OVAL BLOOD CELLS IN HUMAN SUBJECTS TESTED FOR LINKAGE WITH TASTE FOR PTC, MID-DIGITAL HAIR, HAIR COLOR, A-B AGGLUTINOGENS, AND SEX

BARBARA S. BURKS
Carnegie Institution of Washington
AND
HELEN WYANDT
University of Nebraska

Received October 18, 1940

A FIELD study was undertaken in 1939 by WYANDT in order to collect human pedigrees showing oval red blood cells. The rare but non-pathological condition of elliptical erythrocytes was apparently known in medicine even before the first published account which we have been able to locate (DRESBACH 1904), but its hereditary nature was not well established until 1929 (HUNTER and ADAMS). CHENEY (1932) first reported the trait to be a simple Mendelian dominant on the basis of a three-generation pedigree, and STRAUSS and DALAND (1937) furnished confirmation from another three-generation pedigree. Several other cases of familial elliptocytosis have been reported subsequently. In a forthcoming paper WYANDT will publish pedigrees on oval cells in three family lines involving more than a hundred individuals. Her investigation confirms the dominant mode of unit inheritance suggested by previous less extensive studies.

During the course of WYANDT'S field study it was possible for her to gather data upon several additional traits that could be used as test factors in a search for linkage.

Taste for PTC (phenyl-thio-carbamide) was investigated by the use of filter paper dipped into a saturated acetone solution of the compound, dried, and cut into pieces 1 cm square, excepting in a family represented by two sibships to whom PTC crystals on the tip of a moistened tooth pick were presented.

The presence or absence of hairs over the middle phalanx of the fingers (exclusive of thumbs) was determined by examining both hands in daylight (with the exception of one sibship which was examined by electric light). An effort was made to detect fine, nearly invisible hair and to note the presence of hair follicles. When follicles were seen, hair was reported as present even if no hair was visible.

Hair color was matched at the back of the head, close to the scalp, to hair samples of standard shades (the "Shur-on" scale manufactured by Schwerner, Oppenheim Co., New York). Examination was made by daylight (except for one sibship which was seen by electric light).

Eye color was matched to the Martin-Schultz scale (J. F. Lehmanns Verlag, Munich). However, since only five families containing seven pairs of offspring were "crucial,"—namely, heterozygous families in which linkage could be expected to appear if it exists,—linkage tests for eye color could not be made with these data.

Classification into blood group A, B, or O was made from suspensions of cells in two percent sodium citrate in physiological saline, mixed with equal volumes of standard Group A and Group B sera in hanging drop preparations.

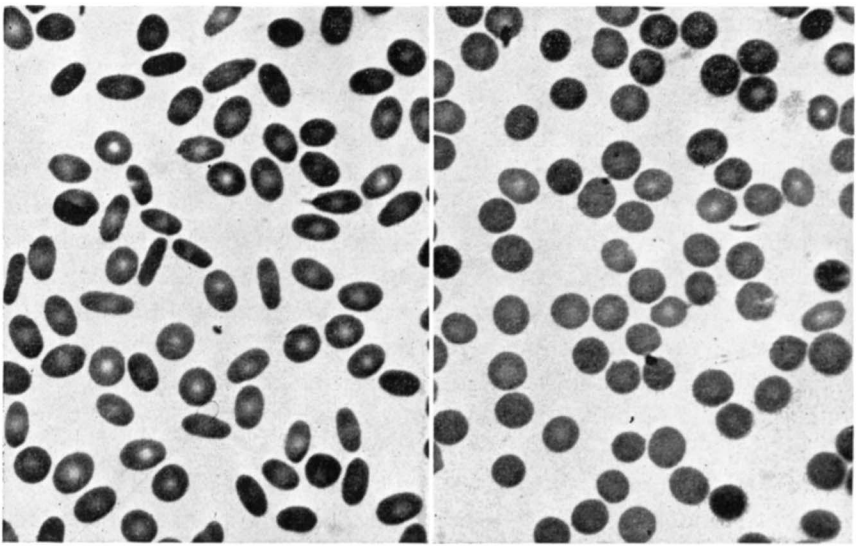

FIGURE 1.—Left, oval cells. Right, round cells.

