Shope (1) found that domestic rabbits bearing his papilloma were generally immune to reinoculation with the virus, an observation that has been amply confirmed by Kidd, Beard, and Rous (2, 3) and by Ladewig (4). Where the animals were not totally resistant the presence of a partial immunity was indicated by the development of but few warts at the site of the second inoculation and by an increase in the length of the incubation period.

Immunity was attributed by Shope to antibodies in the blood serum which generally neutralized the virus completely. According to Kidd, Beard, and Rous (3) these protective agencies develop within a few weeks after the warts appear and are roughly proportional, on the whole, to the area of skin involved. They prevent reinfection, but do not inhibit papillomas that are already established, the occasional regression of a wart being ascribed by Rous and Beard (5), and by Kidd (6), to some influence directed against the cell rather than against the virus itself.

No domestic rabbit was found naturally refractory to inoculation of the virus on scarified skin, either by Shope or by Beard and Rous (7).

Attempting to induce resistance in the absence of a growing tumor, Shope (8) injected extracts of his papilloma into the peritoneal cavity, among other sites, for the virus elicits a neoplasm only in the epidermis. Two c.c. of a 5 per cent extract, repeated eight days later, caused partial or complete resistance to the virus when this was rubbed into the abraded skin twenty days after the second immunizing injection. But despite the precaution of a fresh needle for each animal, in order to avoid infecting the epidermis at the inoculation site, 1 of the 3 treated rabbits developed papillomas at the point where the needle had entered the skin. The injections resulted in immunity, but it was impossible to decide whether this had been elicited by the virus introduced into the peritoneal cavity or by papillomas accidentally induced during the procedure.

In subsequent experiments the dilemma was solved by excising the skin immediately surrounding the needle puncture two days after each injection.

This procedure, though effective, is laborious, and it was with the object of simplifying the technic that the experiments which are to be described in the following paragraphs were undertaken.

Although the work of previous investigators, whom they quote, had suggested that a virus made avirulent by ultraviolet light loses its immunizing power, Hodes, Lavin, and Webster (9) found that when rabies virus was exposed for from forty-five to sixty minutes to a mercury vapor lamp at a distance of 12.5 cm. infectivity was abolished without entire loss of antigenicity,
and it was determined to see whether this would hold true also for the Shope papilloma virus.

According to Baker (10), viruses differ considerably in their resistance to the ultraviolet ray. For the Shope papilloma virus the period required to abolish infectivity has been set by Kidd (11) at sixty to ninety minutes for a 5 per cent extract, with a Hanovia Alpine sun lamp 48 cm. distant, while Yun, Choi, Ryang, and Kim (12) found the infectivity of a similar extract greatly diminished after it had remained for thirty minutes under a Burdick Radio Vitant lamp 25 cm. away. Our own experience has been more or less similar, exposure to a Cooper-Hewitt 110 volt 4 ampere lamp, in a preliminary test, having destroyed the infectivity of a 5 per cent extract after one hour.

As a thermometer placed on the bench next to the container in which virus was irradiated never registered higher than 41.5°C, and thirty minutes at about 70°C is required to render the virus non-pathogenic, according to Shope (1) and to Kidd (13), it was thought highly improbable that infectivity had been destroyed by the heat alone. The belief was strengthened by Kidd’s experience (11), in which the virus was deprived of infectivity after from sixty to ninety minutes under an ultraviolet lamp, though the temperature of the surrounding air did not go above 28°C. Nevertheless, this possible source of error was eliminated in Experiment 3.

**Experimental**

In the experiments about to be described, glycerolated papilloma from wild cottontail rabbits was washed thrice with physiological saline and then ground with sand in sufficient Locke-Ringer solution (without glucose) to make a 10 per cent extract. Exposure took place in an open Petri dish, where the extract formed a layer about 0.5 cm. deep, at a distance of 20 cm. from the lamp.

The rabbits employed were young adults from 2 to 2.5 kg. in weight.

**Experiment 1:** Feb. 24, 1938. After having been irradiated for eighty minutes the extract was placed in the ice-box for use on the following morning. The temperature on the bench under the lamp was 27°C at the beginning and 38.2°C at the end of the exposure.

The 6 rabbits intraperitoneally injected, each with 0.5 c.c. of this extract, were given a similar treatment with 2 c.c. four days after the first, the extract having been kept meanwhile in the ice-box.

