attained with LSD in this study may be due to a direct anti-serotonin effect on the cerebral vessels or within the parenchymal elements. The possibility of a specific action of LSD other than its anti-serotonin property can not be excluded.

Summary. Lysergic acid diethylamide (LSD) was administered to guinea pigs that had received subcutaneous injections of brain homogenate. Dosages of 20 or 50 μg/kg triweekly reduced the incidence of paralysis as well as mortality rate. In animals that had demonstrable histopathologic lesions after the drug, the severity of the lesions was reduced.

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Accentuation of Plaques of Myxoma and Fibroma Viruses by Immune Serum.* (27833)

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Production of plaques on cell monolayers by myxoma and fibroma viruses has been described(1). This report deals with the influence of specific rabbit antiserum on the appearance of these plaques. It was found that when antiserum was incorporated in the agar overlay, the plaques produced by both viruses were not reduced in size or number, were made much more distinct and, without preliminary cell staining, could be counted earlier.

Materials and methods. The Moses strain of myxoma virus (MV) and the Patuxent strain of fibroma virus (FV) were used in all experiments. The origin of the viruses, the details for cultivation of the viruses, preparation of monolayers of secondary rabbit kidney (RK) cells, and composition of the routine nutrient agar overlay (RO) were the same as previously described(1) with the exception that the virus was centrifuged onto the cell monolayers according to the methods of Padgett and Walker (to be published). In brief, monolayers of secondary RK cells grown in 1-oz prescription bottles were placed cell side out in a basket rotor of an International centrifuge (size 1, model CM) and centrifuged at 3500 rpm for 30 minutes at room temperature. The inoculum was removed and the cell sheet covered with 5 ml of the

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routine agar overlay or routine agar overlay containing antibody (AbO). All plaque bottles were inverted and incubated at 35°C. No staining of the cell sheet was necessary when AbO was used or with FV under RO. Myxoma plaque bottles containing RO were stained with neutral red after 5 days incubation.

Antisera. Viruses used in the preparation of antisera were grown intratesticularly in domestic rabbits and used as a 10% homogenate of tissue in saline. *Anti-fibroma serum.* Rabbits convalescent from fibroma virus infection were given additional antigenic stimulation at 2-week intervals for 6-8 weeks by intradermal and intramuscular injections of the viral antigen. The intradermal injections consisted of 0.1 ml in each of 4 sites. The intramuscular injections consisted of 1.0 ml in each of 2 sites of an emulsion of equal parts of complete Freund's adjuvant and viral antigen. *Anti-myxoma serum.* Rabbits convalescent from an initial fibroma virus infection were challenged with a single injection of live, virulent myxoma virus. This resulted in generalized myxomatosis from which the rabbit recovered. Further antigenic stimulation was given by intradermal and intramuscular injections of the myxoma testicular homogenate as described for anti-fibroma serum. Rabbits were bled 2 weeks after the last injection; the serum was collected, heated at 56°C for 30 minutes and stored at −20°C until used.

Sometimes, for reasons as yet unknown, rabbit serum, either normal or immune, caused destruction of the RK cell monolayers. Therefore, all sera were absorbed with the primary RK cells that had been used for planting plaque bottles. One volume of cells to 30 volumes of serum incubated in a 37°C water bath for 1 hour was sufficient to remove this killing effect.

Results. Appearance of plaques under AbO. When antibody was incorporated into the overlay medium, MV plaques could be detected as opaque white foci within 48 hours after inoculation of cell monolayers. The plaques increased in density and whiteness as flat lesions with irregular diffuse edges which reached 2-3 mm in diameter by 4 days. FV

