the intestinal tract as evidenced by significant blood levels. 10,000 u kg per day was sufficient to produce blood levels as high as 0.085 u/cc of plasma 1 hour after administration. 5. Additional evidence of absorption was obtained by the finding of high levels of bacitracin in the urine. The highest obtained with 10,000 u was 0.22 u/cc and with 20,000 u 1.65 u/cc of urine.

16497 P

Attenuation of Vaccinial Virus by Interfacial Adsorption.*

J. M. JOHLIN.

From the Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tenn.

Previous mention has been made of the possibility of attenuating the virus of vaccinia by interfacial adsorption.[1] The data obtained at that time are those given in the present report. They illustrate the attenuation of this virus so that it will no longer produce the lesions which are characteristic of untreated vaccinia when injected endermically into rabbits but will nevertheless produce an immunity to the action of the untreated virus in these animals.

The method of attenuation employed was the same as that which was used in inducing the clotting of non-clotting blood plasmas[2] and in the attenuation of insulin[3,4] and of toxins such as ricin,[1] tetanal toxin,[1] staphylococcal toxin,[5,6] and snake venom.[1] It was shown there that, while emulsified chloroform is on the whole to be preferred over other adsorbing agents, inert emulsified gases are in some instances equally efficient in bringing about an attenuation.[6]

The virus† used in the present experiments was produced in chick embryos and suspended in saline. The saline suspension was treated by shaking with an equal volume of chloroform for 24 hours and all chloroform subsequently removed by evaporation under reduced pressure at a temperature below 40°C. Newly acquired animals which had not been exposed to vaccinia were used and each animal was isolated from all others during the period of treatment.

After completing these experiments, the writer found that Kelser[7-10] had prepared a rinderpest vaccine by allowing a saline suspension of the finely ground tissue from animals killed in the acute stages of rinderpest to stand in the ice box for a few hours in the presence of .75% chloroform. Kelser later prepared a rabic vaccine by the similar treatment of a 33⅓% fixed-virus brain-emulsion in physiological saline in the presence of 1.0% chloroform.

The writer subsequently found that Kelser's method could be applied to vaccinia to attenuate it sufficiently so that it will produce no lesions when administered endermically. Long standing of toxins with chloroform had,

* Aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

1 Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 569.

2 Johlin, J. M., *J. Biol. Chem.*, 1929, **81**, 99.

3 Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 524.

4 Johlin, J. M., *Endocrinology*, 1941, **29**, 574.

5 Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 135.

6 Johlin, J. M., and Rigdon, R. H., *J. Immunol.*, 1941, **41**, 233.

† The writer is indebted to Dr. John Buddingh for the virus preparations used in these experiments.

7 Kelser, R. A., *Philippine J. Sci.*, 1928, **36**, 373.

8 Kelser, R. A., Youngberg, S., and Topacio, T., *J. Am. Vet. Med. Assn.*, 1929, **74**, 28.

9 Kelser, R. A., *J. Am. Vet. Med. Assn.*, 1930, **77**, 595.

10 Kelser, R. A., *Mil. Surgeon*, 1931, **69**, 34.

on the other hand, not been found to have any appreciable effect in attenuating these substances. The effect of Kelser's treatment would seem to be also that of interfacial adsorption.

In the present experiments each of a series of 3 rabbits was given 6 endermical injections widely distributed, totalling 1.5 cc in each case, of the treated viral suspension. The injections were repeated at 4-day intervals, 2 rabbits receiving 3 additional series of injections and the third receiving but a second series. No symptoms followed the first 2 series of injections in any animal. In the case of the 2 animals receiving a third and fourth series of injections, an area of slight induration, about 15 mm in diameter, without erythema, developed within 24 hours at each site of injection and lasted about 2 days. After a rest period of 7 days following the last injection, each animal received 2 injections of 0.1 cc each of the untreated viral suspension. Of the 2 animals which had received the longer series of injections of the treated virus, one developed areas of edema, 30 × 50 mm and 30 × 40 mm accompanied by a faint erythema, at the sites of injection of the untreated virus, within 24 hours. The smaller of these areas disappeared after 4 days and the other after 7 days. The other animal developed areas of slight induration, 18 × 20 mm and 15 × 20 mm accompanied by a faint erythema, at the sites of injection of the untreated virus, within 24 hours. These reactions disappeared within 3 days after the injections and, like those which developed after the third and fourth series of injections of the treated virus, may have been due to an acquired protein-sensitivity.

The third rabbit, which had received but 2 series of injections of the treated virus, developed areas of slight induration, 15 × 30 mm and 30 × 40 mm, accompanied by a faint erythema, at the sites of injection of the untreated virus, within 24 hours. These reactions disappeared within 3 days after the injection of the untreated virus. None of these animals developed lesions. The average increase in weight of the 3 rabbits during this course of treatment was 55 g.

A control animal received 2 injections of 0.1 cc each of the untreated virus and at the same time received 2 similar injections of the treated virus. The treated virus produced no symptoms while the untreated virus produced its usual symptoms. Ten days later this rabbit again received 2 similar injections of the untreated virus. Areas of slight induration and faint erythema developed within 36 hours but disappeared within 3 days after the injections.

Summary. Vaccinia can be attenuated by interfacial adsorption so that it will no longer produce the lesions of this virus when injected endermically into rabbits but will produce an immunity to the action of the untreated virus.

16498

Effect of Medium on Resistance of *Staphylococcus aureus* to Quaternary Ammonium Compounds.*

W. L. MALLMANN, ANITA LEAVITT, AND DWIGHT A. JOSLYN.

From the Department of Bacteriology and Public Health, Michigan State College, and the Research Department, Parke-Davis and Company, Detroit.

A comparison of data obtained by two of the authors on a quaternary ammonium compound with *Staphylococcus aureus* (No. 209 F.D.A.) consistently revealed strikingly different results. It was found that the culture used by one author was grown in standard F.D.A. broth while the other author used Difco "disinfectant testing" broth. A study

* Journal Article No. 952 (N.S.) from Michigan Agricultural Experiment Station.