Genetic recombinations between influenza viruses have been studied frequently. Viruses of the same [4-7] or different [2, 8] serological types have been used as models in such studies. In these studies it became possible to obtain different recombinations of original strains. Some of these proved to be stable; however, in the course of subsequent passages, other strains reverted to the original virus types. All these experiments have been carried out with paired live viruses. Subsequently, it has been possible to demonstrate recombination of 2 viruses, one of which was inactivated by ultraviolet rays or heat [3, 9]. These experiments are interesting in that they demonstrate the preservation in the inactivated virus of ability of partial reproduction in a cell and attest to the possibility of inclusion of this virus material in the simultaneously developing active virus.

A more complete study of recombination between the influenza viruses has been carried out in this investigation.

METHODS

The following virus pairs have been used: duck influenza virus A [Koshitse-550] inactivated by heating at 37° for 5-6 days and live viruses A2pal/60, isolated in China, and B [Moscow-39] 59, isolated in Moscow. Sera produced in rats have been used. These have been prepared by the usual method (1) by immunization of animals with the original strains and recombinations. The antisera titers varied from 1:320 to 1:1280. Hemagglutination and hemagglutination-inhibition reactions have been carried out in the usual manner with 4 AE viruses, using 1% chicken and rabbit RBC suspensions [1].

Hemagglutination-inhibition reactions with normal rabbit serum allowed determination of sensitivity to the inhibitors.

Electron microscopic studies have been carried out with electron microscope JAM5y using 80 kV.

The viruses were adsorbed on chick erythrocytes. Phosphotungstic acid was used for shadow casting.

The preparations were examined and photographed in the electron microscope at magnification of 10,000 to 15,000.

EXPERIMENTAL RESULTS

We tried to select clearly different strains of viruses (markers), for setting up recombination experiments. The A group viruses, which were used for basic determinations, had the following characteristics (Table 1).

Prior to the study, virus A (duck) was passed 12 times in chick embryos in our laboratory, in addition to the passages following the initial isolation. Consequently, this virus could be considered completely adapted to chicken embryos.

The 3rd passage of A2pa1/60 was used in the experiments.

In the first set of experiments 0.2 ml of inactivated group A virus, previously concentrated on chick erythrocytes and having the hemagglutination titer of 1:10,000, was inoculated into chick embryo allantoic cavity; the
TABLE 1. Characteristics of Parental Virus Types

<table>
<thead>
<tr>
<th>Characterizing marker</th>
<th>Virus</th>
<th>duck</th>
<th>pa1/60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>A</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td>Agglutination of rabbit RBC</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sensitivity to inhibitors</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td>Threads</td>
<td>Spheres</td>
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</tr>
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</table>

live virus A2 (with agglutination titer of 1:160) was introduced in 10^-2 (100 ID50) dilution simultaneously, and after 1 and 24 h. After 48 h the allantoic fluid was removed and analyzed for the presence of active virus. Chick embryos inoculated either with live virus A2 served as control.

In killed virus A controls no virus was detected in the allantoic fluid during subsequent passages. In controls with live virus A2 and in experiments with mixed live and killed viruses, the 1st transfer of virus showed the usual titer.

Equal volume of 1:2 and 1:10 dilutions of anti-A2 serum was added to the first passage virus, and the mixture was inoculated into chick embryo allantoic cavity. The virus-containing allantoic fluid obtained after 48 h was divided into 3 parts: one part was used for passage when mixed with anti-A serum, the 2nd for passage with anti-A2 serum and the third for passage with homologous serum. After this, 2 of the obtained lines were passed 6 times in chick embryos. The mixture of virus with homologous serum was not passed, since the virus was completely neutralized. The control was subjected to the same treatment (the mixture of live A2 virus with homologous serum).

As can be seen from the data shown on Fig. 1, two recombinants have been obtained, one of which contained the antigen of virus A and the other recombinant that of virus A2. Both of them differed from the parent viruses, demonstrating the combination of their properties. Both variants were more sensitive to the normal serum inhibitors than the parent variant A2.

In the subsequent passages the first variant retained its properties, a somewhat higher ability to agglutinate rabbit RBC, remaining highly sensitive to inhibitors and resembling morphologically the A2 virus; the 2nd variant lost the ability to agglutinate rabbit RBC, however, retaining the morphology peculiar to virus A and a high degree of sensitivity to inhibitors. Subsequent studies of these variants (18 passages) did not alter their properties. Figures 2 and 3 demonstrate the morphology of the parent virus A2 and its recombinant. (See page 977).

As stated above, the indicated experiments were carried out by simultaneous infection with killed and live viruses as well as by alternating injections of these agents. When the live virus was introduced 24 h after injection of the dead virus, the recombinants have been obtained with difficulty and irregularly; apparently interferon activity played a role. Experiments in recombination carried out in tissue cultures gave similar results.

In the described experiments the variants were selected from a mixed population by means of suppression of one of the variants with homologous sera. It was of interest to study the behavior of a mixed population after serial passages. With this in mind, the virus population from the 2nd passage (designated on Fig. 1 by a cross) was passed 6 times in chick embryos. Samples from the 3rd and 6th passages have been studied for the presence of S- and V-antigens of A and A2 viruses.

As it can be seen from Table 2, both V-antigens have been found in the mixed population from the 3rd passage, however, in the population from the 6th passage only V-antigen of A2 virus, i.e., a component of the live virus, was present.

In experiments described above, in addition to the genetic markers, the selected strains differed in that the inactivated virus has been adopted to chick embryos, while the live virus was passed only 3 times in chick embryos. It was interesting to establish the possibility of formation of recombinants of the 2 viruses adopted to chick embryos. For this purpose, influenza virus A2 was passed 18 times in chick embryos, after which time experiments described above for recombination have been repeated. It was not possible to obtain any recombinants. The virus obtained after infection of embryos with living and inactivated viruses was of one type, identical with the live virus.

Analogous experiments have been carried out with another pair of viruses— with inactivated influenza virus A (duck) and live influenza virus A (strain 539), isolated from swine and passed 2 and 25 times in chick embryos. These viruses differed in several respects. Recombinants have been obtained with the live virus, regardless of the number of passages it had in the chick embryos, i.e., 2 or 25.

Recombination experiments were carried out also with influenza viruses belonging to different serological groups—with inactivated influenza virus A (duck) and live, freshly isolated influenza virus B. No recombinants were obtained. The progeny were found to be analogous to the live influenza virus B.