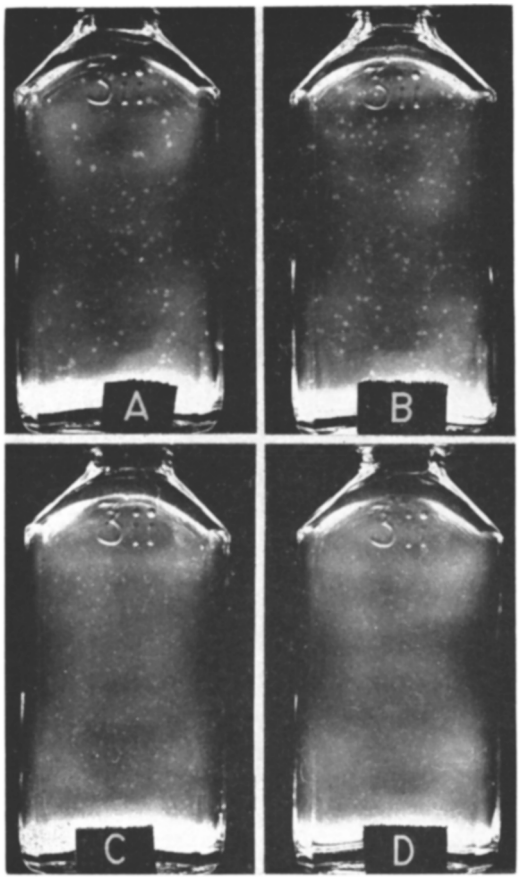

FIG. 1. Myxoma virus plaques on monolayers of rabbit kidney cells under an agar overlay containing immune rabbit serum. Unstained. A. 5% antiserum. B. 2½% antiserum. C. 1% antiserum. D. 5% normal rabbit serum (no antiserum).

plaques under AbO were first seen at 72 hours becoming 1-2 mm in diameter by the sixth day. It was noted that MV plaques continued to increase in size as long as the cell sheet remained intact (10-14 days). The FV plaques, however, reached their maximum size by the sixth day, although there was subsequent increase in the density of the plaque. Comparison of concentrations of 10%, 5%, 2.5% and 1% antiserum in the agar overlay showed no marked effect on the size of the plaques produced by either virus, but the higher concentrations did intensify the whiteness of the focus thereby making them easier to recognize (Fig. 1). All sera gave excellent results at the 5% level. This concentration, therefore, was used for the remainder of the experiments.

Microscopic examination of a MV or FV plaque showed a greyish-white precipitate covering the entire plaque area, and the cells involved in the focus appeared to have increased in number when compared with the surrounding uninfected monolayer. The precipitate associated with a myxoma plaque was more concentrated in the center of the plaque, becoming less dense toward the periphery, and extending beyond the area of obvious cytopathic effect. The precipitate around a fibroma plaque was limited to the immediate area of what appeared to be a small pile of intact cells. This plaque was smaller, more opaque, and more sharply outlined than the myxoma plaque. If the agar was removed from the cells, most of the precipitate surrounding the plaque could be seen embedded in the agar giving an appearance very similar to that seen in a typical antigen-antibody reaction in agar.

When normal rabbit serum or rabbit anti-vaccinia serum was used in place of immune serum, there was a definite thickening suggesting cell proliferation at the focus of infection, but no precipitate was associated with the plaques. This suggested that the increase in thickness of the cell layer in the area of the plaque was related to some effect of rabbit serum, perhaps nutritional, but that the precipitate was associated with the specific antibody content of the immune serum. Efforts to confirm this by classical antibody absorption methods were unsuccessful because significant reduction of antibody levels in the high-titered antisera could not be accomplished with the antigen concentrations that were available. However, supporting evidence was found when the total percentage of rabbit serum was held constant but decreasing amounts of antiserum were added. The resulting amount of precipitate associated with the plaques was found to be directly proportional to the final concentration of antiserum in the overlay although the number of cells involved in the lesions appeared to remain fairly constant.

Time of AbO addition. To determine how soon after virus adsorption the AbO could be added without resulting in a reduction of the number of plaques, plaque bottles that had been inoculated with MV or FV were centrifuged and then placed at 37°C to allow virus penetration. Eight bottles of each virus were removed at 1-, 2-, and 3-hour intervals, and the inoculum was poured off, and the monolayers covered with either AbO or RO. Results (Table I) indicated that both myxoma and fibroma viruses require 2 hours for all of the adsorbed virus particles to penetrate beyond the neutralizing effect of the antibody. FV, unlike MV, required 1 hour to reach maximum plaque numbers even if no antibody was added. Padgett and Walker (unpublished) have shown that centrifuging the virus onto the cell sheet yields higher virus titers of myxoma virus than do other adsorption methods. Therefore, it is unlikely that the difference between the penetration time of the 2 viruses is due to additional adsorption of FV while at 37°C. It is more likely that FV initially attaches more loosely to the RK cell than does MV and early in the infection process can be washed off or disturbed by removal of the inoculum and/or addition of the agar overlay.

