Ann. Hum. Genet., Lond. (1962), **26**, 47
Printed in Great Britain

Differences between families in the amount of salivary H substances*

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Schiff & Sasaki (1932) demonstrated that the secretion of the blood group substances in the saliva was inherited as if due to a single gene *Se*, with the homozygous recessive individuals, *sese*, being non-secretors. This hypothesis has been verified since that time by several investigators and is now generally accepted.

Even though it is quite certain that the secretor trait is dominant over the non-secretor, little experimental evidence has been advanced with respect to the inheritance of the amount of substance secreted and the possible effect of the blood-group genes on the substance secreted.

McNeil, Trentelman, Kuntzer & Fuller (1957) showed that certain A individuals secrete only the A substance and not the H, and likewise some B secrete only the B substance without H. Our experiments (Plato & Gershowitz, 1961) demonstrated that the H substance is secreted in much higher titre in O group individuals than in individuals of the three other ABO groups. The A_2 group secrete less H than the O, but considerably more than the A_1. The B group secrete little H substance and the AB the least. We also advanced the possibility that at least two different types of H substance, with differences in specificities, are present in the saliva.

With the above results at hand, the present study was undertaken with the objectives: (1) to determine whether quantitative differences in the secretion of H substance are genetically determined, and (2) to investigate the role of the ABO genes with respect to the type of substances to be secreted in the saliva.

Materials. Two types of anti-H materials were used. One was extracted from the seeds of *Ulex europaeus* (anti-H_u) and the other from the seed of *Cytisus sessilifolius* (anti-H_c). The method of extraction and the specific differences between these two lectins were described in our previous report (1961).

Data. Twenty-seven sibships (with ninety-eight sibs) were selected. All parents were blood group O and have had at least two secretor children. Sixteen of these families involved secretor × secretor matings and eleven secretor × non-secretor. A second sample of ten families was selected in which one of the parents was group B and the other group O, each family having produced one or more secretor children of each of the parental groups. The non-secretor sibs were excluded from the analyses of variance in all families. The selection of families in which one parent was B was based on the fact that persons of B blood group secrete only small quantities of H substance and persons of O blood group secrete large quantities of H substance. These differences make the detection of fluctuations more reliable.

Methods. Fourfold dilutions of each saliva specimen were prepared in sets of five tubes (1/4, 1/16, 1/64, 1/256, 1/1024) in duplicate. The first set of saliva dilutions was neutralized by equal amounts of 1/50 dilution of the *Cytisus* anti-H extract (anti-H_c), while the other set was

* This investigation was supported by grants from the Kellogg Foundation and the United States Atomic Energy Commission (project AT(11–1)–405).

neutralized by 1/20 dilution of the *Ulex* anti-H (anti-H_u). The tubes were left to stand for 20 min.; one drop of 2% suspension of O cells was added and the tubes read 20 min. later, after centrifugation. In cases of saliva from group B individuals, B substance was also titrated using a 1/16 dilution of commercial anti-B. Any plus (+) agglutination in the anti-H_u and anti-B inhibitions was considered as the neutralization end point. Because of the threshold effect of the anti-H_c extract (1961) a '2' agglutination or higher was taken as the end point. This threshold effect made *Cytisus* extract more reliable for comparative studies since it eliminated the subjective judgement involved in the discrimination between the negative and weak agglutinations. For computational convenience, the neutralization end points (1/4, 1/16, 1/64, 1/256, and 1/1024 and over 1/1024) were coded by the scores 1, 2, 3, 4, 5 and 6 respectively. Any person whose saliva inhibition was scored as 1 (inhibition titre 1/4 or less), was considered as non-secretor. The frequencies of the various scores for both the *Ulex* and *Cytisus* extracts were tested for normality. They fall within the normal distribution and agree quite closely with the frequencies reported by Matsunaga & Suzuki (1958).

DISCUSSION AND RESULTS

The data were statistically processed by the use of analysis of variance and intra-class correlation. K_0 values for the estimate mean squares were calculated by using Snedecor's (1946) formula

$$K_0 = \frac{1}{n-1}\left[\Sigma K - \frac{\Sigma K^2}{\Sigma K}\right].$$

In genetically determined quantitative traits one would expect significant variation between families on one hand and a high intra-class (intra-family) correlation on the other. Sibships in which both parents were of blood group O were the most appropriate for this comparison since they can only have group O offspring and will secrete only H substance. The results of the analysis of variance and intra-sibship correlation are summarized in tables 1 and 2 for the anti-H_c and anti-H_u respectively.

Table 1. *Analysis of variance for the inhibition scores of the anti-H_c* (Cytisus) *extract*

Source	D.F.	S.S.	M.S.	Estimate M.S.
Total	97	94·40	—	—
Between families	26	63·53	2·4435	$\sigma^2 \times k_0\sigma_m^2$
Error	71	30·87	0·4348	σ^2

$$F = \frac{2{\cdot}4435}{0{\cdot}4348} = 5{\cdot}62^{**} \text{ (26, 71. D.F.).}$$

Intra-class correlation, $r_1 = 0{\cdot}5614$.

