Table 1. Frequency distribution of children in reciprocal mating A × O.

Number of children	Observed number of families	
	♀A × ♂O mating	♀O × ♂A mating
0	No record	No record
1	82	95
2	48	48
3	32	29
4	14	15
5	8	8
6	2	3
7	3	2
8	1	1
Total	190	201

critical studies in order to eliminate the possibility of differential fertility in A and O mothers, and of reproductive compensation concurrent with the effect of incompatibility. From the data of Matsunaga and Itoh (*2*) and of Haga (*3*), the following values are derived for the average number of children of the O × O, ♀ A × ♂ O, and ♀ O × ♂ A matings (abbreviated C_{OO}, C_{AO}, and C_{OA}, respectively); the average number of pregnancies (P_{OO}, P_{AO}, and P_{OA}); and the proportions of prenatal deaths per pregnancy (PP_{OO}, PP_{AO}, and PP_{OA}): C_{OO}, 2.5388 ± 0.1151; C_{AO}, 2.5070 ± 0.1055; C_{OA}, 2.2958 ± 0.1136; P_{OO}, 3.1187 ± 0.1443; P_{AO}, 3.0775 ± 0.1320; P_{OA}, 3.2667 ± 0.1501; PP_{OO}, 0.0761 ± 0.0101; PP_{AO}, 0.0778 ± 0.0091; and PP_{OA}, 0.1314 ± 0.0121.

The values of *C*, *P*, and *PP* are nearly the same in O × O and ♀ A × ♂ O matings, indicating that there is no difference in the fertility of A and O mothers. The reduction in fertility of the incompatible ♀ O × ♂ A matings as compared with the ♀ A × ♂ O matings is apparent, and there is in fact some indication of reproductive compensation, since $P_{OA} > P_{AO}$, although the difference is not statistically significant. However, in view of the fact that the prenatal deaths due to ABO incompatibility often begin with the first pregnancy, it is suspected that the differences in the values of *C*, *P*, and *PP* between the reciprocal matings would disappear if we chose families having, say, at least three children. Unfortunately, about half the original records of Matsunaga and Itoh have been lost; the remaining data, plus the data of Haga, are used in the following analysis. After the selection for family size has been made, values are obtained as follows: $C_{AO} = 4.0909 \pm 0.1538$ and $C_{OA} = 3.9565 \pm 0.1592$; $P_{AO} = 4.9487 \pm 0.2226$ and $P_{OA} = 4.9130 \pm 0.2113$; $PP_{AO} = 0.0415 \pm 0.0101$ and $PP_{OA} = 0.0531 \pm 0.0122$. Thus, the two matings now give nearly the same values for *C*, *P*, and *PP*. This suggests that the reduction in viability of the incompatible AO children occurs mostly in small families and that the effect of incompatibility becomes smaller as family size increases. This also suggests that there is little chance for reproductive compensation in the families selected for having at least three children. We may expect, then, that the average number of O children in the two kinds of mating will be almost the same, unless the segregation ratio of the O allele from the AO fathers differs from 0.5.

Some of the data used in our earlier report did not provide a record for each family; therefore they are excluded from the analysis presented here. The distribution of the number of children in the reciprocal O × A matings is presented in Table 1.

The distributions in the two kinds of mating are nearly the same when we choose only families having at least three children. For such selected families, $C_{AO} = 3.8833 \pm 0.1596$ and $C_{OA} = 3.9138 \pm 0.1580$. The average number of O children in ♀ A × ♂ O matings is 1.4833 ± 0.1655, while the average number in ♀ O × ♂ A matings is 1.9655 ± 0.1755. The difference between the two values is statistically significant (the distribution of O children is presented in Table 2).

This result strongly suggests that prezygotic selection favoring the O allele is operating in the AO fathers. However, the data available for the present discussion are rather incomplete, and we hope for more complete data in order to obtain conclusive evidence of prezygotic selection.

Table 2. Frequency distribution of O children in reciprocal matings A × O for families having at least three children.

Number of children	Observed number of families	
	♀A × ♂O mating	♀O × ♂A mating
0	15	11
1	19	10
2	13	15
3	10	15
4	2	6
5	0	1
6	1	0
Total	60	58

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References

1. E. Matsunaga and Y. Hiraizumi, *Science* **135**, 432 (1962).
2. E. Matsunaga and S. Itoh, *Ann. Human Genet.* **22**, 111 (1958).
3. H. Haga, *Japan. J. Human Genet.* **4**, 1 (1959).

