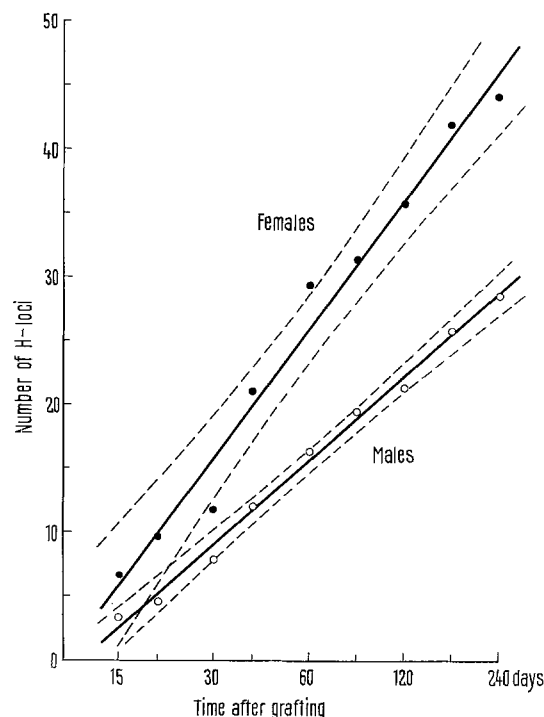


for the regression lines and the corresponding correlation coefficients were:  $Y = -23.14 + 21.75 X$  ( $r = 0.997$ ) in males and  $Y = -33.30 + 33.30 X$  ( $r = 0.989$ ) in females. In short, one clear conclusion emerges: the number of H loci at which two strains differ is not a fixed one but depends on the time elapsed between



Relationship between the number of H loci and  $\log_{10}$  of the time in days after grafting, in male and female  $F_3$  hybrids grafted with DBA/2 ear skin. The broken lines represent the confidence limits of the regressions for 99/100.

grafting and observation. Moreover, if the strength of H loci is measured in terms of survival time, our results confirm the presence of a continuous spectrum of H loci<sup>10</sup> whose strength is geometrically degrading.

In the second place, it is apparent from results that allograft rejection was slower in males than in females, in the same way for all the loci. The lines expressing the values of males show, in fact, a smaller slope than that of females, and the difference between the two corresponding regression coefficients is highly significant ( $P < 0.001$ ). Our evidence of a greater immunological responsiveness of females agrees with the findings of other authors that females of a given strain of mice often reject skin allografts more rapidly than males<sup>10, 13, 14</sup>, and are less susceptible to the growth of certain transplanted tumors<sup>15, 16</sup>.

*Riassunto.* I dati esposti indicano che l'incremento del numero dei loci dell'istocompatibilità ai quali differiscono i due ceppi di topi «inbred» DBA/2 e C57BL/6 è inversamente proporzionale al tempo trascorso dal trapianto, ed è maggiore nelle femmine.

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<sup>12</sup> N. T. J. BAILEY, in *Statistical Methods in Biology* (The English University Press Ltd., London 1968), pp. 24, 95.

<sup>13</sup> W. S. LAPP and J. Q. BLISS, *Immunology* 12, 103 (1967).

<sup>14</sup> M. GALTON, *Transplantation* 5, 154 (1967).

<sup>15</sup> L. GROSS, *Proc. Soc. exp. Biol. Med.* 49, 67 (1942).

<sup>16</sup> G. D. SNELL, *J. natn. Cancer Inst.* 20, 787 (1958).

## Cellular Antigens in Myxo- and Paramyxoviruses as Revealed by Immunodiffusion Methods

Antigens of cellular origin have been revealed in the envelope of myxo- and paramyxoviruses which appeared to be species-specific<sup>1</sup>, group-specific<sup>2</sup> and heterogeneous<sup>3</sup>. Our attention was drawn to immunodiffusion methods which have been explored in the study of antigenic pattern of some viruses<sup>4</sup>.

