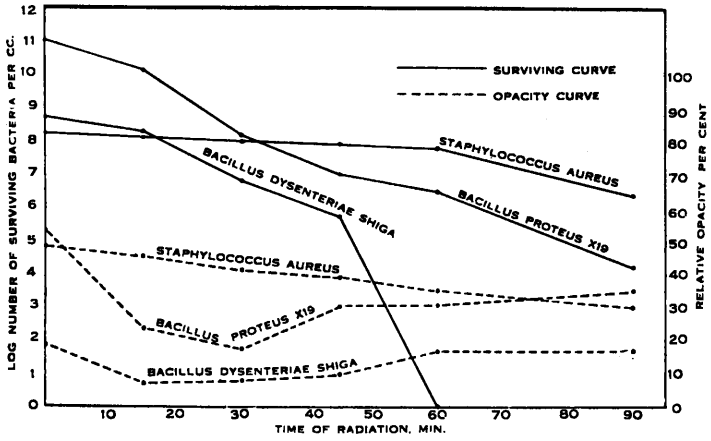

FIG. 2.

Relation between relative opacity and number of surviving bacteria. Volume of bacterial suspension used = 5 cc.

On longer exposure the number of surviving bacteria continued to decrease while the opacity increased to a definite level and then remained practically constant. This increase of opacity is probably due to the coagulation[2] of the cell proteins after lysis.

This study shows that exposure to supersonic waves brings about the killing and dissolution of various bacteria and that on prolonged treatment coagulation of the dissolved bacterial protein may be effected.

7526 C

Variation in Potency of Vaccinia Virus in Tissue Cultures.

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The possibility of employing vaccinia virus in pure culture for prophylactic vaccination against small-pox has been indicated by Rivers,[1] Herzberg[2] and Stevenson and Butter.[3] This leads to investigation of the behavior of such cultures, especially their immunizing and infective properties. Therefore a study was undertaken

1 Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

2 Herzberg, K., *Klin. Wochenschr.*, 1932, **11**, 2064.

3 Stevenson, W. D. H., and Butter, G. G., *Lancet*, 1933, **21**, 228.

to determine the effect of repeated passages in chick-embryonic tissue culture on the potency of the vaccinia virus.

The initial virus was prepared by passing the calf-lymph vaccinia virus supplied by the National Epidemic Prevention Bureau, Peiping, through the testicles of a normal rabbit. The bacteria-free vaccinia virus thus obtained was cultivated in the chick embryonic tissue according to the method described by Rivers.[1] Transfers of the virus to fresh chick embryonic tissue were made every 5 days. After each transfer the titre of the virus, *i. e.,* the lowest dilution which still gave the typical skin lesion was determined by intradermal injections of 0.25 cc. of varying saline dilutions of the virus culture into the shaved skin of albino rabbits. The first virus culture gave a titre of 1:1,000 which rose to 1:100,000 on the 3rd and 4th transfers and reached the peak of 1:1,000,000 on the 5th transfer after which it declined as subsequent transfers were made so that by the 25th transfer the titre had dropped to 1:1,000, equaling that of the initial virus, and by 30th transfer it had fallen to 1:100. From this point on the titre remained at a level between 1:50 to 1:100 when carried up to the 40th transfer. As no further drop in titre was observed, it was concluded that the virus culture had reached a stage of biological stability at the low titre of 1:50 to 1:100.

Since it is well known that the calf-lymph vaccinia virus of low titre can be increased by interposing a rabbit passage, it appeared interesting to see whether passage of this low titre vaccinia virus through a rabbit testicular tissue culture could raise the titre of the virus. The virus cultures after 30 and 40 transfers in chick embryonic tissue were inoculated separately into minced young rabbit testicular tissue cultures in Tyrode's solution. After the first passage, the titre immediately rose to 1:1,000 and by the third passage in the testicular medium it became 1:100,000. On reinoculating these virus cultures to chick embryonic tissue, the titre of the virus remained at the high level of 1:1,000, 1:10,000, 1:100,000 respectively after the 1st, 2nd, and 3rd transfers. Thus, it was clearly shown that the vaccinia virus of low titre after repeated passage in chick embryonic tissue culture could be made to regain its high titre by an interposing passage through a rabbit testicular tissue culture.