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Individuality of Human Serum by Immunoelectrophoresis

Abstract. A method is described which implements the direct comparison of serum samples from different individuals by simultaneous electrophoresis followed by simultaneous diffusion and precipitation by anti-human horse serum. Results have been evaluated, with an arbitrary reference grid, by comparison of the numbers of precipitin bands present in the paired immunoelectrophoretograms. Graphic representation of these differences distinguishes serum samples of like from those of unlike origin.

In the search for the laws and generalizations which govern the physical world, the scientist's preoccupation with similarities has often led to the neglect of the opposite side of the coin—the individuality of objects and organisms. As applied to human beings, individuality is not significantly less important than similarity, inasmuch as it influences all aspects of life and is of direct interest in many fields, including medicine and criminalistics. Numerous studies have been made of the individuality of certain features of human blood, one of which is the subject of this report.

Both qualitative and quantitative differences in the distribution of serum protein fractions, as revealed in electrophoretic studies of one type or another, have been noted by a number of investigators (*1*). However, though electrophoresis has been a powerful tool in showing variations in the separated protein components of normal blood, the demonstrable differences have been less definitive than is required for discrimination among large numbers of individuals.

The development of highly refined techniques for immunoelectrophoresis (*2*) leads naturally to the application of this method to further differentiation among the serum proteins and the consequent establishment of individual differences in the blood of healthy persons. In this study a number of blood sera from healthy individuals of college-student age were subjected to direct comparison by immunoelectrophoresis. The technique employed is a modification of that described by Grunbaum and

Dong (*3*) with cellulose acetate membrane as the electrophoretic supporting medium, and with an equine antihuman serum from the Pasteur Institute in Paris to induce the precipitation of the individual proteins contained in the electrophoretically separated protein fractions. Two essential alterations of method were made: two serum origins were placed on each membrane, one close to each edge and midway of its length; a single narrow strip of filter paper was laid along the longitudinal midline of the membrane for the application of the equine antiserum. This arrangement made it possible to run two simultaneous electrophoretograms on one membrane and then to subject the resulting protein fractions to simultaneous diffusion and immune precipitation. It was thus possible to assess the reproducibility of the method by placing serum from the same individual on both origins, and also to judge its discriminatory value by placing serum from two individuals opposite each other on the membrane.

Figure 1 illustrates the comparison of serum proteins from two individuals. Serum samples from each of them were analyzed in duplicate, the results being shown in the upper two immunoelectrophoretic membranes. The third membrane shows the analysis of serum samples from the same two people, the samples being run opposite each other on a single strip. The differences are not immediately apparent to the eye,

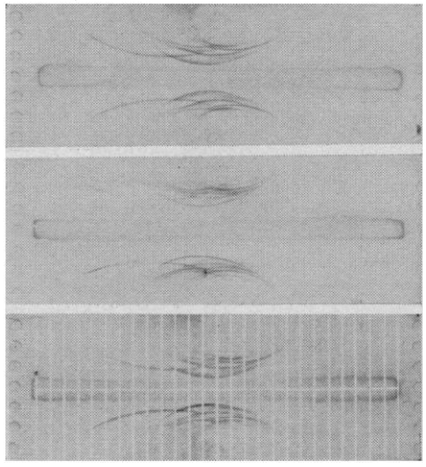

Fig. 1. Three immunoelectrophoretograms. (Top) Two samples from individual A. (Middle) Two samples from individual B. (Bottom) comparison of samples from both A and B run simultaneously, shown with the reference grid superimposed. The origins (locations at which the serum samples were applied) appear as small circles close to the edges of the membranes, midway of their length.

though patient examination will reveal them. Examination with the assistance of the superimposed reference grid makes the differences immediately perceptible.

Previous efforts to achieve reproducible results by repeated runs of serum from the same individual under carefully controlled conditions had demonstrated that the extreme complexity of the precipitin-band pattern (sometimes as many as 35 lines) (*3*) made satisfactory comparison of separate membranes impossible. For purposes of determining individual differences—in the sense of the presence or absence of specific fractions, or of quantitative differences which would affect visibly the strength or length of a precipitin band—identical running conditions are essential. Such conditions are assured by the simultaneous treatment of both samples on the same membrane.

Blood serum from nine individuals was tested, each individual being "run against himself," several of them more than once, to establish reproducibility. A total of 14 such like-like comparisons were made. Intercomparative runs were made with blood sera from 29 pairs of individuals.

