergillus niger* and since then has been found in bacteria, fungi, yeast, the serum of certain fishes, and in insects (Kalf and Reider, 1958). In mammals trehalase has been found in the intestinal mucosae of hog (Dahlqvist, 1960) and man (McMichael, Webb, and Dawson, 1965), human blood serum (Courtois, Debris, and Georget, 1962), and human kidney cortex (Lukomskeya and Tarasova, 1965; Courtois and Demelier, 1966; Sacktor, 1968; Van Handel, 1968, 1969).

It was observed by Courtois *et al.* (1962) that the serum trehalase activity of an individual human subject varied very little from one day to another but that there was a large variability between healthy subjects.

The object of the present research was to evaluate possible causes for this large intersubject variability in human serum trehalase activity.

Materials and Methods

$\alpha\alpha$ Trehalose (B.D.H. Biochemical Reagent); other chemicals were B.D.H. 'Analar' Reagents; acetate buffer; acetic acid/sodium acetate, pH 5.2, 0.5 M.

Solutions for 'Technicon' Autoanalyser version of the method of Hoffman (1937) for estimating glucose concentrations were as given in the 'Technicon' Autoanalyser Manual.

Serum samples. 10 ml. venous blood were collected from British White volunteer subjects, except where otherwise stated. The blood was allowed to clot, and following retraction the clear serum samples were stored at -20°C . to await analysis. The clot was used for red cell ABO grouping.

Saliva samples. Saliva samples collected for ABH secretor typing were obtained by having the subject spit into a test-tube. The quantity collected varied from 2 to 5 ml. Artificial stimuli to salivation were not used. The samples were boiled for 10 minutes on a water bath within one hour of collection, centrifuged at about 2000 r.p.m. for 5 minutes, and the supernatants were stored at -20°C . to await testing. ABO blood grouping was carried out by means of the standard tube techniques (Lawler and Lawler, 1957).

Determination of salivary ABH secretor status. All salivas were tested for H, A, and B activity quantitatively by means of doubling dilutions haemagglutination-inhibition technique with 0.145M sodium chloride as diluent. Standard salivas from known O, A, and B secretors and non-secretors were included in each day's estimations.

Estimation of serum trehalase activity was performed by a modification of the method of Courtois *et al.* (1962). Two incubation mixtures, the test and control, were prepared from each serum sample (Table I).

TABLE I
SOLUTIONS FOR SERUM
TREHALASE ESTIMATION

Constituent	Incubation Mixture (ml.)	
	Test	Control
0.05 M trehalose	0.2	Nil
1.0% sodium fluoride	0.2	0.2
Acetate buffer pH 5.2, 1.0M	0.1	0.1
Serum sample	0.2	0.2
Distilled water	0.3	0.5

Both incubates were kept at 37°C . in an air oven for six hours. The hydrolysis of trehalose was halted by placing the tubes at -20°C . to await assay. Sodium fluoride prevents glycolysis of glucose either present originally in the serum or liberated by hydrolysis of trehalose.

Glucose assay was performed using the technique of Hoffman (1937) as adapted to the 'Technicon' Autoanalyser. Pump speed was 40 samples per hour. A water cup was placed following each sample cup. An

array of standards at 25, 50, 100, 150, 200, and 250 mg. glucose per 100 ml. concentration was run before and after each batch of unknowns. The difference between test and control incubates indicated glucose liberated by trehalase activity. The enzyme activity is expressed according to the rules established by the International Union of Chemistry and Biochemistry: 'One unit of trehalase activity is equal to one micromole of trehalose hydrolysed in one minute by one litre of serum.'

Random subjects. Donors of blood for transfusion were asked to provide a specimen of saliva. At the end of blood donation a 10 ml. sample of blood was obtained from each subject before the venous cannula was removed. Serum samples from the first 20 male O secretors, the first 20 male O non-secretors, etc. were eventually analysed for trehalase activity. The experiment was terminated when 20 persons in each of the 8 phenotypic categories shown in Table II had been examined.

Subjects for investigation of effect of food on serum trehalase activity. These subjects were university laboratory and other staff and hospital staff whose ABO blood group and salivary ABH secretor status was already known.

