ERRATA

In the article entitled “Incorporation of Parental DNA into Genetic Recombinants of E. coli” by Obaid H. Siddiqi, which appeared in the May issue of volume 49 (1963), the last line of paragraph 3 on page 589 should read “it is S’”; and in the legend to Figure 1 on page 591 “Hfr DNA” should appear as “F− DNA.”

In the article entitled “Synthetic Polynucleotides and the Amino Acid Code, IX” by Albert J. Wahba, Robert S. Miller, Carlos Basilio, Robert S. Gardner, Peter Lengyel, and Joseph F. Speyer, which appeared in the June issue of volume 49 (1963), pages 880–885, Lys should be substituted for Leu in the amino acids listed in Table 6 under the heading “Triplet composition 2A1U.”

In the article entitled “Fluorogenic Substrates for β-D-Galactosidases and Phosphatases Derived from Fluorescein (3,6-Dihydroxyfluoran) and Its Monomethyl Ether” by Boris Rotman, John A. Zderic, and Marvene Edelstein, which appeared on pages 1–6 of volume 50 (1963), the following data should be inserted on page 4 between the two paragraphs of the section entitled “Fluorescein-3-O-methyl-6-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranoside) (IIg):”

Analysis calculated for C_{35}H_{32}O_{14}: C, 62.1; H, 4.8; O, 33.1. Found: C, 62.5; H, 4.8; O, 32.7.
INCORPORATION OF PARENTAL DNA INTO GENETIC RECOMBINANTS OF E. COLI

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Two kinds of mechanisms have been considered to account for genetic recombination in bacteriophage and bacteria. One involves exchange of pre-existing genetic material between homologous chromosomes (breakage and reunion), and the other implies exchange of genetic information without exchange of pre-existing genetic material (copy choice).1 Experiments by Meselson and Weigle2 and Kellenberger, Zichichi, and Weigle3 have shown that when a strain of bacteriophage λ containing labeled DNA is crossed with an unlabeled and genetically distinguishable strain, the genetic recombinants arising from such a mating contain discrete amounts of the parental DNA. We present evidence which indicates that recombinants arising from the mating of an Hfr with an F- strain of Escherichia coli inherit labeled DNA from the F- parent.

Bacterial conjugation involves a progressive transfer of genetic markers from the Hfr to the recipient F-.4 There is a concomitant transfer of DNA from the Hfr to the F-5. The recipient subsequently gives rise to recombinants. The present procedure for detecting the incorporation of parental DNA into recombinants involves the mating of a T6-resistant Hfr to a T6-sensitive F- carrying specifically labeled DNA. When the mated cells are exposed to a high multiplicity of the phage T6, the sensitive cells are rapidly lysed. The T6-resistant F- recombinants can then be separated by filtration and examined for the presence of the parental label.

Materials and Methods.—Bacteria: The F- is a derivative of a thymine-requiring strain which originated in the laboratory of Dr. F. Ryan and was obtained through the courtesy of Miss E. Fox. Its pertinent characters for the present experiment are Thy- (requirement for thymine) and S+ (resistance to streptomycin). The Hfr is resistant to phage T6 (T6r) and transfers the phage resistance marker at about 5 min after mating; it is S-.

Phage: Wild-type coliphage T6 was used. High-titer phage suspension (5 × 10^11 particles/ml) was prepared according to the methods of Adams.6

Medium: The broth medium contained Bacto Tryptone 10 gm, yeast extract 5 gm, NaCl 5 gm, dextrose 1 gm, and distilled water one liter. The pH was adjusted to 7.4 with NaOH.