For classifying individuals with respect to oval and round red blood cells, moist preparations were inspected under No. O cover slips, using small enough drops of blood so that a single layer of cells was obtained. One thousand cells were tabulated for each subject. The percentages of oval cells gave a dichotomous distribution, one group of subjects classified as "oval" showing percentages that varied from 72 to 96 and another group classified as "round" showing percentages that varied from zero to 10. Figure 1 shows micro-photographs of oval cells and round or normal cells.

The main method used for tests of linkage was that of "like" and "unlike" sibling pairs (BURKS and TOLMAN 1932, BURKS 1938, PENROSE 1935). FISHER'S (1934–35) $\underline{u}$ statistic, although more sensitive, at least in the "single back-cross" mating, did not in general meet the requirements of the present study because of the irregularity of the mode of transmission of

the test factors (other than A-B-O groups and sex). The method which was used examines, in effect, the fourfold correlation between sibling-pair likeness-unlikeness on two traits and can be applied even if data from only a single generation are available. The method becomes more sensitive, however, if "non-crucial" sibships may be discarded, and consequently no sibships were retained that were clearly unsuited to test a linkage hypothesis (namely, families in which an oval-celled parent showed the recessive phenotype in the test factor), although sibships have been tabulated which may or may not be crucial (namely, a few sibships in which there is no offspring recessive for the test factor to prove the oval-celled parent doubly heterozygous).

In table 1 the data for the linkage tests consist of the "observed" entries, which show the number of sibling pairs who are "alike" on both traits, "unlike" on both, and "alike" on one but "unlike" on the other. Clearly in the absence of linkage, the ratio of the entries in the first two columns should equal the ratio of the entries in the third and fourth columns. The significance of deviations from proportionality was tested by chi-square (using FISHER's short-cut formula for the fourfold table and applying YATES's correction for continuity). The degree of deviation—in a direction favorable or unfavorable to a linkage hypothesis—was also calculated by the BOAS-YULEAN ϕ for a fourfold point distribution.

The data have also been examined as to evidence for "non-linkage," since failure to establish linkage in small population samples does not *ipso facto* disprove its existence. The tendency in human genetics has perhaps been too readily to label as negative data which do not stand up to tests of significance, without inquiring whether the data are adequate for establishing positive results if present. Though admittedly loose linkages would take more data to become established than we ordinarily obtain in field studies of human populations, it evidently has "mop-up" value to inquire whether pedigree findings are definitely inconsistent with close linkages. To this end we have set up "expected" distributions on certain assumptions with respect to recombination.

For simplicity's sake we have assumed zero crossing over in the case of mid-digital hair, for which both parents are often heterozygous, since under this condition (even if there is no recombination) sibling pairs will appear in all the four "expectation" categories of table 1 and thus make the chi-square test applicable. It is clear from the results of table 1 that even if recombinations significantly higher than zero were tested for,—for example, .10 or higher,—the results would still constitute strong evidence against linkage.

In the case of hair color a "non-linkage" test was not made, because the mode of inheritance of hair-color is not yet well enough agreed upon to

justify the setting up of an "expected" linkage distribution. With the remaining traits, for which only one parent is frequently heterozygous, we have set up expected distributions on the assumption of 25 percent re-

TABLE 1

Oval blood cells tested for linkage and non-linkage with five normal traits.

