Reports and Letters

Genetic Recombination in Bacteria

In the issue for 12 Aug. *Science* (p. 278) reported on the work of Wollman and Jacob on genetic recombination in *Escherichia coli*. This work concerned the effects of mechanical disruption of mating cultures on the patterns of segregation. The authors interpreted their results in terms of fractional fertilizations by broken gametic units, whose passage into the fertilized bacterium is specifically polarized. The news report further correlated this effect with virus-mediated genetic transduction. These findings are extremely interesting, but a report on a single article cannot take the space to discuss diverse interpretations. This danger is underlined by the fact that previous news summaries [*Science* **118**, 66 (17 July 1953)] have reported claims that recombination in *E. coli* K-12 depends on an F+ "virus" that acts as the vector; the present article refers to "mating pairs" and could not discuss the evolution in hypotheses. This letter is intended as an extension of the remarks of the news report that presents a more complete picture.

Transduction, as the term was defined when first introduced, is the transfer of a fragment of genetic material by phage or any other agency; fertilization implies the union of whole genomes or nuclei. Intermediate categories of genetic exchange may be found to occur naturally, or as suggested by the current article, may be artificially produced. For example, fractional fertilizations (or postzygotic losses with the same effect) have been indicated in frog eggs by sperm treated with toluidine blue [R. Briggs, *J. Gen. Physiol.* **35**, 761 (1952)] and this might be speculated on as one approach to the achievement of genetic transduction in higher organisms. However, the artificial interruption of fertilization serves, if anything, to emphasize the completeness of the normal process and the consequent distinction between fertilization and transduction mechanisms.

Unfortunately, the true constitution of the fertilized cell cannot be inferred with certainty from data on haploid segregants, owing to peculiarities of the meiotic mechanism in *E. coli* K-12. In the cited experiments, fertilization might have been incomplete, or it might have been complete with later disturbances of chromosome pairing to account for the segregation effects. Furthermore, if fertilization were fractional, the gradient of recovery of various loci might be due, as proposed, to the preferential orientation of gametic chromosomes, or to dependence on one locus (a centromere?) for the completion of synapsis, crossing-over and segregation.

These limitations of inference are equally applicable to undisturbed matings and have provoked a diversity of hypotheses that share the concept that the Hfr or F+ gametes are normally incomplete and variable in their genetic content. However, all the experimental data on haploid segregants are equally consistent with a second hypothesis that fertilization is regularly complete, but is coincident with chromosome breakage at specific, predetermined points on the F+ chromosome, so that certain segments are deleted after meiosis. Even small deletions would be lethal in haploid segregants and would influence the entire segregation pattern.

The most direct means of analyzing the intermediate processes of recombination and deciding between these conflicting viewpoints lies in the behavior of nondisjunctional types, which have been found both for sexual fertilization (heterozygous diploids) and a phage-mediated transduction (heterogenotes) in *E. coli* K-12. Thus the heterogenotes have helped to clarify the nature of the fragments in transduction, while the heterozygotes support the second hypothesis that in sexual recombination, the losses are postzygotic. The evidence is that the losses sometimes involve segments of the F- rather than the F+ or Hfr chromosome, or even cross-overs between them, and must therefore be preceded by complete fertilization, synapsis, and crossing-over. When the chromosomes are first broken is not known, and this may occur in the Hfr gamete, although the losses ordinarily (perhaps not always) occur later, after meiosis. However, the heterozygotes have so far shown no evidence of variability in the loci of breaks, for some loci are regularly eliminated—that is, they appear in the hemizygous state—while others are always intact—that is, heterozygous or, by crossing-over, homozygous).

In this light, mechanical stresses might be thought to create additional breaks in the migratory chromosomes, to accelerate the loss of previously broken segments, or to alter their pairing ability. The concept of mechanical breakage of gametic chromosomes in transit is eminently plausible and deserves to be tested in other experimental materials. However, in view of the ambiguity of haploid analysis, nondisjunctional types should be looked for. If the hypothesis of fractional fertilization is correct, interrupted matings should lead to "diploids" that carry variable fragments of the Hfr genome, in contrast to the regular elimination types found from undisturbed matings. The documentation for these remarks may be found in another review [*J. Cellular Comp. Physiol.* Suppl. 2, 75 (1955)].