Preparation of labeled F- and mating procedure: An overnight culture of the F- was diluted one hundredfold into 0.5 ml of broth to which 10 microcuries of tritiated thymidine (specific activity

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8 Reich, E., R. Franklin, A. Shatkin, and E. Tatum, these PROCEEDINGS, 47, 1212 (1961).
The cells were incubated at 37°C for 3 hr, centrifuged, washed thrice with a large excess of cold thymidine, and reincubated for 20 min in 1 ml of broth. After this second incubation, the labeled cells were resuspended in fresh broth prewarmed to 37°C. The optical density of the suspension was 0.15. The Hfr was grown to an optical density of 0.1, centrifuged, and resuspended in one fourth of the original volume of broth. The cells were mixed as follows: 1 ml Hfr + 1 ml F− + 1 ml fresh broth + unlabeled thymidine to yield a final concentration of 50 μg/ml. The mixture was spread on a Petri dish and kept at 37°C with occasional gentle swirling. After one hour, the mixture was diluted 20-fold in broth to which 100 μg/ml of streptomycin and 50 μg/ml of cold thymidine had been added. The diluted culture was incubated on a rotary shaker at 37°C. At desired intervals, samples were removed for determining the titer of phage-resistant cells and for radioactivity measurements as described in the following section.

Separation of recombinants: In order to separate the T6+ recombinants from the nonresistant F− cells, an aliquot of the diluted mating mixture was infected with about 300 phage particles per cell. This resulted in a rapid lysis of the sensitive cells. After incubation with 20 μg/ml of deoxyribonuclease for 30 min to degrade the DNA liberated from the lysed cells, 0.5 ml aliquots of the mixture were filtered through a bacterial filter (Halibut ultrafilter) and extensively washed with water. The unlysed cells remained on the filter paper. It is important to ensure that the filtration is rapid and clogging does not occur. For a suggestion of this method the author is indebted to Dr. S. D. Silver.

Measurement of radioactivity: The filter papers were dried and warmed in counting vials with 1 ml of hydroxide of Hyamine (obtained from Packard Instrument Co.) at 65°C for 1 hr. Ten ml of scintillation liquid containing 0.5% PPO (2,5-Diphenyloxazole) and 0.05% dimethyl POPOP (1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene) in toluene were then added, and the samples were counted in a Packard liquid scintillation spectrometer.

Measurement of tritium in the DNA: The tritium in the unlysed cells was fractionated according to the method described by Hershey and Melechen. After the mated F− cells had grown for 8 hr, they were exposed to phage and incubated with deoxyribonuclease for 30 min. The unlysed cells were centrifuged and washed repeatedly to remove the DNase and precipitated in cold 0.3 molar TCA. The precipitate was resuspended in 1 molar KOH and kept at 37°C for 15 hr to solubilize RNA. The suspension was chilled and neutralized with HCl, and TCA was added to a final concentration of 0.3 molar to precipitate DNA. The precipitate was resuspended in 0.05 molar phosphate buffer at pH 7 containing 0.003 molar MgSO4 and was incubated at 37°C with DNase for 20 min. The suspension was again precipitated with cold TCA and centrifuged. The tritium activity in the supernatant was used as a measure of the amount of label in the DNA.

Results.—As a preliminary experiment to test the efficacy of the technique, T6-sensitive cells were labeled with tritiated thymidine and grown in broth. Radioactivity measurements at different times showed that, during the course of the experiment, the label was conserved in the cells and essentially all of it was precipitable with TCA. When the culture was filtered through a bacteria-retaining filter, the radioactivity remained on the filter paper. Treatment of the cells with T6 phage and DNase before filtration rendered most of the radioactivity filterable. The result of such an experiment is given in Table 1.

In the mating experiment, the F−S'T6+ strain, labeled with tritiated thymidine, was mixed with the Hfr S'T6+ strain. After a period of 1 hr at 37°C, the cells were

<table>
<thead>
<tr>
<th>Treatment of sample</th>
<th>Radioactivity Remaining on Filter Paper</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>940</td>
<td>98.5</td>
</tr>
<tr>
<td>Precipitated with cold TCA</td>
<td>900</td>
<td>94.1</td>
</tr>
<tr>
<td>Exposed to T6 phage and DNase</td>
<td>4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

See text for details of the experiment.
diluted into broth containing streptomycin and incubated at 37°C. At different times thereafter, aliquots of the mated cells were lysed and filtered as described in the section on Methods, and the radioactivity remaining on the filter paper was measured. Viable counts of the S' and T6S' cells were obtained by plating suitable dilutions on broth containing, respectively, streptomycin or streptomycin and T6 phage.

