are interesting, for example, the oxalates of Li, Na, K, have little or no hemolyzing power but cause extensive necrosis of the skin. Thirdly, the precipitation of staphylococcus toxin by certain of these salts, such as $(NH_4)_2 SO_4$, the salts of Ba and of Sr, and more particularly the precipitation by calcium chloride and the recovery of the toxin with K_2Ox, which will be subsequently reported, are worthy of note.

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Attempts to Demonstrate Virus-Neutralizing Substances in Saliva and Serum from Mumps Immunes.*

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Active immunity to experimental mumps parotitis in *Macacus rhesus* has been studied by Johnson and Goodpasture,[1] with the following conclusions: "The only reliable experimental method at present of inducing active immunity to mumps in monkeys is by causing a unilateral or bilateral clinical or subclinical specific parotitis by intraparotid inoculation." They failed, except rarely, to demonstate any passive immunity to mumps in monkeys previously injected with the serum of persons immune to this disease, or any marked virus-neutralizing action of human convalescent serum.

As a further step toward understanding the mechanism of active immunity to experimental mumps, the present study has been made concerning the virus-neutralizing properties of human and monkey saliva and of monkey serum.

For this purpose it was decided to inject a number of normal *Macacus rhesus* monkeys by the usual transductal route with variously treated mixtures of mumps virus (saline suspensions of infected glands) and saliva (or serum) from immune or normal animals and human beings. One parotid would receive the immune substance, the other the normal, and the presence or absence of virus-neutralizing substances would be shown by the presence or absence of or difference in the mumps "takes" on the 2 sides, with respect to time of onset and size of gland.

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1 Johnson, C. D., and Goodpasture, E. W., *Am. J. Hygiene*, 1936, **23**, 329.

Several monkeys were actively immunized by unilateral of bilateral injections, into the parotid duct, of centrifuged saline suspensions of the parotid glands of monkeys in the active stage of experimental mumps. The virus used had been recently obtained from the saliva of a boy in the first day of clinical mumps, and had undergone several typical monkey passages, with characteristic clinical signs and gross and microscopic changes in the glands.

When the inoculated animal developed, on the 6th to 8th day, fever, swelling of the parotid gland or glands, with or without edema of the overlying tissues, and enlargement of the adjacent lymph-nodes, it was assumed, on the basis of the constantly positive results obtained by Johnson and Goodpasture, that it had mumps, and would subsequently be immune. It did not, therefore, seem necessary to test each animal's immunity by reinoculation at a later time.

Saliva was obtained from immune and from normal monkeys under nembutal anesthesia (Nembutal 30 mg. per kilo, intraperitoneal), by administering pilocarpine hydrochloride (one mg. per kilo) and collecting the fluid which drooled from the mouth. Attempts to obtain the parotid secretion by itself, through suction cups and cannulas, were unsuccessful. The saliva was centrifuged to get rid of solid particles, and certain samples were then filtered through a Berkefeld V candle. Others were used unfiltered.

The saliva-virus mixtures consisted of equal parts of saliva and of a 20% saline suspension of the infectious glands, giving a final tissue percentage of 10%, which concentration had been consistently found sufficient by itself in 2 cc. amounts, to cause mumps. The dose of the virus-saliva mixtures was 3.5 cc.

Human saliva, from immunes and normals, was employed in the same manner.

Except in 2 experiments, in which the mixed inoculums were placed in the icebox overnight, and one in which the inoculum was heated to 55°C. for ½ hour, all the virus-saliva mixtures were held at 37°C. for 2 hours before injection, to give presumably enough time for antibody-virus combination.

Monkey serums were obtained from either the saphenous vein or the heart, mixed in equal parts with virus suspensions, and heated, as above, at 37°C. for 2 hours.

Both salivas and serums of immune monkeys were taken between one and 2 months after inoculation with mumps. Human salivas were taken one month and 2 months after the onset of the disease.