Although a little experience in the unaided visual examination of the resulting immunoelectrophoretograms makes some of their distinguishing features recognizable, an adequate differentiation awaits the development of a suitable scanning device. In the interim, in order to appraise this preliminary phase of the study, a simple numerical comparison was made of the numbers of precipitin bands derived from the serum of each individual. This was done by superimposing upon the immunoelectrophoretogram a transparent grid plate oriented so that its zero line passed through the centers of the two serum origins, the grid zones being at right angles to the long axis of the membrane. The number of precipitin bands on each side of the membrane in each grid zone was determined by visual observation, transmitted light and a × 9 binocular magnification being used. Though this kind of evaluation is not free from subjective variability, its application to these immunoelectrophoretograms did discriminate between those made from like and from unlike sera.

Ideally an immunoelectrophoretogram made from two samples of the same serum should give the same precipitin-band count in each grid zone for both sides of the membrane, each line on one side having its counterpart directly opposite to it. It was found that differences of one line were not infrequent. These were considered as resulting from errors inherent in the procedure, and were compensated for, in the making of graphs, by subtracting 1 from all differences in the readings. On this basis the 14 like-like immunoelectrophoretic comparisons showed no differences in band count. On the other hand, except for one instance, the 29 intercomparisons of unlike sera showed numerous differences of band count, in most cases differences of more than 1 and in several grid zones. In this one exception there was a difference of only 1 in only one grid zone.

Figure 2 shows a sampling of typical graphs derived from comparative values determined by visual assay with the arbitrary reference grid. For taking

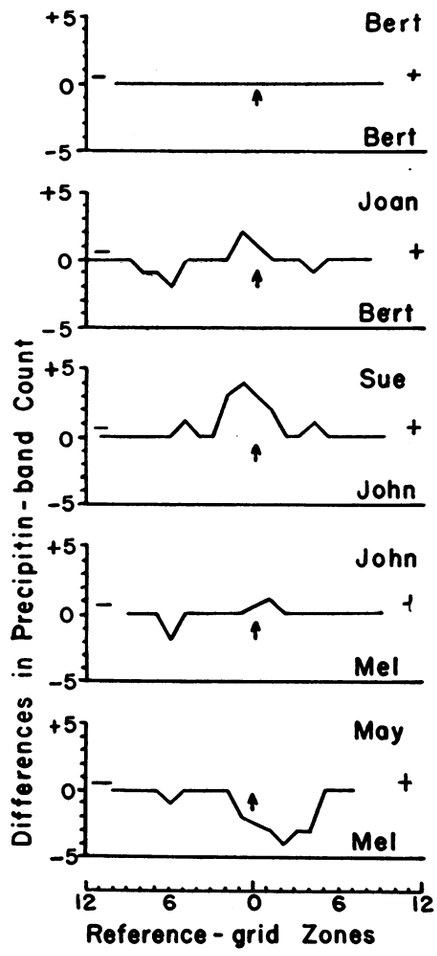

Fig. 2. Graphs showing differences in the number of precipitin bands in successive zones of an arbitrary reference grid. Straight-line graphs were always obtained from immunoelectrophoretic comparisons of serum samples from any given individual. Arrows represent the positions of the origins.

readings the electrophoretograms were all oriented alike (with the cathode end at the left) and the precipitin-band count of the lower run was subtracted from that of the upper run. In every case straight-line graphs resulted from the comparison of serum samples from the same individual. Comparisons between sera of different individuals never resulted in straight lines. However, the particular shapes and slopes of the intercomparative graphs have no known significance.

This study, designed to reveal inherent differences in the protein fractions of serum from different individuals, does not necessarily promise direct applicability to the practical determination of an individual source of a blood sample. It does not, for example, provide a classification of individuals based upon immunoelectrophoretic patterns, since it is applicable only to pairs of individuals whose serum is examined under identical conditions.

In the field of criminal investigation blood—often contaminated and usually dry—is frequently important evidence. However, before this method can be adapted to the practical problem of blood identification, it is essential to establish that the serum proteins in the fresh blood of different persons respond differently to immunoelectrophoretic treatment, and that this differentiation is reproducible. Preliminary work with sera extracted from dry blood indicates that, although the system operates at a somewhat reduced sensitivity, the results are essentially comparable with those obtained from fresh serum (*4*).