The results are presented in Figure 1. The radioactivity on the filter paper is taken as a measure of the label in the T6-resistant recombinants. It will be seen that the counts on the filter paper rise in parallel with the proportion of the T6-resistant recombinants among the F−, and the two values reach a maximum at about the same time.

In control experiments, the addition of the T6' Hfr was omitted but six per cent unlabeled F−S'T6' cells were added to the labeled cells. In this case, there was no increase in the counts on the filter paper. It would thus appear that the unlysed T6' cells contain the label only when they arise from the labeled F− cells. In other words, the labeled DNA in the F− is transmitted to its recombinant progeny. More than 90% of the tritium activity in the resistant cells was located in the DNA.

The proportion of S'T6' recombinants among the F− cells varied in different experiments from 5% to 6.5% while the proportion of the total radioactivity in recombinants was 4% to 5%.

Discussion.—Sensitivity to T6 is dominant over resistance. In order to be phenotypically T6-resistant, a bacterial cell needs not only to incorporate the resistance gene but also to lose the existing receptor sites. Consequently, a resistant bacterium cannot be heterozygous or heterokaryotic for T6', and the resistance marker must be fully integrated with the genome. The presence of labeled DNA in the T6-resistant cells, therefore, demonstrates the association of new genetic information with the old DNA.

Some limits may be placed on the mode of transmission of the parental DNA to the recombinant progeny by the quantity of the label present in the recombinants. If only one of the two DNA strands is involved in this transmission, the proportion of the parental label in the recombinants can be no more than half of the proportion of recombinants among the F− cells. As the recombinants inherit a part of their genome from the Hfr, this proportion will, in fact, be less. In our experiments, the amount of label present in the T6-resistant cells is well above this value, indicating that both strands of DNA must be transmitted from the labeled F− to the recombinants.

The S'T6' recombinants constituted 5%-6.5% of the F− cells and contained 4%-5% of the total tritium activity. More than 80% of the DNA from the
individual F- parents must, therefore, have been transmitted to the recombinants. Since spontaneous interruption of conjugation can occur before the entire chromosome has been transferred, the contribution of the F- parent to the recombinant chromosome is expected to be predominant. The amount of the DNA transmitted by the F- to its recombinant progeny thus appears to parallel its genetic contribution.

I wish to express my gratitude to Dr. Alan Garen for his help and advice.

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2 Meselson, M., and J. J. Weigle, these PROCEEDINGS, 47, 857 (1961).
9 Lederberg, J., these PROCEEDINGS, 35, 178 (1949).

GENETICS OF A GAMMA GLOBULIN ISOANTIGEN (ALLOTYPE) IN THE MOUSE*

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In man and the rabbit, isoantigens on gamma globulins have proved invaluable as structural markers in the purification and characterization of gamma globulins and their subunits.1-3 In the mouse, in addition to these uses, the isoantigens are potentially useful as genetic markers of the whole animal and of transplanted or in vitro cultured lymphoid cells for genetic studies of somatic cells.4

In this publication, we describe studies of a mouse gamma globulin isoantigen (appearing in strains different from those already reported by Kelus5) and show that this isoantigen is present on antibody molecules of two different antibody specificities. We show further that the inheritance of this antigen is controlled at a single genetic locus.

Materials and Methods.—Mouse strains and sources: A/J, C57BL/6J, from Jackson Memorial Laboratories; BALB/c.CrglKa, C57BL/Ka, from Department of Radiology, Stanford University; C3H/SnHz, C3H.H-2b/SnHz, C57BL/10SnHz, from Department of Genetics, Stanford University; (101/RI X C3H/RI)F1, T (noninbred tester stock), from Biology Division, Oak Ridge National Laboratory.

Isoimmunization with mouse gamma globulin: BALB/c mice, males and females, were immunized with a 50% ammonium sulfate precipitate from a pool of C57BL/6 retired breeder sera which represented more than 50 animals of mixed sex. The primary injection, 2.3 mg globulin protein per mouse, was given in Freund’s adjuvant intraperitoneally and subcutaneously (1 vol of