TABLE I. Effect of the Application of Antibody-Containing Overlay at Various Times after Viral Adsorption.

Hr after adsorption	Fibroma virus plaques		Myxoma virus plaques	
	RO	AbO	RO	AbO
0	80*	67	84	46
1	110	88	90	72
2	116	109	84	84
3	114	116	88	97

* Means counts of 4 assay bottles.

Comparison with standard assay. Forty bottles of RK cells were inoculated with a single dilution of MV and 20 bottles were inoculated with a single dilution of FV. After 2 hours at 37°C, the inoculum was removed and AbO was added to half and RO added to the remainder. MV-inoculated bottles that had received AbO were counted on days 4 through 6; those bottles containing no antibody had a second overlay of neutral red added on day 5 and were counted only on day 6, since plaques were too small to allow accurate counting prior to this day. FV-inoculated bottles under AbO were counted on days 6 and 7; those with RO on day 7 only.

By the 4th day, the mean number of countable plaques of MV under AbO had reached a number equal to the 6-day count under RO. Since the mean did not increase significantly on the subsequent days, the 4-day count appeared to be a satisfactory measure of the viable particles, thus allowing the time required for reliable counting (from inoculation to reading) to be reduced by one-third. Similarly, counts of FV plaques under AbO made on the 6th day were equal to 7-day counts under either AbO or RO. The results of several experiments indicated that the time after inoculation at which MV or FV plaques could be counted with reliability was dependent on the serum used (presumably on its antibody content), since with use of certain high-titered antisera plaques of FV could readily be counted 4 days after inoculation.

Differentiation of mixed populations of MV and FV with AbO. When artificial mixtures of 40 pfu each of MV and FV were inoculated onto a RK cell sheet and covered with overlay containing 5% anti-fibroma serum, the plaques of each virus could be recognized clearly. The FV plaques are small, dense and very white foci while the MV plaques are larger, fainter and more diffuse (Fig. 2). If anti-myxoma serum was used, the contrast between the plaques was less distinct because both plaque types were equally white and selections had to be based upon plaque size only. This was to be expected since it is consistent with other experience that MV has greater cross-reaction with FV than *vice versa* (unpublished observations).

Discussion. Much of the striking appearance of MV and FV plaques under AbO appeared to be a result of the precipitation by antibody of soluble antigens in the agar around the focus of infection. Both of these viruses produce abundant quantities of several soluble antigens that readily diffuse through agar(2). In addition, however, normal rabbit serum alone in the overlay caused a noticeable increase of the plaque density suggesting that an additional factor was operating in the accentuation of plaques by antiserum. This second factor appeared to cause increased multiplication of cells in the foci, particularly in the FV foci, but in the present study it was not possible to verify this by quantitative data. This effect is under further study.