TEST FACTOR	BLOOD CELLS ALIKE		BLOOD CELLS UNLIKE		LINKAGE TEST: DEV. OF OBSERVED DATA FROM CHANCE DISTRIBUTION			NON-LINKAGE TEST: DEV. OF OBSERVED DATA FROM LINKAGE EXPECTATION	
	ALIKE	UNLIKE	ALIKE	UNLIKE	ϕ	χ^2 (n=1)	P	χ^2 (n=2)	P
Taste for PTC									
Observed	5	1	7	1	−.06	.30	.50–.70		
Expected (most fav. to linkage)	3.75	2.25	3.00	5.00				9.64	<.01
Expected (least fav. to linkage) (if c=.25)	5.53	.47	7.59	.41				1.57	.30–.50
Mid-digital hair									
Observed	10	10	18	10	−.14	.48	.50		
Expected (if c=0)	17.50	2.50	14	14				28.00	≪.01
Hair color									
Observed	10	8	13	11	.01	.05	.80–.90		
A-B-O groups									
Observed	16	13	17	24	.14	.79	>.30*		
Expected (if c=.25)	18.34	10.66	16.25	24.75				.88	.60–.70
Sex (oval fathers)									
Observed	9	10	18	12	−.12	.33	.50–.70		
Expected (partial sex if c=.25)	11.88	7.12	11.25	18.75				8.34	.01–.02
Sex (oval mothers)									
Observed	6	4	10	7	.01	.12	.70–.80		

* When the families contributing to the test of oval cells×A-B-O groups are separated into double and single-back-cross families and tested by FISHER'S u_{11} and u_{31}, the fiducial chance of the results observed becomes approximately .06.

combination. As in the case of mid-digital hair, the chi-square test was used to test the observed data for their deviation from the hypothetical.

Though several of the fourfold distributions were based upon too few cases to permit a rigorous application of the chi-square test, the general conclusion reached by the chi-square test in such cases is probably sound: that the data neither establish nor disprove genetic linkage.

TASTE FOR PHENYL-THIO-CARBAMIDE

The data of BLAKESLEE and SALMON (BLAKESLEE 1932, BLAKESLEE and SALMON 1931) and of SNYDER (1931, 1932) give satisfactory evidence that inability to taste phenyl-thio-carbamide is transmitted as a simple recessive. The presence of modifiers, however, is suggested by the fact that "tasters" and "non-tasters" of the crystals or of filter paper treated with a saturated solution vary within their groups in thresholds of sensitivity to PTC solutions and show some overlapping between their groups (SETTERFIELD, SCHOTT and SNYDER 1936, HARTMANN 1939[1]). While occasionally the threshold of an individual changes significantly from one test to another (SALMON and BLAKESLEE 1935), PTC data offer results sufficiently clear-cut on the whole to permit tests for the detection of linkage.

Five sibships containing 13 offspring (none of whom happened to be young children) were used in the tests of linkage with oval cells. These yielded the 14 sibling pairs distributed in table 1 and showed no evidence of linkage.

The five sibships were then retabulated to show expectations when the distribution of oval- and round-cell offspring remains the same in each sibship but with a recombination ratio between the factors for oval cells and taste for PTC of .25. Since there were three families which could not be proved to have crucial genetic composition (even when data for parents were available), distributions were calculated upon hypotheses "most favorable" and "least favorable" to detection of linkage. For example, in one sibship the most favorable supposition was that the oval-cell parent was heterozygous for taste; the least favorable supposition, that he was homozygous dominant for taste.

When the "most favorable" supposition is used, the probability of a chance deviation from the linkage distribution of a magnitude as great as that obtained is less than .01. But when the "least favorable" supposition is used, the probability reaches the .30 to .50 range. In view of the small number of cases and the equivocal results, we should list this pair of traits as among those for which close linkage is neither established nor disproved.

[1] HARTMANN presents a bimodal curve for PTC threshold and shows that while all her series of non-tasters of crystals reach or exceed the threshold for solutions represented by the minimum frequency on the curve, so do one fifth of her series of tasters of crystals.

PRESENCE OR ABSENCE OF MID-DIGITAL HAIR

DANFORTH (1921) infers from his data that absence of hairs over the middle phalanx of the fingers may be due to one primary recessive. A forthcoming paper from the Genetics Record Office supports this view.

No restrictions as to age of subjects were made and none seem necessary, since the youngest subjects (age two or three) were found to have mid-digital hair present.

Ten usable sibships contained 33 offspring yielding 48 pairs as distributed in table 1.