*Materials and methods.* Experiments were conducted with fowl plague virus (FPV, strain Weybridge), Newcastle disease virus (NDV, strains Tomilin and Herfordshire), and Sendai virus (strain 960) grown in chick embryos and purified in DEAE cellulose<sup>5</sup>. S and V antigens of FPV were obtained by the method of HOYLE<sup>6</sup>. Immune sera were obtained by quadruple intramuscular inoculation of purified virus preparations with one-week intervals. S and V immune sera were obtained by the method of LIEF and HENLE<sup>7</sup>. Cellular extracts (10%) in saline were used as cellular antigens, extracts of guinea-pig kidney and rabbit erythrocytes were used as Forssmann's antigen, human group A erythrocytes were used as group-specific antigen.

The following immunodiffusion methods were used: double diffusion<sup>8</sup>, immunoelectrophoresis<sup>9</sup>, and immunosmophoresis<sup>10</sup>.

*Results and discussion.* In double diffusion experiments both virus-specific and cellular antigens were revealed in purified virus preparations. Figure 1 shows the results of an experiment in which purified concentrated FPV (III, VIII) was subjected to interaction with FPV (1, 10, 11, 13) and NDV (23, 24) immune sera. It is seen that both sera interact with FPV antigen as well as anti-charioallantoic serum (18) and group B human serum (26), the latter specifying the presence of group A antigen in

<sup>1</sup> P. N. KOSYAKOV and Z. I. ROVNOVA, *Vop. Virus.* (Russian) 10, 17 (1965).

<sup>2</sup> G. F. SPRINGER and H. TRITEL, *Science* 138, 687 (1962).

<sup>3</sup> R. DRZENIEK, M. S. SOBER and R. ROTT, *Z. Naturforsch.* 21b, 254 (1966).

<sup>4</sup> H. W. I. RAGETTI and M. WEINTRAUB, *Science* 144, 1023 (1964).

<sup>5</sup> G. K. S. CHEPULIS, A. F. BOCHAROV and V. M. ZHDANOV, *Vop. Virus.* (Russian) 4, 439 (1967).

<sup>6</sup> L. J. HOYLE, *Hygeia* 50, 229 (1952).

<sup>7</sup> F. S. LIEF and W. HENLE, *Virology* 2, 753 (1956).

<sup>8</sup> O. OUCHTERLONY, *Ann. N.Y. Acad. Sci.* 121, 640 (1953).

<sup>9</sup> G. GRABAR and C. A. WILLIAMS, *Bioch. biophys. Acta* 10, 193 (1953).

purified FPV virions. FPV serum (II) also interact with guinea-pig kidney extract (VI) thus specifying the presence of Forssmann's antigen in the virions. The latter is demonstrated in Figure 2 where guinea-pig kidney extract (VI) interacts with rabbit hemolytic

standard serum (8) and FPV serum (10) which does not interact with the antigen (VI) after exhaustion with antigens of normal chorioallantoic membrane (10a).

Similar data were obtained in experiments with immunoelectrophoresis. Figure 3 shows the results of an

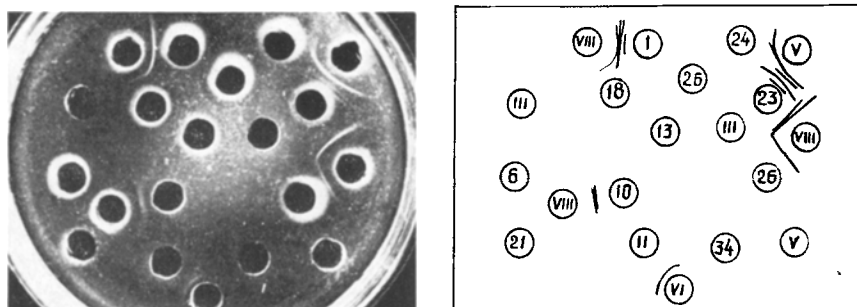


Fig. 1. Double diffusion test. Antigens: purified FPV concentrated 3 times (III) and 8 times (VIII), normal chorioallantoic membrane (V) and extract of guinea-pig kidney (VI). Sera: FPV (1, 10, 11, 13), NDV (23, 24), anti-normal chorioallantoic membrane (18), human group B (26), normal rat (6), guinea-pig (21) and rabbit (34).