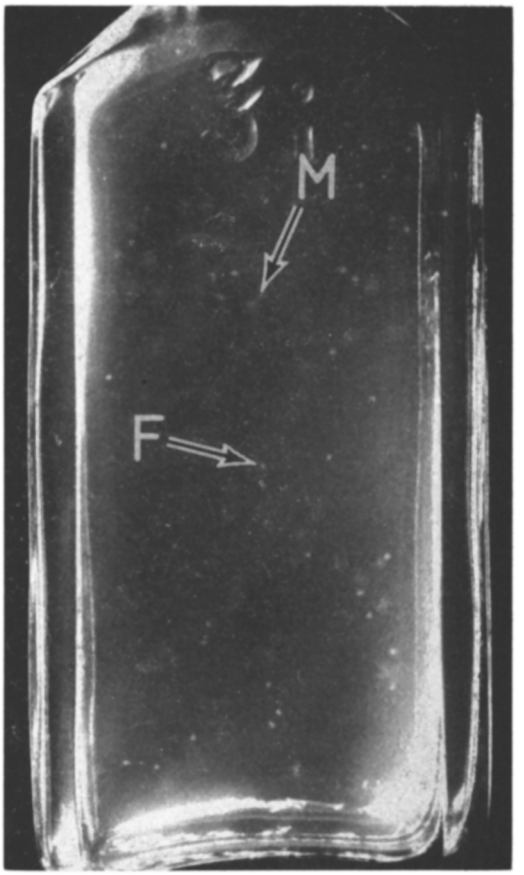

FIG. 2. A mixture of myxoma and fibroma virus plaques on a monolayer of rabbit kidney cells under an agar overlay containing 5% anti-fibroma rabbit serum. F points to a typical fibroma virus plaque; M to a typical myxoma virus plaque. Unstained ×2.

The use of antibody in the agar overlay might prove useful for improving the visualization of plaques produced by viruses other than myxoma or fibroma. Several conditions would appear to be necessary before one should expect the enhancement seen with MV and FV. First, the virus should be one that produces copious quantities of diffusible antigens against which high titered antisera can be produced. Second, the virus would have to be one that is not inhibited in its peripheral spread from an initial focus by the presence of antibody in the medium. The importance of this is emphasized by Wecker's report(3)

that the plaques of polio virus are completely inhibited or markedly reduced in size by specific antibody in the agar overlay. On the other hand, Black and Melnick(4) found that while the spread of poliovirus was inhibited by antibody under liquid overlay, the spread of herpes B virus was not inhibited. Such viruses as those of the herpesvirus group, as well as the poxviruses, may be ones for which this technic would be useful.

Summary. Incorporation of specific immune serum in the agar overlay does not inhibit the appearance or normal development of myxoma or fibroma virus plaques. On the contrary, these plaques are clearly visible on an unstained cell sheet; thus enabling an accurate and sensitive plaque count to be made with greater ease at least 2 or 3 days earlier than assays using the standard overlay. The accentuation of the plaques appeared to be due to 2 factors: (1) a precipitate in the agar around the plaque resulting from antibody-antigen reaction, and (2) an increased thickening of the cell layer at focus of infection. The former was directly proportional to the concentration of antibody in the overlay while the latter was related to the percentage of rabbit serum, either normal or immune, in the overlay. A clear distinction between plaques resulting from inoculation of an artificial mixture of myxoma and fibroma viruses was easily provided when fibroma antiserum was present in the overlay since the plaques differed both in size and density. It is suggested that technics reported here might be applicable to other viral assay systems.

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Serum Protein Synthesis by Embryonic and Neonatal Chicks. (27834)

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The chick embryo is an ideal organism for the study of serum protein synthesis since it is separated from the maternal circulation during its development. Heim and Schechtman(1) and others(2,3) have indicated that both qualitative and quantitative changes occur in the sera of developing embryos and neonatal chicks. It has not been determined what part, if any, of these changes is the result of protein synthesis by the animal, and what part is the result of absorption of proteins from the yolk(4). This problem has been investigated in our laboratory, using incorporation of radioactively labeled amino acid as a criterion for protein production, and autoradiography of immunoelectrophoretic patterns(5) to identify the labeled proteins.

Materials and methods. Animals. Seventeen- to nineteen-day-old chick embryos were given 100 μc ($\sim$50 μg) S-35 methionine intravenously; 3-day-old and 12-day-old chicks received 200 μc intraperitoneally. All animals were bled one day following injection.

Immunoelectrophoresis and autoradiography. Microimmunoelectrophoretic patterns(6) of experimental sera alone and mixed with chicken globulin (obtained by salt fractionation) or with adult chicken serum were prepared. Rabbit antisera against chicken globulin or against whole chicken serum were employed to develop the precipitation lines. The mixtures of experimental sera with "carrier" protein solutions were used in order to obtain reproducible immunoelectrophoretic patterns. It will be seen that the patterns obtained with experimental sera varied with the age of the animals. Autoradiographs were made as previously described(5).

Results. The antisera employed demon-