No evidence in favor of linkage appears. The sibships were retabulated to show expectations when the distribution of oval- and round-cell offspring remains the same in each sibship, but with zero recombination between the factors for oval cells and mid-digital hair. When there was doubt as to the genetic composition of a family, the formula consistent with the distribution of offspring but least favorable to detection of linkage was assumed. For example, in one sibship of five offspring it was assumed that the mother, who was not seen, was heterozygous rather than recessive for mid-digital hair, since this would dilute the evidence obtainable from the doubly heterozygous father. Oval-cells and mid-digital hair were considered as alternating between coupling and repulsion in the doubly heterozygous parents of successive families, which presumably would yield an equitable result in the frequencies of "like" and "unlike" sibling pairs for a group of families even if not for individual families.

Since the expected frequency of the second entry is less than five, there is question as to the large contribution which this entry appears to make to chi-square. If we should arbitrarily reduce this contribution to the amount contributed by column 1, P would still be in the neighborhood of .01. Close linkage between oval cells and mid-digital hair, the more so because the "expected" values represent those least favorable to the detection of linkage, seems therefore to be ruled out; rarely would data in the presence of close linkage deviate so far from expectation by chance.

HAIR COLOR

Although the mode of transmission of hair color, and even the classification of hair color, are by no means generally agreed upon, the schema used by one of us in an earlier study of linkage in man (BURKS 1938) gave results sufficiently promising to justify its continued use for linkage tests until a better formulation is established through further research.

Only the dark-light series, presumably due mainly to melanic pigment, is here considered. Red shades are discarded, being too rare in this material to warrant separate treatment. Red tints are disregarded in brown hair,

the hair samples being judged only for darkness and lightness. Instead of classifying individuals upon an absolute scale, which would be an arbitrary procedure with a trait like hair color showing continuous variation in the general population, we (quoting from BURKS 1938) "followed the principle of classifying sibling pairs as 'similar' in hair color if their shades were so close together on the chart that discrimination between the two shades of hair was doubtful or even liable to a reversal of direction if repeated on another day. All other sibling pairs were counted 'dissimilar'." The previous result of using such a classification was consistent with the hypothesis that the dark-light series of hair color is mainly determined in a single gene locus corresponding to a number of non-dominant alleles, giving "(1) in families with two heterozygous parents, four potential phenotypes corresponding to four genotypes [H_1H_3, H_1H_4, H_2H_3, H_2H_4] and (2) in families with one homozygous parent (or families in which certain phenotypes are not readily distinguished), two potential phenotypes. . . ."

No offspring younger than fifteen years were used in the tabulations, since up to about this age rapid changes in hair color are taking place. Individuals with gray hair were of course not included. Eight sibships containing twenty-seven usable offspring yielded the forty-two pairs distributed in table 1.

Like the previous tables of observed data, this one gives no evidence in favor of linkage. For reasons already explained, hair color was not tested for "non-linkage."

A-B AGGLUTINOGENS

The generally accepted theory of the main blood groups as due to three allelemorphs, A and B dominant with respect to their absence, but not with respect to each other, has been employed in the interpretation of data. Siblings were classified as "alike" or "unlike" in the blood agglutinogens, but only with respect to the alleles possessed by their doubly heterozygous parent. For example, in one sibship the oval-celled parent was B, the round-celled parent A, so A was merely treated as "not-B" and would have been grouped with O if any O's had appeared. Since more families were available here than were available for the other test factors, only families known to be "crucial" (by the criteria of parental combinations and traits appearing in offspring) were included in the tabulations.

Using twelve sibships, containing 37 offspring yielding 44 pairs, gave the data shown in table 1.

This is the first indication of a positive test; although P is not very low, we should bear in mind that the probability of getting a chance deviation in a direction favorable to linkage is approximately half this magnitude.

A procedure similar to that used with the other traits was employed to

build up a hypothetical linkage table for a recombination ratio of .25. Most of the usable families were "double back-cross"—that is, had one doubly heterozygous parent and one doubly homozygous parent—which greatly simplified the calculations. In the three families having both parents heterozygous for blood type A, the first was assumed to be "most favorable" (in coupling and repulsion conditions) for the detection of linkage, the second, "least favorable," and the third, "most favorable."