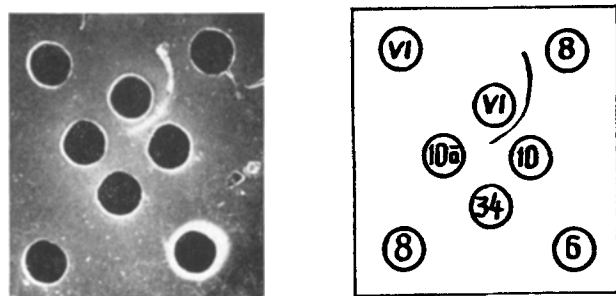


Fig. 2. Double diffusion test. Antigen: extract of guinea-pig kidney (VI). Sera: FPV (10), FPV exhausted with normal chorioallantoic membrane antigens (10a), rabbit hemolytic standard serum (8) and normal rabbit serum (34).

experiment in which FPV (XI) and normal chorioallantoic membrane (V) antigens were subjected to interaction with a set of virus specific and tissue specific sera. It is worthwhile to note that virus-specific antigens have cathode mobility (Figure 3, g, h) while tissue-specific antigens are mostly anode-mobile (Figure 5, e, f).

Figure 4 shows the results of an immuno-osmophoresis experiment in which cellular antigens are revealed in FPV virions by heterologous sera (Figure 4, a) and vice-versa (Figure 4, c). The multiple antigenic composition of FPV virions is revealed in experiments with biphasic immuno-osmophoresis, one of which is shown in Figure 5. In this experiment, 11 antigenic components are revealed, including species specific and Forssmann's antigens.