To determine further the immunizing power of the virus cultures after repeated passages in chick embryo, the following experiment was performed. Several rabbits were divided into 3 groups and each group of animals was immunized by intradermal vaccinations. The first group was given intradermal injection with a virulent virus

culture (titre 1:1,000,000) obtained after the 5th passage in chick embryonic tissue medium, the second group with a virulent virus culture (titre 1:10,000) grown in rabbit testicular tissue medium and the third, with a virus of low virulence (titre 1:100) obtained after the 35th passage, in chick embryonic tissue culture. The test for immunity was performed one month later by intracutaneous injections of varying dilutions of a virulent vaccinia culture. The subsequent observation of all these animals clearly demonstrated that no appreciable differences in the state of immunity could be detected among the 3 groups. It was found that each group of animals showed immunity reaction with the dose equal to 1:100 dilution or lower of the virulent culture, while an entirely negative response was observed with a 1:1,000 dilution of the culture. Control test with normal rabbits showed that typical skin lesions were produced when a 1:10,000 dilution of the same culture was inoculated.

The result suggests that in rabbits the production of vaccinia immunity does not necessarily require the use of a highly virulent culture. Adequate immunity in rabbits can easily be created with a virus of low virulence. Therefore in human practice of prophylactic inoculation against small-pox, the use of a vaccinia virus of low virulence may present several advantages, one of which concerns the existence of post-vaccinal encephalitis. Without going into the details of this particular problem, one may, on the basis of the theoretical conception, assume that such a pathological condition can more successfully be brought about when a virus of high potency is used. To substantiate this assumption we have performed an experiment in which the invasive power of a vaccinia culture of high and low potency has been determined. Several rabbits were injected either intratesticularly or into the brain, using a desired quantity of the respective culture and it has been found that 2 rabbits injected into the brain and 2 rabbits injected into the testicles with a highly virulent culture succumbed to the infection; out of 4 rabbits similarly inoculated with a slightly less virulent culture only one animal survived; while, on the other hand, all 6 rabbits which received the intracranial or intratesticular inoculations of a low virulence culture survived. It would appear definite that the attenuation of the vaccinia virus cultivated for 30-40 generations in the chick embryonic tissue culture is associated with the entire loss of its invasive potency, while as shown above it still retains its protective power.

There may be objections to the practical employment of the vaccinia virus of low virulence considering the question of the "take".

This important question can be solved only by the actual test on human beings. It seems to us, however, that this objection is partially obviated by the observation that for many years the attenuated calf vaccine has been found satisfactory as regards its "take."

7527 C

A Simple Method for Aseptic Grinding of Bacteria.

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For the mechanical disintegration of bacteria Macfadyen and Rowland[1] employed a highly-complicated machine in which the organisms are pulverized in a frozen condition between a rapidly rotating metal pestle and a stationary metal receptacle immersed in liquid air. The bacteria are rendered very brittle by this intense chilling and are rapidly disintegrated by the grinding process. The amount of bacteria dealt with is not large, varying from 0.5 to 1 gm. of the washed organism. Barnard and Hewlett[2] introduced a ball mill capable of grinding bacteria in a moist pasty condition. Thomson[3] devised a vaccine churn that is capable of smashing bacteria in emulsion form with some success. Recently, Krueger[4] described a type of ball mill for disintegrating bacteria and tissues under aseptic conditions.

The grinding machines above described are generally very costly to construct, and are not all uniformly successful. For grinding small quantities of bacteria it appears that an inexpensive and equally efficient apparatus may be used with advantage. A simple method in which bacteria may be disintegrated in a moist condition is here described.

The grinding chamber consists of a large-size pyrex test tube measuring approximately 20 cm. long and 3 cm. wide. Into this are placed a number of rustless steel balls, 8 mm. in diameter, sufficient to fill one-third of the test tube. The tube is tightly closed, plugged by means of a rubber cork and inserted into an outer metal tube 23 cm. long and 3.2 cm. wide. The test tube is held firmly in position

1 Macfadyen, A., and Rowland, S., *Centralbl. f. Bakt.*, 1903, **34**, 765.

2 Barnard, J. E., and Hewlett, R. T., *Proc. Roy. Soc. B.*, 1912, **84**, 57.

3 Thomson, D., *Annals Pickett-Thomson Res. Lab.*, 1924, **1**, 275.

4 Krueger, A. P., *J. Inf. Dis.*, 1933, **53**, 195.