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References and Notes

1. P. Bernfeld, V. M. Donahue, F. Homberger, *Proc. Soc. Exptl. Biol. Med.* **83**, 429 (1953); J. Kohn, *Nature* **181**, 839 (1958); O. Smithies, *Biochem. J.* **61**, 629 (1955).
2. P. Grabar and C. A. Williams, Jr., *Biochim. Biophys. Acta* **17**, 67 (1955); P. Grabar and P. Burtin, *L'Analyse Immunoelectrophorétique; Ses Applications aux Liquides Biologiques Humains* (Masson, Paris, 1960); J. Kohn, *Nature* **180**, 986 (1957); C. Wunderly, A. Hässig, F. Lottenbach, *Klin. Wochschr.* **31**, 87 (1953).
3. B. W. Grunbaum and L. Dong, *Nature* **194**, 185 (1962).
4. This work was supported by grants from the National Institutes of Health, U.S. Public Health Service (RG-4848), and from the Research Committee, University of California, Berkeley.

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Gas Spitting by Alarmed Fish Disturbs Their Hydrostatic Equilibrium

Abstract. Besides maintaining hydrostatic equilibrium, the physostomatous gas bladder of fish has the broader function of adapting the density of the fish to momentary needs.

Physostomatous teleosts can decrease the amount of gas in the gas bladder by "spitting" bubbles. This "gas spitting reflex" is generally assumed to be controlled by proprio- or exteroreception of ascent to higher levels in the water, for instance, during the ascent phase of vertical migration (*1*, *2*). With the release of gas the volume of the gas bladder is kept constant during ascent, and the hydrostatic equilibrium of the fish is maintained by keeping the density of the fish constant. In order to maintain equilibrium during descent, the amount of gas in the gas bladder must be increased. The means by which this is accomplished is not quite clear. A possible, but rather peculiar, method might be by swallowing air at the surface before descending. Another method, secretion of gas from the blood into the gas bladder, is very slow (*2*).

During experiments into the occurrence of an alarm substance in some North American fish (*3*) gas spitting related to downward movements was observed in the hornyhead chub (*Hybobsis biguttata* Kirtland, Cyprinidae) and in the white sucker (*Catostomus commersoni* Lacépède, Catostomidae). Release of gas bubbles was first observed when the fish were disturbed optically or mechanically. Both species are rather shy. Other cyprinids such as the creek chub (*Semotilus atromaculatus* Mitchell) and the bluntnose minnow (*Pimephalus notatus* Rafinesque) were tame within 1 week after collection by seining. But even 3 weeks after collection the hornyhead chub and the white sucker became very frightened if noise or visible movement occurred near their aquaria.

Ten hornyhead chubs (total length 8 to 12 cm) were kept in a 200-liter tank; ten white suckers (total length about 15 cm) were kept in a similar tank.

If undisturbed the fish swam quietly in the aquarium, and searched sporadically for food along the bottom. Sometimes one swam in its own direction, at other times all fish swam together in a polarized group [aggregating or schooling according to the definitions of Breder (*4*)]. In an undisturbed state the fish seemed to be in perfect hydrostatic equilibrium and were able to swim at any level with minimal correcting fin movements. When a noise occurred, such as the banging of a door, or when I approached their aquarium, they immediately dashed along the bottom, concentrated in a corner, and formed a tight "pod" (*4*). At one time they lined up nose-by-nose, at another time they spaced themselves more randomly. Gas spitting started almost immediately after the disturbance, or after some tens of seconds. Without further disturbance the fish would stay in a stationary pod and continue gas spitting for up to 20 minutes. After spitting, the fish were completely out of hydrostatic balance, and were too heavy to ascend from the bottom without great effort.

While studying whether a similar behavior pattern is elicited by the administration of alarm substance (aqueous skin extract), I took care to avoid frightening the fish by other means. The fish were observed through a peep-hole in a screen. The skin extract was put in the aquarium through a tube. Control fluid (aquarium water) was introduced in the same way in order to check whether the fish reacted to other stimuli accompanying this procedure. In view of the extreme variability of the response of the suckers, a definite decision with respect to their reactivity to skin extract does not seem justified. In the chubs, however, the responses to skin extract—forming of a pod and gas spitting as described previously—were convincing.

Another indication of alertness after disturbance in both species was the erectness of the fins while the fish were lying on the bottom of the aquarium. I noticed that frequently the pectorals were spread wide, with the caudal margin turned upward in a position so that the surface of the fins made an angle of about 45° with the bottom. The results of some improvised experiments in flowing water tentatively suggest that the fish were pressed down toward the bottom by the force of flowing water on the pectoral fins. Together with the increased density of the body, a result of excessive gas spitting, this attitude of the fins would enable the fish to remain motionless in a current. By this method they would avoid the production of key stimuli (movements) which would release attack behavior in several predators.

This type of gas spitting may be more widespread. Dijkgraaf (*5*) observed gas spitting resulting in sound production in the European minnow (*Phoxinus*