TABLE II

MEAN, STANDARD DEVIATION (SD), AND STANDARD ERROR OF MEAN (SEM) OF $\log_{10}$ ($10 \times$ SERUM TREHALASE ACTIVITY IN UNITS) IN VARIOUS PHENOTYPIC CATEGORIES (20 SUBJECTS IN EACH)

Phenotype	Male			Female		
	Mean	SD	SEM	Mean	SD	SEM
O secretor	2.07	0.34	0.075	1.83	0.44	0.098
O non-secretor	1.94	0.40	0.089	1.66	0.40	0.089
A secretor	2.09	0.30	0.068	2.02	0.29	0.064
A non-secretor	2.06	0.33	0.074	2.05	0.32	0.072

TABLE IIa

GEOMETRIC MEAN AND FACTORS CORRESPONDING TO SD AND SEM OF SERUM TREHALASE ACTIVITY IN UNITS, OBTAINED BY TAKING ANTILOGARITHMS OF RESULTS GIVEN IN TABLE II

Phenotype	Male			Female		
	Geom. Mean (units)	SD Factor	SEM Factor	Geom. Mean (units)	SD Factor	SEM Factor
O secretor	11.75	2.18	1.19	6.79	2.76	1.25
O non-secretor	8.70	2.52	1.23	4.57	2.52	1.23
A secretor	12.27	2.02	1.17	10.54	1.94	1.16
A non-secretor	11.55	2.15	1.19	11.28	2.12	1.18

First test meal. 10 ml. venous blood were taken from the fasting subject who then consumed a standard breakfast meal between 8 a.m. and 9 a.m., consisting of two slices of toast spread with marmalade (but no butter) and one cup of tea containing a minimal amount of milk. Venous blood was taken at 2 and 4 hours after the meal, the subject having taken no other food or drink apart from tap water during this time.

Second test meal. On another occasion, at least a week later, 10 ml. venous blood were taken from the fasting subject, who then consumed a standard breakfast meal between 8 a.m. and 9 a.m., consisting of two slices of toast thickly spread with butter, with marmalade, and tea. Venous blood was taken at 2 and 4 hours after the meal, the subject again having taken no further food or drink apart from tap water.

Family studies. Twenty-nine two-generation family units (28 British, 1 African) comprising mother, father, and a variable number of offspring have been studied. Propositi were healthy university staff and friends, and none was ascertained because of being known to have any disease condition. Non-fasting venous blood samples were taken when convenient to the subjects concerned. Only one observation was made on each individual in the family study. *Statistical methods*

FIG. 1. Frequency distribution histogram of serum trehalase activities in the 160 subjects recorded in Table II.

TABLE III
ANALYSIS OF VARIANCE OF LOG_{10} ($10 \times$ SERUM TREHALASE ACTIVITY)

Item	Degrees of Freedom	Sums of Squares	Mean Squares	Variance Ratio
Blood group*	1	1.3111	1.3111	10.2430
ABH secretor status	1	0.2235	0.2235	1.7461
Sex	1	0.8807	0.8807	6.8805
Blood group $\times$ secretor status	1	0.2236	0.2236	1.7469
Sex $\times$ blood group*	1	0.4864	0.4864	3.800
Secretor status $\times$ sex	1	0.0005	0.0005	—
Blood group $\times$ sex $\times$ secretor status	1	0.0231	0.0231	—
Residual	152	19.4552	0.1280	—
	159	22.6041		

* Blood group (A or O).

were standard (Snedecor, 1956). The computations were done on the logarithm, to the base 10, of ten times the serum trehalase activities (units). This transformation normalized the data (see Fig. 1) and also resulted in reasonably constant variances in the various groups (see, for example, Table II). The factor 10, by which the measured values in the standard units were multiplied before taking the logarithms, avoided the introduction of

negative logarithms. It has no effect on the variances. Four figure logarithms were used to compute Tables III and V, but have been contracted to two places of decimals in Tables II and IV.

Results

Random subjects. The arithmetic means, standard deviations, and standard errors of the

TABLE V
HIERARCHICAL ANALYSIS OF VARIANCE OF LOG_{10} ($10 \times$ SERUM TREHALASE ACTIVITY IN UNITS) FROM THE FEEDING EXPERIMENT

Source of Variation	Degrees of Freedom	Corrected Sum of Squares	Mean Square	Contribution to Variance	Percentage of Variance
Phenotype	3	1.3398	0.4466	0.16904	98.51
Persons, within phenotypes	20	22.1212	1.1061		
Occasions or diets, within persons	24	0.1393	0.0058	0.00163	0.95
Times, within diets and persons	96	0.0892	0.0009	0.00093	0.54
	143	23.6895		0.17159	100.00

TABLE IV

LOG_{10} ($10 \times$ SERUM TREHALASE ACTIVITY IN UNITS) BEFORE AND AFTER EATING IN SUBJECTS OF VARIOUS PHENOTYPIC CATEGORIES (EACH ROW REPRESENTS ONE INDIVIDUAL)