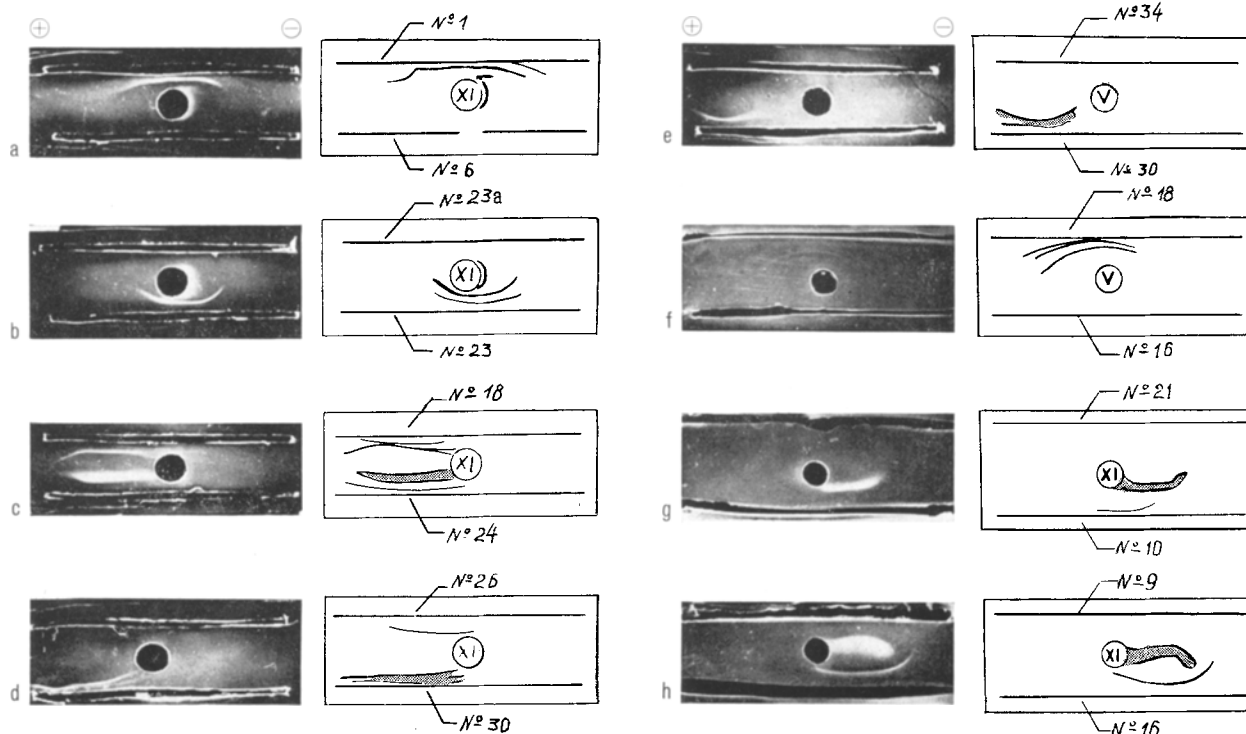


Fig. 3. Immunelectrophoresis test. Antigens: purified FPV concentrated 11 times (XI) and normal chorioallantoic membrane (V). Sera: FPV (1, 9, 10, 30), NDV (23, 24), NDV exhausted with normal chorioallantoic membrane antigens (23a), FPV S serum (16), anti-normal chorioallantoic membrane (18), human group B (26), normal rat (6), guinea-pig (21) and rabbit (34).

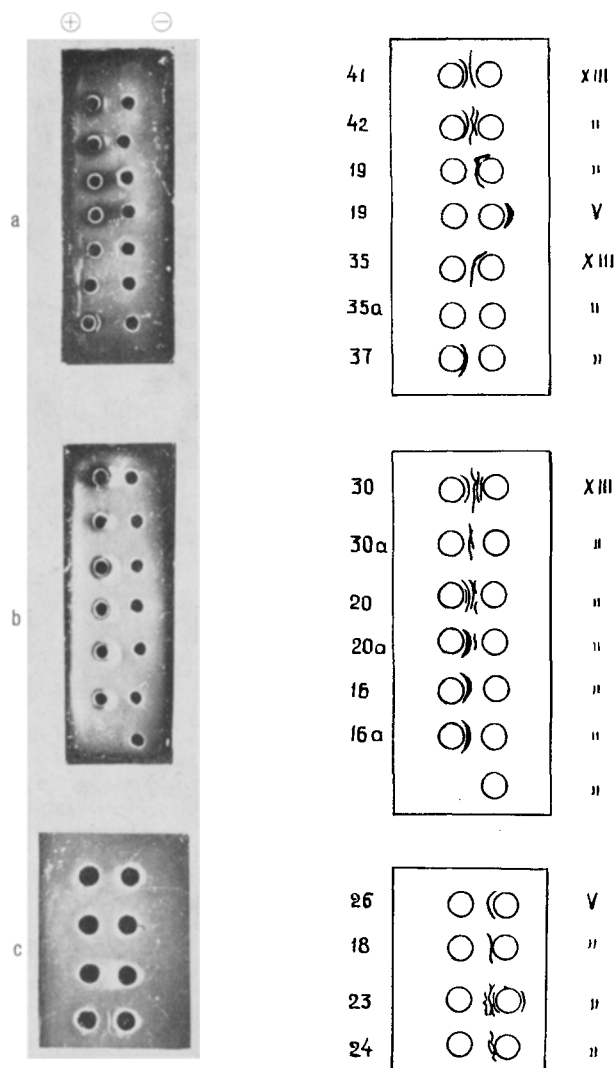


Fig. 4. Immuno-osmophoresis test. Antigens: purified FPV concentrated 13 times (XIII) and normal chorioallantoic membrane (V). Sera: FPV (30), FPV V (20) and S (16), NDV (23, 24), Sendai (35, 37, 41, 42), normal chorioallantoic membrane (19), human group B (26); index 'a'-exhausted with normal chorioallantoic membrane antigens.