Twenty-five days after their first immunizing injection, the treated rabbits, and 3 untreated controls, were inoculated in separate shaved and scarified areas on the flank by rubbing in a fresh 5 per cent extract or a 1:1,000 or 1:10,000 dilution of it.

Forty-eight days after this inoculation the inoculation site in all 6 immunized rabbits appeared normal, and no tumor could be found at the point where the needle had passed through the epidermis. The 3 controls, on the other hand, had papillomas averaging 2.5 X 3.5 cm. in diameter as a consequence of the introduction of undiluted extract; in the 1:1,000 area there were warts which averaged 0.75 cm. in diameter in 2 animals, while in the third rabbit this site was negative. In none of these 3 untreated controls had the 1:10,000 dilution given rise to a tumor.

**Experiment 2:** June 27, 1938. Here the exposure was but forty minutes, with a new Victor mercury arc lamp. The temperature was 27°C at the beginning and 32°C at the end of the period. The extract was stored over night in the ice-box.

On the following day 6 rabbits were injected intraperitoneally with 0.5 c.c. each of the irradiated extract.
Seven days later the 2 survivors were injected again by the same route, each with 2 c.c. of the extract, which had been stored during the interim in the ice-box.

Twenty-eight days after their first immunizing injection these 2 treated rabbits, together with 3 untreated controls, were inoculated by rubbing a fresh 5 per cent extract, or a 1:1,000 or 1:10,000 dilution of it, into abraded areas on the flank.

Seventy-eight days after this inoculation all 3 inoculation sites appeared normal in the immunized rabbits, and no growths could be found at the point where the immunizing injections had been made. In contrast with this, the 2 surviving controls had tumors averaging 1.2 × 1.4 cm. in diameter at the site of inoculation with the full strength extract. Neither dilution had given rise to a papilloma.

**Experiment 3: May 22, 1939.** The extract was exposed for ninety minutes to the lamp employed in the first experiment. The temperature was 23.2° C. at the beginning and 41.5° at the end of the exposure.

As a control, 10 c.c. of the extract was kept in a water bath, the temperature of which was maintained parallel with the rising temperature under the lamp. This sample was inoculated to see whether its infectivity had been destroyed by the heating.

Five rabbits were injected intraperitoneally, each with 0.5 c.c. of the irradiated extract. Eight days later this treatment was repeated with 1.0 c.c., and fifteen days after the first immunizing injection a third, of 1.0 c.c., was administered.

Twenty-six days after their first immunizing treatment these 5 rabbits, and 2 untreated controls, were inoculated by means of a tattooing needle with a 10 per cent fresh extract of papilloma.

Forty-two days after this inoculation the inoculation site appeared normal in the 4 surviving imunes, save for a 1 mm. papule in one of these animals, whereas in the 2 normal controls the entire area was covered by innumerable small warts. As in the preceding experiments, injection of the rayed virus elicited no papillomas at the point where the needle entered the epidermis, nor were any discovered at autopsy in the peritoneal cavity.

This experiment was more fully controlled than the first two. The rayed virus used for immunization was tested for infectivity by inoculation into the epidermis of 3 rabbits, and found to be inert in this respect, an unnecessary precaution, it may be, since rayed virus had never given rise to a papilloma in the needle track. Again, because virus maintained at a temperature equalling that under the lamp produced a growth 2 × 3.5 cm. in diameter, the abolition of infectivity in rayed virus was clearly due to the ultraviolet light itself and not to the heat of the lamp.

**DISCUSSION**

The fact that the Shope papilloma virus will lose virulence yet retain antigenicity under ultraviolet irradiation offers a method of inducing immunity to this growth without eliciting a tumor at the same time. Another means of attaining the same end might be to employ extracts from non-infectious warts, for Shope found these as efficacious as similar preparations from papillomas rich in virus. This has not been employed in a practical way, however, and in any case the procedure described in the foregoing paragraphs is quicker and less expensive than the testing of extracts for infectivity.

The authors wish to thank Dr. Shope for his generosity in keeping them supplied with virus.

**CONCLUSION**

Of 12 rabbits intraperitoneally injected with an ultraviolet-irradiated extract of the Shope papilloma, 11 proved totally immune to subsequent inoculation with the virus of this growth and 1 virtually so, while all 7 untreated controls developed the tumor.
BIBLIOGRAPHY