Sex	ABO Blood Group	Salivary ABH Secretor Status	LOG_{10} ($10 \times$ Serum Trehalase Activity in Units)					
			First Fasting	2 Hours After Non-fat Meal	4 Hours After Non-fat Meal	Second Fasting	2 Hours After Fatty Meal	4 Hours After Fatty Meal
Male	O	Secretor	1.82	1.76	1.82	1.67	1.79	1.73
		"	2.00	1.97	1.97	1.91	1.97	1.95
		Non-secretor	1.98	2.00	2.03	1.89	2.02	1.97
		"	2.51	2.50	2.51	2.53	2.52	2.56
		Mean	2.08	2.06	2.08	2.00	2.07	2.05
Male	A	Secretor	1.19	1.19	1.19	1.43	1.36	1.29
		"	2.25	2.23	2.25	2.22	2.21	2.25
		"	1.95	1.93	1.95	1.91	1.97	1.89
		"	1.89	1.86	1.91	1.89	1.89	1.89
		Non-secretor	2.57	2.58	2.56	2.56	2.57	2.56
Female	O	"	0.89	0.89	0.89	1.06	1.06	1.06
		"	2.48	2.49	2.47	2.46	2.46	2.51
		"	2.05	1.95	1.98	2.00	1.91	1.97
		Mean	1.91	1.89	1.90	1.94	1.93	1.93
		Secretor	1.70	1.67	1.73	1.67	1.67	1.70
Female	O	"	2.12	2.09	2.12	2.10	2.14	2.13
		"	2.41	2.39	2.39	2.35	2.36	2.39
		"	2.09	2.09	2.13	2.13	2.09	2.12
		Non-secretor	1.54	1.54	1.63	1.63	1.63	1.59
		"	1.59	1.63	1.63	1.63	1.54	1.54
Female	A	"	1.95	1.84	1.87	1.84	1.82	1.84
		"	2.12	2.09	2.15	2.12	2.15	2.13
		Mean	1.94	1.92	1.96	1.93	1.93	1.93
		Secretor	2.47	2.38	2.49	2.46	2.47	2.48
		Non-secretor	2.06	2.05	2.05	2.06	2.08	2.08
Female	A	"	2.09	2.03	2.05	2.06	2.09	2.09
		"	1.19	1.19	1.19	1.29	1.29	1.29
		Mean	1.95	1.91	1.95	1.97	1.98	1.99

logarithmic serum trehalase values in the various phenotypic categories are shown in Table II. The estimate of the standard deviation within phenotypic categories is 0.358. Table IIa shows the anti-logarithms of the results calculated in Table II.

An analysis of variance has been performed and the results are shown in Table III. Sex and blood group are significant factors, males tending to have higher values than females and A subjects higher levels than O subjects (see Table II). Salivary ABH secretion is not a factor of significance.

From the analysis in Table III it can be estimated that of the total variance of the selected population tested in this experiment about 10% is due to blood group (O/A) and about 6% is due to sex.

Effect of diet. The results are presented in Table IV. Simple inspection of the data reveals how constant the serum trehalase activity is in a given individual and that it is not affected in any systematic manner by ingestion of either non-fatty or fatty foods. This view is borne out by analysis of variance.

The sex of the subjects was not taken account of in the design of the feeding experiments, and in consequence the phenotypic groups contained varying numbers of the two sexes. Though there was an indication in the previous experiment (Table III) of a sex factor effect, it was not felt to be sufficiently large to warrant a more complicated analysis than is shown in Table V.

The main conclusion from this experiment was that the 'repeatability' (see Falconer, 1960) without reference to phenotype was roughly 98.5% (Table V). Other main factors and interactions investigated in this experiment yielded no significant contributions to the total variance.

Since sex was included as a source of variation between persons, the 'repeatability' would presumably have been even higher if the analysis had been performed separately for males and females.

Heredity. There is a significant regression of uncorrected mean-offspring value on mid-parent value of $\log_{10} (10 \times \text{serum trehalase activity})$ in 29 families (Fig. 2). The regression coefficient $b = 0.58$, standard error of $b = 0.22$, intercept a (i.e. y when $x = 0$) $= 0.74$, correlation coefficient $r = 0.45$, t to test significance of b or r is 2.62 and $p < 0.02$. The regression coefficient gives an estimate of 'heritability' of 58%.

Serum trehalase activity appeared to rise up to the age of about 20 years. This observation is borne out by finding a significant linear regression of serum trehalase activity on age (up to 20 years) in

Fig. 2. Regression of mean offspring values of serum trehalase activity upon the mid-parent value [both in the transformation $\log_{10}(10 \times \text{serum trehalase activity in units})$].

both male and female offspring. Whether this relation was due to body weight rather than to age could not be investigated as weights were not recorded during this study. No significant regression of trehalase activity upon age was found in either male parents or female parents. An age-corrected trehalase activity was computed for offspring aged 20 years and below. The score for each offspring was adjusted to 20 years of age, using the calculated regression of male and female offspring $\log_{10} (10 \times \text{trehalase activity}) = y$, on age in years $= x$; $n = 54$, $b = 0.025$, $a = 1.54$, $r = 0.48$, $t = 3.94$, $p < 0.001$. This age-corrected value was used in computation and was found to make little difference to the heritability regression ($b = 0.57$).