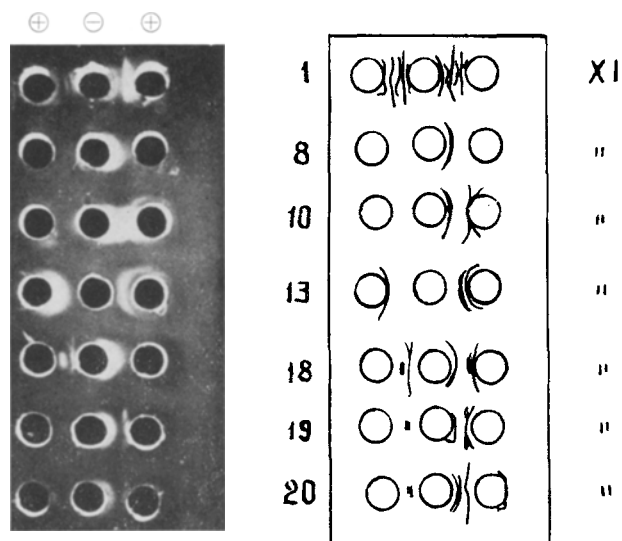


Fig. 5. Biphasic immuno-osmophoresis test. Antigen: purified FPV concentrated 11 times (XI). Sera: FPV (1, 10, 13) and FPV V sera (20), normal chorioallantoic (18, 19) and standard hemolytic sera.

Выводы. Для выявления клеточных антигенов в составе вирусов классической чумы птиц, болезни Ньюкасла и Сендай были применены методы диффузии в агаре, иммуноэлектрофореза и иммуноосмофореза. С помощью этих методов удалось выявить в составе вирионов несколько клеточных антигенов, включая видоспецифический, группоспецифический А и антиген Форсмана.

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<sup>10</sup> J. NASZ, J. CSERBA and K. ROZSA, Z. Immunforsch. exp. Ther. 134, 225 (1967).

## Experimental Myocarditis: Enhancement by the Use of Pertussis Vaccine in Lewis Rats<sup>1</sup>

It has been shown that Lewis rats are more susceptible than other strains to a number of auto-immune disease<sup>2</sup>. In addition, pertussis vaccine is known to accelerate and intensify certain experimental auto-immune diseases when given in addition to the antigen adjuvant emulsion<sup>3-6</sup>.

In previous studies, 28% of Hebrew University stock rats immunized with heart extracts in complete Freund's adjuvant, developed myocarditis<sup>7</sup>. The present experiment was designed in order to establish whether Lewis rats were more susceptible to the development of myocarditis than the Hebrew University stock rats and whether pertussis vaccine has any augmenting effect on this process.

**Materials and methods.** *Animals:* Inbred Lewis rats (Microbiological Associates, Bethesda, Md.) and 'Sabra'

rats of the Hebrew University outbred stock, of both sexes and weighing 150-200 g, were used.

**Antigens:** Rabbit and rat hearts were stored at -20°C, thawed before use and a 33% homogenate in saline prepared in a Sorvall omnimixer at 4°C. The homogenate was filtered through 2 layers of gauze and the protein content determined. The antigens were mixed in equal volumes with Freund's complete adjuvant (Difco) enriched with 4 mg/ml of *M. tuberculosis* human type C, DT, PN (kindly supplied by the Ministry of Agriculture, Fisheries and Food Control Veterinary Lab., Weybridge, Surrey, England). The final protein content of the adjuvant emulsion was 20-25 mg protein/ml.

**Pertussis vaccine** was kindly supplied by RAFA Ltd. Laboratories, Jerusalem, and contained  $228 \times 10^9$  *Bordetella* per ml.