It is most promising to study the mechanism of genetic recombination on the simplest models, namely, intracellular phage development and bacterial transformation. The work on phage showed that recombination takes place according to a break-reunion mechanism (Meselson and Weigle 1961; Kellenberger, Zechin and Weigle 1961; Ihler and Meselson 1963). Similar results were obtained in transformation studies. It was shown that a short time after contact of DNA with recipient cells was first established, the genetic markers introduced by transforming donor DNA become linked with adjacent markers of the recipient chromosome. Since this process is possible under conditions of cell starvation when DNA synthesis is stopped (Fox and Hotchkiss 1960; Voll and Goodgal 1961), the transforming DNA molecule must be incorporated directly into the chromosome of the recipient cell. Thus, the problem of the molecular mechanism of genetic recombination in transformation is reduced to establishing of the mechanism of DNA incorporation into the chromosome. There have been several investigations of this mechanism, but the results are contradictory. Herriott (1961) studied the transformation of Hemophilus influenzae for two linked markers, using molecular hybrids of DNA carrying both transforming loci on different polynucleotide strands. It was found that in this case the number of double transformants greatly surpasses their number in a control using a mixture of both types of donor DNA. This was taken as a proof for the incorporation of both DNA strands into the chromosome. Later, however, Kent, Roger and Hotchkiss (1963), Marmur, Round and Schalkraft (1963), who worked with pneumococcus, and Nester and Stocker (1963), using Bacillus subtilis, could not find the effect originally observed by Herriott. Hence, they concluded that only one of the DNA strands is integrated into the cell chromosome. Lacks (1962) came to the same conclusion, using chromatography of cell lysates of Pneumococcus that had previously interacted with extracellular DNA. In our previous work (Bresler, Kreneva, Kushev and Mosvitskiif 1964) we have shown that in transformation of B. subtilis cells auxotrophic for two linked markers (indole and histidine dependence) by means of DNA molecules carrying transforming indole and histidine loci on different strands, the number of double transformants (prototrophic cells) is higher by an order of magnitude than the background of random coincidences, just as in Herriott's experiments with H. influenzae.
In the present work we compared the transforming activity of hybrid and native DNA in *B. subtilis* transformation. We have studied also the segregation of genetic characters in the progeny of transformed cells. On the basis of these experiments a recombination mechanism is proved that assumes independent interaction of both strands of transforming DNA with the cell chromosome and equal probability of their integration into the chromosome.

**Materials and Methods**

We used several strains of *Bacillus subtilis*; their genetic characteristics are listed in Table 1. The isolation of DNA, the thermal hybridization of DNA and the transformation of bacteria were conducted according to previously described procedures (Bresler, Kreneva, Kushev and Mosevitski 1964).

**Experimental Results**

1. **The influence of hybridization on the transforming activity of DNA**

After hybridization of transforming DNA with homologous but genetically inert DNA the number of molecules carrying active genetic markers should increase (Marmur and Lane 1960). As a limit, their number ought to be doubled by hybridization. This can be obtained by hybridization of genetically active DNA with a great excess of inert DNA. If double stranded DNA molecules are incorporated into the chromosome during transformation, we must expect the molecular heterozygotes to be as effective in transformation as ordinary molecules. In an experiment especially designed to detect this effect DNA isolated from a prototrophic strain of *B. subtilis* SHgw (DNA_SHgw) was hybridized with a DNA preparation from one of the strains auxotrophic for one marker: indole (DNA_{168}), histidine (DNA_{H25}) or tyrosine (DNA_{SB65}). The ratio of DNA_{SHgw} to DNA of the auxotrophic strain during the hybridization procedure was 1:10. In these conditions about 90% of the DNA_{SHgw} strands should be transferred into hybrid molecules (Fig. 1). As a control an equivalent mixture of both DNA preparations was used after their separate thermal denaturation and renaturation. Transformation was carried out with the *B. subtilis* strains SB 25 and SB 29 auxotrophic for two linked markers (Table 1). The active marker common to both

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHgw</td>
<td>prototroph</td>
</tr>
<tr>
<td>SB 25</td>
<td>ind⁻ his⁻</td>
</tr>
<tr>
<td>SB 29</td>
<td>ind⁻ tyr⁻</td>
</tr>
<tr>
<td>H 25</td>
<td>his⁻</td>
</tr>
<tr>
<td>SB 70</td>
<td>tyr⁻ his⁻</td>
</tr>
<tr>
<td>168</td>
<td>ind⁻</td>
</tr>
<tr>
<td>SB 65</td>
<td>tyr⁻</td>
</tr>
</tbody>
</table>

Fig. 1. Scheme of DNA hybridization. ⚫, ⚫ active (prototrophic markers); ◦ auxotrophic marker.