There was no significant correlation between female parent enzyme activity and male parent activity ($n = 29$, $r = -0.24$, $t = -1.13$, $p > 0.10$).

There is evidence that the 24 subjects for the feeding experiment were drawn from the population as represented by Table II. The arithmetic mean of the $\log_{10} (10 \times \text{serum trehalase activity})$ of the first fasting value for the 24 subjects is 1.95, and the estimate of the mean for the population is 1.97 and the variance estimates are, respectively, 0.19 and 0.14.

The family parents are a sample of unrelated adults and the mean serum trehalase activity for these 58 subjects is 1.92 and the variance 0.13. The families can, therefore, be considered to be representative of the general population.

Discussion

The results clearly indicate that the genetic characters, sex, and ABO blood group made small but significant contributions to the variance of serum trehalase activity in the human population. Salivary ABH secretor phenotype has not been shown to exert a significant effect.

Feeding fatty or non-fatty food had no significant effect upon the serum trehalase activity under the conditions of the experiment. Though the effect of feeding was only followed for 4 hours, it seems unlikely that fasting (or feeding) status would have influenced the variance of the serum enzyme activity observed in the population survey. Nevertheless, no systematic observations have been undertaken by us to establish the constancy of serum trehalase activity over a long period of time of months or years, and it is possible that the relative levels of activity in different people could change substantially over such a period.

Trehalase is found in the jejunal mucosal epithelial cell (Borgström and Dahlqvist, 1958; McMichael *et al.*, 1965). Alkaline phosphatase from this same location is found in the sera of blood group O and B (but not A) subjects, and in the sera of secretors but not of non-secretors; and its concentration rises after fat ingestion (Langman *et al.*, 1966). It has been suggested that this rise is due to a greater permeability of the cell membrane allowing enzyme molecules to escape more easily from the cell (Langman *et al.*, 1966). The fact that trehalase did not behave in a similar manner is evidence against the hypothesis.

The regression coefficient computed for mean-offspring serum trehalase activity (y) upon mid-parent serum trehalase activity (x) gives an estimate of 'heritability'. Heritability has been defined as 'the ratio of additive genetic variance to phenotypic variance' (Falconer, 1960). Since the families appear to be representative of the population it seems that about 58% of the variance observed for this serum enzyme activity in the population is due to additive genetic factors. About 1% ($0.95\% + 0.54\%$; Table V) of the variance observed for this serum enzyme activity in the population is associated with temporary (short-term) 'environment' or errors of measurement. The remaining approximately 40% could be due to: (1) longer term 'environmental' changes within individuals and (2) non-additive genetic differences between individuals (Falconer, 1960).

Sex and ABO blood group have been shown to be minor components of the polygenic system controlling serum trehalase activity, but the other components are as yet unknown. The kidney cortex

contains the highest activity of trehalase in many mammals including Man (Courtois and Demelier, 1966), and it would be of interest to know if the enzyme at that site exhibits genetic polymorphism.

Summary

Trehalase is an enzyme which splits the disaccharide trehalose yielding glucose. This enzyme is found in human serum and there is a large variability between subjects but little variation in one subject from one day to another.

Three experiments have been performed to try and find out why this serum enzyme activity varies so much between subjects. First, to see if any of the three factors sex, ABO blood group, and salivary ABH secretor status make any significant contribution to the variance. The first two factors were found to make a small but significant contribution but secretor status did not. Secondly, to see if eating fatty or non-fatty foods significantly affected the serum trehalase activity. No significant effect was observed. The short term 'repeatability' of serum trehalase activity within an individual was 98.5%. Thirdly, families have been studied to see to what extent serum trehalase activity is a genetically determined character. A heritability of about 58% was found.

It was concluded that serum trehalase activity was under polygenic control; and that two genetic factors making small but significant contributions to this polygenic system were sex and ABO blood group.

The authors acknowledge the following. The United Liverpool Hospital Medical Research Committee (Chairman, Professor C. A. Clarke, C.B.E.) and the Nuffield Trust (via Professor C. A. Clarke, C.B.E.) for generous financial support. Dr. D. Lehané, Director, Liverpool Regional Blood Transfusion Service, for providing access to blood donors. Miss M. F. Bullen, S.R.N., for performing the feeding experiment. Mr. W. T. A. Donohoe, for secretor typing and blood grouping. Miss J. Lomath and Miss R. Hillary for technical assistance.

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