Whether or not a test animal had a "take" was determined entirely on clinical grounds, by swelling, with or without edema, of

one or the other parotid gland. The animals were not killed, both for reasons of economy and because it was necessary to observe them for longer than the usual period to detect possible delayed "takes".

Of 12 monkeys receiving either human or monkey immune saliva and virus on one side and normal saliva and virus on the other, only 2 showed a better "take" in the normal saliva parotid than in the immune saliva parotid. In one, which had received mixtures whose saliva had been heated to 55°C. for ½ hour, there was no "take" on the immune-injected side, and a moderate enlargement of the normal-injected side. In the other both glands showed some swelling, but it was much more marked on the side which had had the normal saliva mixture. A third monkey showed a slight delay in the onset of swelling on the immune side, but the ultimate maximum swelling was the same in both glands.

The rest of these 12 monkeys either showed the greater enlargement on the immune-saliva side (in 2 cases) or showed no distinct difference between the 2 glands.

Of the 4 monkeys injected with immune serum and virus in one parotid and with normal serum and virus in the other, one had equal enlargement on the 2 sides, one showed no swelling one either side, a third had a small "take" only on the immune-serum side, and the fourth had just the opposite, a "take" only on the normal side.

One monkey was used as a control. It received normal and immune monkey salivas without virus, and was negative both clinically and microscopically.

The results of these experiments are obviously indecisive and fail to demonstrate the presence of any antiviral substance in either the saliva or the serum of monkeys, or in the saliva of human beings recently recovered from mumps. The variations in reaction with the different mixed inoculums are no greater than might occur accidentally, especially if one considers the summer decline in mumps "takes" which has been repeatedly observed, and which may have affected a few of the later experiments in this series.

One comes to the same conclusion as Johnson and Goodpasture, that the study of immunity to mumps requires a more delicate test of the presence of antibodies than is provided by intraparotid inoculations. If there were some other, less direct way, as yet undiscovered, of inducing the specific parotitis, which would give a more delicate balance between the infecting agency and the immunizing substance, significant experiments might be performed. It is also most important to develop a way of measuring and standardizing the virus content of the tissues used for inoculums. Without having a

minimal infective dose, it is impossible to detect small amounts of immune substances in fluids or tissues.

Summary. 1. The parotid glands of monkeys were injected with various mixtures of mumps-virus-containing gland suspensions and of saliva or serum of mumps-immune and normal monkeys, and saliva from immune and normal human beings. 2. The results of these injections give no evidence for the presence of any considerable amount of antiviral substance in either serum or saliva. 3. More delicate and accurate techniques are needed to demonstrate the nature of antiviral immunity to mumps.

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Attempted Chemical Isolation of the Virus of Poliomyelitis.

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The demonstration of the chemical nature of the tobacco mosaic virus by Vinson and Petre,[1] its recent isolation as a crystalline protein by Stanley,[2] and the experiments of Howitt[3] indicating that the virus of poliomyelitis may be also a chemical entity, have led us to employ Stanley's procedure in an attempted isolation of the virus of poliomyelitis in the form of a crystalline chemical substance.

This attempt was not successful but, because the results are of interest in that they afford added evidence for the theory of the chemical nature of viruses, they are briefly reported herewith.

One hundred grams of spinal cords from monkeys infected with the M.V. strain and developing typical paralysis in from 7 to 11 days after intracerebral inoculation, were finely ground with sand and extracted overnight in the refrigerator with 500 cc. of a solution of disodium hydrogen phosphate at pH 8. The thoroughly mixed suspension was poured onto a fine wire screen and the retained precipitate again extracted for 24 hours with 500 cc. of the phosphate solution, poured through the screen and the combined milky suspensions filtered by gravity through coarse fluted filter paper. A monkey inoculated with 0.5 cc. of this filtrate developed typical paralysis in 9 days.

1 Vinson, C. G., and Petre, A. W., *Bot. Gaz.*, 1929, **87**, 14.

2 Stanley, W. M., *Science News Serv.*, 1935, **81**, 644.

3 Howitt, B., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 158.