The calculations indicate that in spite of the possible exaggeration of the requirements for close linkage, the probability that distributions consistent with close linkage would deviate through chance by an amount as great as that observed is .60 to .70. Thus the two complementary sets of calculations are both consistent with close linkage, although they by no means establish it.

The results looked interesting enough (and the segregation of oval cells and of A-B agglutinogens regular enough) to warrant a reworking of the data by FISHER'S (1934–35) $\underline{u}$ test—namely, for the double back-cross families having an expected 1:1:1:1 ratio of offspring in the absence of linkage:

$$u_{11} = (a-b-c+d)^2 - (a+b+c+d)$$

and for the single back-cross families having an expected 3:3:1:1 ratio of offspring:

$$u_{31} = (a-b-3c+3d)^2 - (a+b+9c+9d)$$

where a, b, c, and d are frequencies of offspring of four types within a family. For twelve double back-cross families the sum of the $\underline{u}_{11}$ was 18 with S.E. 15.9; for three single back-cross families the sum of the $\underline{u}_{31}$ was 22 with S.E. 20.8. Combining the two sets of data gives a $\underline{u}$-score of 40 with S.E. 26.2, the ratio of $\underline{u}$ to S.E. being 1.53. If the sampling distribution of $\underline{u}$ is something like normal, the probability of a chance result as favorable to linkage would be about .06.

To rule out the possibility that correlation between oval cells and the blood groups in the general population might simulate a "linkage distribution," a contingency table was set up for oval and round blood cells against blood groups A, B, and O (there were too few AB's to include). A chance distribution was very closely approximated, chi-square corresponding to P in the range .50 to .70.

INCOMPLETE SEX LINKAGE

Since the appearance of HALDANE'S (1936) article on incomplete sex linkage in man, it has been clear that all genes which appear at first sight to be autosomal must be tested for possible locus in the homologous segment of the X Y chromosome pair. The unique attribute of an incompletely

sex-linked character is that it transmits from the father preferentially to offspring of the same sex as the father's affected parent. When, as in the present data, it is frequently not known which of the paternal grandparents was affected, an indirect test (less sensitive than a direct test) can be made by ascertaining whether individual families show a tendency for affected offspring to be of the same sex.

Only the families in which the father transmits the trait are crucial for detecting incomplete linkage, but we have worked up the data on offspring of oval-celled mothers as well as fathers so that one tabulation could serve as a control upon the other.

With oval-celled father nine sibships containing 32 offspring yielding 49 pairs were available.

With oval-celled mother there were eight sibships containing 24 offspring yielding 27 pairs.

No evidence for linkage appears. The supplementary calculation (for the cases having oval-celled fathers) based on a hypothetical distribution for recombination .25, moreover, leaves us with reasonable assurance that oval-cell is not incompletely sex-linked with a crossover ratio less than .25.

DISCUSSION AND SUMMARY

A systematic collection of data on "test factors" was made during the course of a field study of families characterized by the appearance of oval blood cells. It is obviously a desirable procedure in any study conducted to establish or corroborate the mode of transmission of a human trait to include at the same time a search for linked traits. It is to be hoped that such a search will be carried out routinely in future studies, not only with the test factors here employed, but with a larger number of test factors as these become available.

Tests previously reported for the detection of linkage between random pairs of traits have generally given results consistent with chance recombination—both because recombination is in fact chance (two given traits having only one chance in twenty-four of belonging to the same linkage group) and because the number of pedigrees has often been too small to give clear-cut detection of linkage even if present. The investigations on record have commonly failed to distinguish between the reasons for negative results, with the consequence that many of the data laid aside with "no evidence for linkage" might well be reexamined as to "evidence for non-linkage." In the present study evidence for non-linkage as well as for linkage was considered by testing the observed data for their deviation from hypothetical linkage tables as well as from chance distributions. The hypothetical linkage tables were set up for a recombination ratio of zero excepting when this gave expectations close to zero in certain categories, in

which case a recombination ratio of .25 was arbitrarily assumed. The method could obviously be extended to cover any desired recombination ratio.