For both extracts, there is significant variation at the 0·01 level between families, as shown by the F test values of Tables 1 and 2. The high intra-family correlations of 0·5614 for the *Cytisus* inhibition and 0·3464 for the *Ulex* suggest genetic control. For reasons expressed above the anti-H_c inhibition scores should be considered more reliable than the *Ulex* scores.

Since the O × O sibships of this study involved secretor × secretor as well as secretor × non-secretor matings, an examination was made to determine whether the between family variation (significant at the 0·01 level) observed in Tables 1 and 2 was due to consistently lower scores of

families of the latter mating type. In order to determine whether the mating types had any effect upon the scores of the offspring, the sibships were separated into two 'groups' according to the phenotype of the parents (either secretor × secretor or secretor × non-secretor). Separate analyses of variance were carried out for each group (mating type).

Table 2. *Analysis of variance for the inhibition scores of the anti-H_u* (Ulex) *extract*

Source	D.F.	S.S.	M.S.	Estimate M.S.
Total	97	76·20	—	—
Between families	26	39·34	1·5131	$\sigma^2 + k_0\sigma_m^2$
Error	71	36·86	0·5192	σ^2

$$F = \frac{1{\cdot}5131}{0{\cdot}5912} = 2{\cdot}914^{**} \text{ (26, 71. D.F.).}$$

Intra-class correlation, $r_1 = 0{\cdot}3464$.

The F values of the deviations between families were again significant for both mating types. On the other hand the F tests of the variances of the two groups were non-significant for both the *Ulex* and *Cytisus* extracts. Because of the unequal number of families within each group as well as the inequality of the sibship sizes, these analyses of variance should only be considered as approximations.

The results of the analyses of variance and intra-class correlations suggest, first, that the amount of H substance secreted in the saliva is genetically determined and, secondly, that the two allele system of Schiff is not sufficient to explain this quantitative variation. Clarke, McConnell & Sheppard (1960), in a paper published while the present study was in progress, have reached similar conclusions from data limited to sib-pairs.

Ten B × O matings, with at least one B and one O secretor children, were studied. An effect of the ABO blood groups upon the type of substance secreted in the saliva could not be demonstrated in the statistical sense but certain trends are noteworthy. These are:

(1) Within the same families, the O children secreted much higher amounts of the H substance than the B group sibs. This finding agrees with the results of the population study reported previously (Plato & Gershowitz, 1961), where the B individuals were found to secrete much lower amounts of the H substance than the O group persons.

The fact that the B sibs of the B × O matings secreted much less H substances than the O sibs suggests that the B substance in the saliva is formed at the expense of the H, and especially H_u (the type of H which is inhibited by the *Ulex europaeus* extract). This supports the theory expressed by Watkins (1959) that the A and B substances are formed from a precursor H substance.

(2) The amount of basic substance converted to B seems to be a constant one so that the inhibition titre of the B substance shows little variation between families. To this extent, in some families of low secretion, all the available H substance is utilized towards the formation of B so that no H substance is observable in these B individuals.

In view of the findings of this study a genetic hypothesis may be advanced with regard to the control of the ABO blood group substances in the salivary secretions. This hypothesis in some respects resembles the one proposed recently by Watkins (1959), but allows for the quantitative

variation of the amount of the secreted substance. The factors involved in our hypothesis are first, the classical *se* gene of Schiff which determines whether the basic substance, very likely some type of H closely related to the H_u, will be secreted or not. Secondly, a set of independent modifiers or multiple alleles determines the amount of basic substance to be secreted. Discrimination between the hypotheses of multiple allelism or independent modifiers is not possible at present due to the lack of key families. Clarke *et al.* (1960) have suggested that the inheritance of variable quantities of H substance is due to 'polygenes' or independent modifiers. The results of this study, in which whole families were tested, agree with their findings and so confirm their report. We feel, however, that discrimination between the hypotheses of multiple alleles or independent modifiers as controllers of the level of H substance is not possible at present. Final judgment must be reserved until data on the analysis of many large families can be accumulated. Thirdly, the ABO genes will determine the form in which the basic substance will be expressed (A, B, H or a combination of these). With the above hypothesis it is possible to explain both types of McNeil *et al.* (1957) aberrant secretors. The inability of some A, B, or AB individuals to secrete the H substance is the result of low secretion of the precursor O, basic H substance. Since a certain amount of basic H substance seems to be allocated to A or B or both, very little if any substance is left to be expressed as H. On the other hand, the absence of the A or B substances in the salivas of persons of A or B blood group who do have H substance is probably a result of some form of biochemical block which prevents the conversion of the basic substance into A, B, or AB according to the blood type.

SUMMARY

The saliva ABO inhibition titres of the members of twenty-eight O × O and ten B × O mating types were determined. The results of the examinations suggest that: (1) A precursor 'H' substance is formed by all secretors which is converted into A, B, or AB depending on the individual's blood group. (2) the titres of the H substance secreted by members of the same sibship and of the same blood group are very uniform (high intra-class correlation) indicating genetic control over the amount of the soluble ABO substances secreted. A genetic hypothesis was put forward to account for these findings.

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