plants is probably caused by the loss of their main channels of renal blood supply. On the other hand, in the rabbit, rat and man, certain parenchymal cells which have somehow retained their vascularity, may be rejected very slowly. The importance of the blood supply for rejection of skin grafts has been stressed by Conway and Stark(5) and Rolle, Taylor and Charipper(6).

The surgical procedures developed for transplantation of the rabbit kidney can be adapted to transfer other organs such as segments of intestine or adrenal glands. Wherever a T-section of vessels is available, a plastic tube can be used in the operation to maintain their patency. This simplifies suturing and also insures against the possibility of accidental closing of the lumen.

Summary. Homologous transplants of the rabbit kidney have been carried out by a procedure in which vascular anastomoses of the donor aorta and vena cava were made to the carotid artery and jugular vein of the host. During the operation, plastic tubes were employed to maintain the patency of the vessels. These tubes were removed after completion of the anastomoses. Some nucleated cells can survive 4 weeks in homologous kidney transplants. The destructive process in the rabbit kidney is considerably slower than in the dog. It is suggested that the breakdown in the vascular supply to the transplant may be important in the rejection process.

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Inhibition of Cytopathic Action of Salisbury Virus by Antiviral Agent 1758.* (26531)

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In recent tissue culture studies in work related to the common cold, we have tested the activity of an antiviral agent against the cytopathic action of a Salisbury virus derived from a human cold. The reports of the work of Dr. Andrewes' group in Mill Hill and Salisbury, England(1,2,3), have renewed our interest in cold viruses. Coincidentally, we have experimented with antiviral substances from penicillium molds which demonstrated a considerable spectrum of activity in mice when virus and antiviral agent were administered by separate routes of injection(4,5,6). We are reporting here the results of tests of successful inhibition of the cytopathic action of a Salisbury virus by a penicillium antiviral agent called "1758".

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Materials. Salisbury virus H.G.P. was supplied to us as an ampoule of dried material by Dr. C. H. Andrewes. Properties of this virus have been described(1,2,3). H.G.P. virus was typed during this work with rabbit antiserum of a titer of 1-16 made with an early pool of virus. Antiviral agent 1758 was supplied to us as partially concentrated broth by Lilly Research Laboratories. This agent was derived from tank cultures of Penicillium stoloniferum (group Brevi-Compactum), and was first detected in the Lilly Labs (Powell, Culbertson, Hoehn, and McGuire, unpublished) using the technic found useful in detecting antiviral agent 8450(4). Antiviral

agent 1758 may be of more interest than 8450, since it can be prepared from tank growth, while 8450 must be prepared from shake flask growth by a tedious procedure. Tissue culture for growing virus H.G.P. was second generation or secondary monkey kidney cell cultures as the Andrewes group have described. The virus phase has utilized the low bicarbonate content replacement medium in tubes in roller drums placed at 33°C, both of these conditions comprising the standard Andrewes technic.

Procedure. When reasonably confluent monolayers of these second generation monkey kidney cells were ready, antiviral agent 1758 was either (a) put directly on the cells and the tubes rotated 10 minutes, then replacement medium added and tubes rotated again, or (b) put into the replacement medium as added to the tubes and these rotated. Twenty-four hours after addition of 1758, H.G.P. virus was seeded into all tubes following another replacement of medium, and tubes were rotated again at 33°C. Titers of H.G.P. virus attained in tubes without 1758 were generally over 10^{-4} but under 10^{-5}. Activity of 1758 as judged by the technic used in previous mouse tests(4) was as follows: Of 10 mice injected intraperitoneally with consecutive 0.5 ml doses of 1758 diluted 1-5 at 8 A.M., noon, and 4 P.M. on day 1 and 8 A.M. of day 2 (4 doses in all), and injected subcutaneously at noon of day 2 with 100 LD_{50} of Semliki Forest virus, 4 mice died on days 5, 6, 6, and 8 respectively, and 6 mice survived and were normal on day 14.

In addition to tissue culture tubes treated with 1758 and subsequently seeded with H.G.P. virus, control tubes with tissue alone, tissue and 1758, and tissue and virus but without 1758 were included. Daily microscopic observation of all tubes was made to detect cytopathic effects of the virus. In general there was little difference between the (a) and (b) procedures above, so the (b) procedure was favored, *i.e.*, putting 1758 in the replacement medium with another replacement of medium and addition of virus 24 hours later.