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Chromatographic Fractionation of Normal Adult Oxyhemoglobin

The chromatographic separation of different human carboxyhemoglobins by Prins and Huisman (*1*) and of carboxyhemoglobins of different species by Boardman and Partridge (*2*) has recently been reported. The application of column chromatography to oxyhemoglobin in our laboratory has shown that not only different types of hemoglobin can be separated but also that normal adult hemoglobin itself can be resolved into at least three components (*3*).

Indications of the nonhomogeneity of normal adult hemoglobin have appeared from time to time in the literature. Various techniques, including solubility (*4*), resistance to alkaline denaturation (*5*), and gasometric determinations (*6*), have been used for this purpose. Therefore, it seemed probable that the differences implied by these techniques could be great enough to permit separation of the distinct molecular species in a suitably sensitive chromatographic system. Preliminary

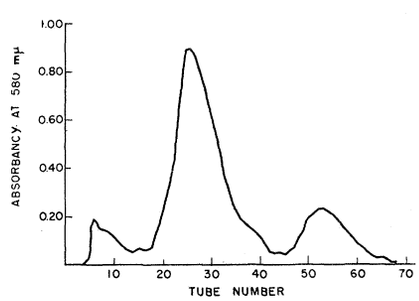

Fig. 1. Chromatogram of normal adult oxyhemoglobin.

nary work in our laboratory (*7*), as well as the work of Boardman and Partridge (*8*), suggested that the ion-exchange resin IRC-50 might be used in such a way.

To prepare the hemoglobin solutions, fresh normal human adult erythrocytes were washed three times with isotonic saline, ruptured with 4 vol of distilled water and 0.1 vol of toluene and centrifuged. The supernatant hemoglobin solution was removed and centrifuged again, this time at 20,000 *g* for 1 hr, in order to insure complete freedom from stroma and other particulate matter. This solution was dialyzed against dilute phosphate-citrate (McIlvaine) buffer (0.03*M*) at *p*H 6.3. One volume of this solution, containing about 50 mg of hemoglobin, was carefully layered on a column of ion-exchange resin IRC-50 that was 50 cm long and 0.9 cm in diameter. The resin had previously been equilibrated with the 0.03*M* buffer. The hemoglobin solution was allowed to enter the resin bed at its own rate of flow. This required 20 to 30 minutes. The hemoglobin was eluted with phosphate-citrate buffer at *p*H 6.3 of constantly increasing strength from 0.03*M* to a maximum of 0.2*M*. The eluant was collected by means of an automatic fraction collector. Each tube collected contained 4.2 ml. The rate of flow was approximately 10 ml/hr. The entire procedure was carried out in a constant-temperature cold room at 4°C.

Figure 1 shows a typical chromatogram of normal human adult oxyhemoglobin. It was obtained by reading successive tubes in a colorimeter at 580 mμ. Each of the fractions gave an absorption spectrum typical for oxyhemoglobin. Estimated total recovery is 95 percent or more. No further pigment removal was seen on washing a column with 2-percent NaOH after passage of the strongest buffer. The first fraction represents about 10 percent of the eluted oxyhemoglobin, and the second and third fractions represent about 84 percent and 6 percent, respectively.

Work with *A* (normal adult), *F* (fetal), and *S* and *C* (abnormal) hemoglobins showed entirely different chromatograms. The major fraction contained in a homozygous *S*-hemoglobin sample moves down the column more slowly than does the major fraction of normal adult hemoglobin, while fetal hemoglobin moves more rapidly than does adult hemoglobin.

The major fractions of the various hemoglobins come off the column in the following order: *F, A, S,* and *C*. This sequence is exactly the same as that reported by Prins and Huisman, using different conditions for carboxyhemoglobin. The use of oxyhemoglobin instead of carboxyhemoglobin for fractionation offers somewhat greater simplicity and, hence, less chance for denaturation of the protein.

