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.

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

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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

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of the 2 media was made to determine the cause of the discrepancy.

Tests were made simultaneously on both media using *Staph. aureus* (No. 209 F.D.A.) with a mixture of alkyl dimethyl benzyl ammonium chlorides.

The culture was transferred daily in each medium for at least 3 successive days to obtain a constant inoculum. The tests were made according to the phenol coefficient procedure recommended by the Food and Drug Administration.[1] The culture grown in Difco "disinfectant testing" broth was adjusted to pH 7.2 before use to eliminate the effect of differences in hydrogen ion concentration. Killing was obtained in 10 minutes in Difco "disinfectant testing" broth in a dilution of 1-4000, whereas in F.D.A. broth the killing dilution was 1-20,000. The same effect was obtained with N(acyl colamine formyl methyl) pyridium chloride. With Difco "disinfectant testing" broth the killing dilution was 1-250, whereas with F.D.A. broth the dilution was 1-1800. No differences in killing dilutions were obtained with phenol. It is apparent from the results obtained that the effect is due to the action of the medium on the resistance of the bacteria.

To further demonstrate that the action is due to a change in the resistance of the test organism, the organism grown in each broth was centrifuged and washed repeatedly to remove the broth. The organisms were resuspended in F.D.A. broth and tested as before. The same results were obtained, namely a high resistance in those organisms grown in Difco broth and a loss resistance in those grown in F.D.A. broth.

In the next series of tests, variations were made in the formulations of Difco "disinfectant testing" broth to determine the specific ingredient responsible for the change in the resistance of the organisms. The formula for Difco "disinfectant testing" broth follows: proteose-peptone Difco 10 g, Bacto beef extract 3 g, Bacto lactose 5 g, sodium chloride 2 g, ascorbic acid 0.25 g, and water 1,000 ml.

Substitutions were made in the kinds of peptone used and formulations were made with and without lactose. The peptones used were Armour's peptone, Bacto proteose peptone No. 3, and Bacto peptone. Two media were prepared using Armour's peptone and proteose peptone respectively but the lactose was not added. The tests were made with 4(1, 1, 3, 3-tetra methyl)butyl phenoxyethoxy ethyl dimethyl benzyl ammonium chloride. It was found that the kind of peptone does not affect the resistance of the organisms. On the other hand, the addition of lactose causes the change in resistance of the organism because media containing lactose gave killing dilutions of 1-1000 to 1-3000, whereas in the absence of lactose, the killing dilutions were 1-20,000 or higher. When other fermentable sugars (glucose and maltose) were substituted for lactose, the results were similar to those obtained with lactose. When lactose was added to the F.D.A. broth, an increase in resistance of the organisms occurred to the same extent as that obtained with Difco "disinfectant testing" broth.

Other gram positive cocci that ferment lactose were tested. *Micrococcus caseolyticus* gave results comparable to those obtained with *Staph. aureus*.

In another series of tests, *Eberthella typhosa*, a gram negative organism, was tested. Because lactose is not fermented by this organism, glucose was substituted. No difference was obtained with the various media. It is apparent that the presence of a fermentable sugar does not change the resistance of *Eberth. typhosa* toward quaternary ammonium compounds. Tests with *Escherichia coli*, a gram negative organism fermenting lactose, gave results similar to those obtained with *Eberth. typhosa*. These observations indicated that the resistance of gram negative organisms is not changed by growing in Difco "disinfectant testing" broth.

Tests were made to determine whether the culture medium used for growing *Staph. aureus* had any effect on other types of disinfectants. Two compounds, Lysol, a cresolic type, and Benchlophen, a phenolic derivative, were studied. No difference was obtained in killing dilutions with these two disinfectants in the F.D.A. broth and Difco "disinfectant testing" broth. It would appear that the

change in resistance is limited largely to the quaternary ammonium compounds or possibly to those disinfectants characterized by long hydrocarbon chains. Further studies along this line are in progress.

Conclusions. Data have been presented to show that the resistance of *Staph. aureus* to quaternary ammonium compounds is materially increased by growing the test cultures in Difco "disinfectant testing" broth as compared with standard F.D.A. broth.

[1] Ruehle, G. L. A., and Brewer, C. A., *U. S. Dept. Agri. Circ.* 198, 1931.

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Gastrointestinal Tract of Guinea Pig and Elimination of Ascorbic Acid Given Intraperitoneally.

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In previous experiments it was found that adult guinea pigs receiving their ascorbic acid intraperitoneally in the proportion of 5 mg per 100 g of body weight excrete about one-fourth of it in the urine. Although some of the vitamin may be used up metabolically in some way in the tissues, it seemed probable that considerable loss due to destruction was occurring elsewhere. The present tests were performed to determine where this destruction, if any, takes place. A preliminary report of the results was made previously.[1]

Method. The investigations were conducted by administering the ascorbic acid intraperitoneally and determining at successive intervals the rate of urinary excretion of the vitamin, the amount present in the blood, liver, kidneys, spleen, and adrenals, and amount present in the contents of 4 different parts of the gastrointestinal tract, then correlating the latter data with observations made in another study[2] on the rate of movement of the contents through the gastrointestinal tract. In these latter studies it was found that in the guinea pig there is a rapid movement of the contents through the stomach and small intestine. Within a period of 2 hours these organs are practically emptied.

[1] Reid, M. E., *Proc. Fed. Am. Soc. Exp. Biol.*, 1947, **6**, 418.

[2] Reid, M. E., and White, W. C., *Am. J. Physiol.*, in press.

The present observations were made on 41 adult guinea pigs of an inbred strain. They were fed a commercial pelleted diet devoid of ascorbic acid. To ensure a sufficient supply of the B vitamins, 1% of powdered yeast was added to the pellets. The 29 male animals used in the tests had an average weight of 921 g, the 12 females an average weight of 771 g. Each animal was injected intraperitoneally each day with ascorbic acid (made up in 0.5% $NaHCO_3$, 15 mg asc. ac./ml) in the amount of 5 mg per 100 g body weight. The animals were maintained on this regime for at least a month before being used in the tests. At periods varying between 1 to 24 hours after injection, blood samples were taken from the heart, following which the animals were killed and the organs quickly removed. The liver, kidneys, spleen, and adrenals were frozen rapidly in dry ice and kept in the frozen state until the extractions with 10% metaphosphoric acid could be made. The contents of the stomach, small intestines, cecum, and large intestine were washed out separately with 10% metaphosphoric acid and centrifuged. The centrifugate was washed with another volume of acid and centrifuged. The first and second extracts were combined and made up to appropriate volumes. In the later phases of the study the washing of the centrifugate was omitted, the contents being made up to appropriate volume