Certain refinements over the method here used would also be useful, although we believe the broad conclusions drawn out of the data are valid. In particular, it would be desirable: (1) to devise a way of dealing systematically with the sibships which may or may not be "crucial" for detecting linkage (as when one parent may be either simplex or duplex for the test factor); (2) to devise a method for allowing for irregularities in the transmission of traits mainly but not wholly attributable to a single gene substitution; (3) to gain more light upon the appropriateness of the chi-square test when sibships supply more than one pair of siblings. It seems clear that in the presence of linkage, sibling pairs in a sibship larger than two cannot be considered as independent. On the other hand it is not immediately clear (though MATHER 1938, p. 118, recommends it) that all pair-combinations provided by sibships larger than two can be treated as independent in the absence of linkage.

If, as we believe, these reservations do not seriously influence the results of the present study, it seems justifiable to conclude that close linkage may be ruled out for oval cells with mid-digital hair and with sex (that is, evidence is against incomplete sex linkage), that the data are equivocal for oval cells with taste for phenyl-thio-carbamide and with hair color (probably negative for the latter), and that the possibility of linkage between oval cells and the A-B agglutinogens deserves further investigation.

LITERATURE CITED

BLAKESLEE, A. F., 1932 Genetics of sensory thresholds: taste for phenyl-thio-carbamide. Proc. Nat. Acad. Sci. **18**: 120–130.

BLAKESLEE, A. F., and M. R. SALMON, 1931 Odor and taste blindness. Eugen. News **16**: 105–109.

BURKS, B. S., 1938 Autosomal linkage in man—the recombination ratio between congenital tooth deficiency and hair color. Proc. Nat. Acad. Sci. **24**: 512–519.

BURKS, B. S., and R. S. TOLMAN, 1932 Is mental resemblance related to physical resemblance in sibling pairs? J. Genet. Psychol. **40**: 3–15.

CHENEY, G., 1932 Elliptic human erythrocytes. J. Amer. Med. Ass. **98**: 878–881.

DANFORTH, C. H., 1921 Distribution of hair on the digits in man. Amer. J. Phys. Anthrop. **4**: 189–204.

DRESBACH, M., 1904 Elliptical human red corpuscles. Science **19**: 469.

FISHER, R. A., 1934–35. The detection of linkage with "dominant" abnormalities. Ann. Eugen. **6**: 187–201.

HALDANE, J. B. S., 1936 A search for incomplete sex-linkage in man. Ann. Eugen. **7**: 28–57.

HARTMANN, G., 1939 Application of individual taste difference towards phenyl-thio-carbamide in genetic investigations. Ann. Eugen. **9**: 123–135.

HUNTER, W. C., and R. B. ADAMS, 1929 Hematologic study of three generations of a white family showing elliptical erythrocytes. Ann. Int. Med. **2**: 1162–1174.

MATHER, K., 1938 The measurement of linkage in heredity. ix+132 pp. New York: Chemical Publishing Company of New York.

PENROSE, L. S., 1935 The detection of autosomal linkage in data which consist of pairs of brothers and sisters of unknown parentage. Ann. Eugen. **6:** 133–138.

SALMON, T. N., and A. F. BLAKESLEE, 1935 Genetics of sensory thresholds: variations within single individuals in taste sensitivity for PTC. Proc. Nat. Acad. Sci. **21:** 78–83.

SETTERFIELD, W., R. G. SCHOTT, and L. H. SNYDER, 1936 The bimodality of the threshhold curve for the taste of phenyl-thio-carbamide. Ohio J. Sci. **36:** 231–234.

SNYDER, L. H., 1931 Inherited taste deficiency. Science **74:** 151–152. 1932 The Inheritance of taste deficiency in man. Ohio J. Sci. **32:** 436–440.

STRAUSS, M. B., and G. A. DALAND, 1937 Hereditary ovalocytosis (human elliptical erythrocytes). Observations on ten cases in one family. New Engl. J. Med. **217:** 1–11.