We believe that this is the first report of a separation of undenatured normal adult hemoglobin into various fractions (*8a*). The differences in these fractions are now under investigation (*9*).

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References and Notes

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2. N. K. Boardman and S. M. Partridge, *Biochem. J. (London)* 59, 543 (1955).
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6. W. N. M. Ramsay, *ibid.*
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8.a *Note added in proof*: After preliminary publication of our results (7) and while the present report was in press, similar findings were published by H. G. Kunkel and G. Wallenius, *Science* 122, 288 (1955).

9. We would like to acknowledge the encouragement and support of Elmer Stotz, chairman of the department of biochemistry, and of William S. McCann, chairman of the department of medicine.

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Radiocarbon Dates for Kara Kamar, Afghanistan, University of Pennsylvania II

Kara Kamar is a rock shelter etched by ancient water action in the face of a mountain of limestone, probably Cretaceous, and characteristic of the northern foothills of the Hindu Kush Mountains. It is situated directly above the Uzbeg village of Sar Kiar, Afghanistan, 8 miles north of Haibak, 3200 feet above sea level, and 450 feet above the plain. The shelter, which faces south, commands a view of three valleys over distances of several miles in each direction. It was excavated in 1954, as shown in Fig. 1.

Henry W. Coulter of the U.S. Geological Survey, who served as expedition geologist, is studying the soil. His preliminary results indicate the following. The entire floor was covered by a thin layer of dung. In Trench A and part of Trench B, the next layer was a deposit of powdered, chalky lime, the product of many hot fires on the limestone of the cave. It contained a mixture of flints and wheel-made pottery. Under this and extending into Trench C was found an undisturbed layer of brown cave earth overlying, in all trenches, a thick deposit of loess, which, in Trenches C and B, overlay a second and terminal layer of brown cave earth. In Trenches C and B the loess may be divided into three naturally demarcated levels on the basis of the relative fineness of the rubbly particles contained in the wind-borne material, with the coarsest on the bottom and the finest on the top. In Trench A, where it is mixed with debris from rockfall, the loess cannot be so divided with certainty, nor is the border between the upper brown earth and the loess a sharp one.

The flints and animal bones from these levels have been tentatively classified into four cultural assemblages. Culture I, found in the upper brown cave earth and in the overlying powdered lime, is typologically Mesolithic. Culture II, from the fine and medium upper half of the loess in Trenches B and C, is a flake assemblage in which the flint has turned white from weathering. Culture III, in the lower half of the medium rubbly loess and the lower loess, is an unweathered assemblage of Upper Paleolithic blade tools accompanied by horse bones. The flints in the loess of Trench A probably belong mostly to Culture III. Culture IV, in the lower brown cave earth, is a small assemblage of weathered flakes more or less similar to those of Culture

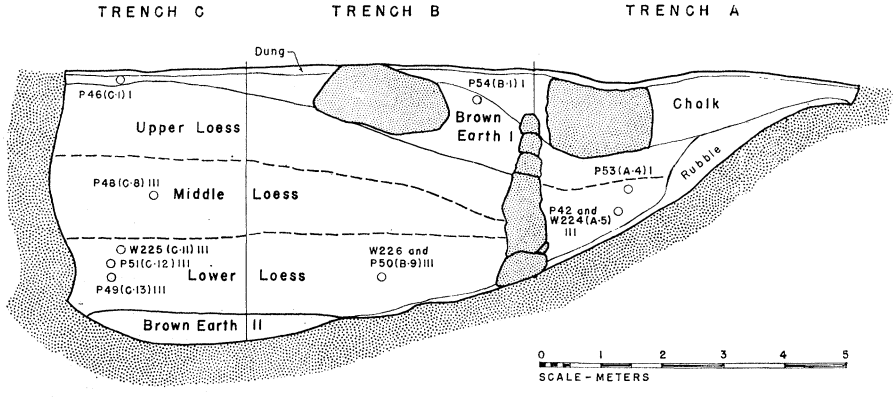

Fig. 1. East-west section, facing north, of Kara Kamar rock shelter.