Results. Application of antiviral agent 1758 to otherwise susceptible tissue culture prevented the appearance of cytopathic effects of H.G.P. virus seeded into the cultures 24 hours later. Early tests utilized 0.1 ml of 1758 diluted 1-10 in replacement medium and 0.1 ml of a 10^{-3} dilution of H.G.P. virus into 4 roller tubes, and these tubes showed no cytopathic effects. This inoculum of virus alone in 4 tubes produced complete damage in 5 days, while the 1758 alone in 4 tubes appeared to be without toxicity. Subcultures of the tubes in which virus damage to cells was prevented by 1758 into new tissue culture tubes without 1758 showed reappearance of virus and damage to the cells on days 5 and 6. The 1758 appears not to destroy or neutralize virus, but rather to act on the tissue culture cells in such a way that virus does not damage them. The antiviral agent after being boiled 5 minutes did not do this. These effects are similar to results of Hull and Lavelle for antiviral agent 8450 and the 3 types of poliovirus(7). Table I shows the results of a somewhat enlarged test including several dilutions of 1758 and different dilutions of H.G.P. virus, all dilutions being made in replacement medium. Cytopathic action of a 10^{-3} dilution of H.G.P. virus was inhibited by increasing dilutions of 1758 up to 1-10. A dilution of 1-16, however, was ineffective. An intermediate dilution of 1758 of 1-8 also inhibited the cytopathic action of virus diluted 10^{-2} and 10^{-4}. 1758 was not toxic to the cells. Boiled 1758 did not inhibit cytopathic action of the virus. Also, when cytopathic action of H.G.P. virus was inhibited by 1758, subcultures of these tubes showed reappearance of the virus and cytopathic effects. The antiviral agent thus appears again to render the cells insusceptible to virus action for a time, without neutralization of the virus. In an additional test parallel with that shown in the upper 8 lines of Table I the (a) variation in technic referred to above, *i.e.*, putting the 1758 on the cells directly and rotating the tubes 10 minutes, then adding the new replacement medium and rotating again, gave the same end point of action as that shown in the Table. Both technics showed 1758 diluted 1-10 was effective, while 1-16 was not effective. In all these tests duplicate tubes of tissue culture were used.

TABLE I. Effect of 1758 on Cytopathic Action of H.G.P. Virus.

Dilution of 1758	Dilution of H.G.P.	Cytopathic readings	Cytopathic reading: Subcultures
Undiluted	10^{-3}	— —	0
1 - 2	10^{-3}	— —	0
1 - 5	10^{-3}	— —	0
1 - 8	10^{-2}	— —	+ +
1 - 8	10^{-3}	— —	+ +
1 - 8	10^{-4}	— —	+ +
1 - 10	10^{-3}	— —	0
1 - 16	10^{-3}	+ +	0
None	10^{-2}	+ +	0
"	10^{-3}	+ +	0
"	10^{-4}	+ +	0
Undiluted	None	— —	0
1 - 2	"	— —	0
Undil. (boiled 5 min.)	10^{-2}	+ +	0
Idem	10^{-3}	+ +	0

Legend: + indicates complete damage to cell layer usually starting on day 5 or 6 and rapidly progressing. — indicates no cytopathic effect. 0 indicates not done. 1758 and H.G.P. were added in volumes of 0.1 ml.

In additional tests using a 10^{-2} dilution of H.G.P. virus throughout, the titer of 1758 was 1-5, instead of 1-10 as shown in Table I. A dilution of 1-8 almost completely, and a dilution of 1-10 partially, inhibited cytopathic action.

Our detection of active virus (lines 4, 5, and 6, Table I) after 10 to 14 days at 33°C in tissue culture protected by 1758 is compatible with some earlier unreported tests we have done on the kinetics of thermoinactivation of H.G.P. virus.

Discussion. The results reported here indicate that an antiviral agent provisionally called 1758, first detected in mouse screening tests and having chemoprophylactic action in mice similar to antiviral agent 8450, inhibits the cytopathic action of Salisbury virus H.G.P. As far as we have noted, the virus itself is not affected directly, but the tissue culture cells are made refractory for an undetermined period, at least 14 days, to the cytopathic action of the virus. In this type of antiviral action as well as in the results of mouse tests, antiviral agents 1758 and 8450 are somewhat similar. Dr. R. N. Hull has recently told us that 1758 like 8450 inhibits cytopathic action of poliovirus. Both these agents are products of penicillium molds. Since 8450 has appeared similar in spectrum to Helenin(6), also a penicillium product, it follows that 1758 is likely similar in this respect to Helenin. It is possible that, due to tank growth of 1758, more ample supplies may expedite further work.

Summary. It has been found that an antiviral agent called 1758, and somewhat similar to agent 8450 in antiviral action in mice, exhibits demonstrable inhibition of the cytopathic action of Salisbury virus H.G.P. This antiviral agent is of penicillium origin, and virus H.G.P. is one of several strains reported of common cold origin.

Addendum: See report of 1758 as Statolon in Probst, G. W. and Kleinschmidt, W. J., *Federation Proceedings,* 1961, v20, 441.

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