TABLE III.
Antibody Response of Persons Receiving Concentrated Influenza Vaccines.

Vaccine	No. tested	Mean titers type A		Mean titers type B	
		before	after	before	after
Alum ppt.	6	28.5	406	16.0	362
Hirst method[2]	8	29.3	430	32.0	234

method the virus is separated from the formaldehyde used in inactivation, and from other extraneous materials. The continued action of formaldehyde apparently tends to diminish the antigenic potency of influenza vaccines. Formalin also produces some discomfort after subcutaneous inoculation. Suspensions of the alum precipitates are relatively stable at 4°C without the necessity of drying. By the combined method, using adsorption and elution from chicken cells and precipitation with alum, vaccines more concentrated than those now in use could be made.

Summary. The virus of influenza grown in allantoic fluid of chick embryos can be precipitated in a stable form with alum and the precipitate redissolved in sodium citrate. This procedure does not alter the infective, antigenic, or hemagglutinating properties of the virus. Adsorption on sterile chicken cells was also used to concentrate the virus before inactivation and precipitation with alum.

The immunizing power of formalinized and alum-precipitated virus in mice was slightly less than that of active fluid. Inactive concentrated alum precipitates inoculated subcutaneously produced a satisfactory antibody response in human beings. The suspensions of alum precipitates freed of formaldehyde and acid retain their antigenicity when stored at 4°C for at least three months.

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Method of Producing Open Skin Wounds Uniformly Infected with Staphylococci, Suitable for Local Chemotherapy Studies.*

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While engaged in the evaluation of various methods of local sulfonamide chemotherapy of infected wounds, the problem arose of producing controlled, equally involved, infected skin wounds, before different methods of local chemotherapy could be compared.

In evaluating the effectiveness of local chemotherapy of infected wounds, quantitative bacteriological studies were first considered. At first thought, it might be believed that the wounds could be excised completely, ground up in a tissue mill, and the degree of infection estimated by bacterial population of the macerates. This method is open to the criticism that in excising the wound, some normal tissue necessarily must be included, making it impossible to obtain quantitatively reproducible results. Moreover, it is extremely difficult to grind the skin of animals either by sterile mechanical mills, or by freezing-grinding methods. It was finally decided that for all practical purposes, epithelization rate, healing time, and qualitative bacteriological studies would be the best ways in which to evaluate the effectiveness of local chemotherapy of infected open wounds.

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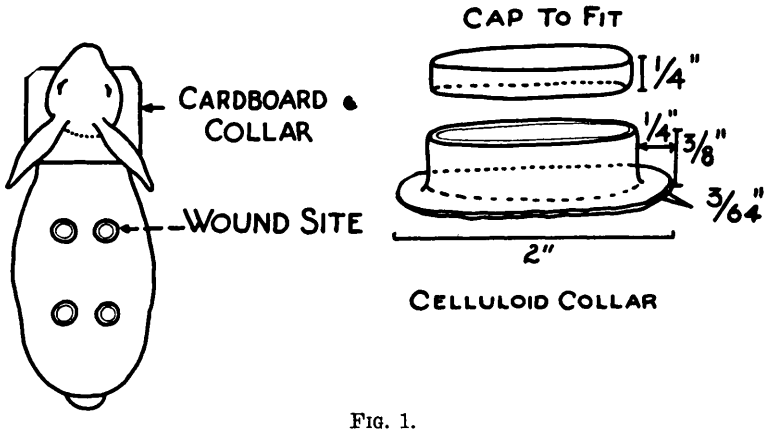

FIG. 1.
Wound dressings and restraining collars.

Previously[1] one of us has briefly described a technic which was developed for studying the rate of granulation and epithelization of clean, open skin wounds in rabbits. The present method is essentially an extension of this method to infected wounds.

Methods. Three to 5-lb healthy rabbits were anesthetized by intravenous sodium pentobarbitol, using picrotoxin if necessary when barbiturate poisoning seemed imminent. The fur along the back and sides was clipped close to the skin with electric hair clippers. The best type found was the Oster No. 2 small animal clippers, with "Angra" (angora) head and blades.† Special celluloid collars open at the top and bottom, and flanged at the bottom, and with separate celluloid caps to fit, were heat moulded. They are illustrated in Fig. 1. The flanges of the collars were cemented to rings of single layers of cotton gauze, with flexible U.S.P. collodion, and allowed to dry. The flanges of the collars and the gauze rings were cemented to the clipped skin of the animals with flexible collodion, the collars being held firmly to the skin with weights until thoroughly dry. Electric hair driers or electric lamps hastened the drying. Such protective dressings could be covered with the removable celluloid caps, which then could be removed easily for inoculation, examination and measurement of the wounds, change of dressings, and taking of smears. The collars remained firmly cemented to the skin for 2 to 3 weeks if properly applied. In rabbits, hair growth was not noticed under the flanges, but it did occur in guinea pigs and subsequently caused the collars to be lifted away from the skin. Hair growth did occur inside the collar around the wound. Cardboard collars, as illustrated in Fig. 1, were placed around the rabbits' necks to prevent tearing at the dressings. These collars were held in place on the animal with adhesive tape, and had to be watched carefully, as some rabbits eventually could remove them. In most cases they did not prevent the animals from feeding and drinking. The celluloid wound dressings demanded frequent inspection, as leaks sometimes developed, especially if the animal freed itself of the cardboard collar and gained access to the wounds. In the event of leaks, patches of gauze and collodion were applied.

The operating room atmosphere was treated with propylene glycol vapors during the operations. The skin area inside each collar was then treated with Novak's germicide solution,‡ and circular skin wounds were made with sterile instruments, cutting down to the panniculus carnosus muscle layer, exercising care to avoid blood vessels. These wounds were approximately 3 to 4 mm in diameter. Small curved scissors and tissue forceps were found

[1] Olson, M., Slider, E., Clark, W. G., and Macdonald, R., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 396.

† John Oster Mfg. Co., Racine, Wisconsin.

‡ 95% alcohol 515 g, acetone 100, water 375, saponated cresol 10, mercuric chloride 0.7, Eosin Y 0.6, acid fuchsin 0.08.

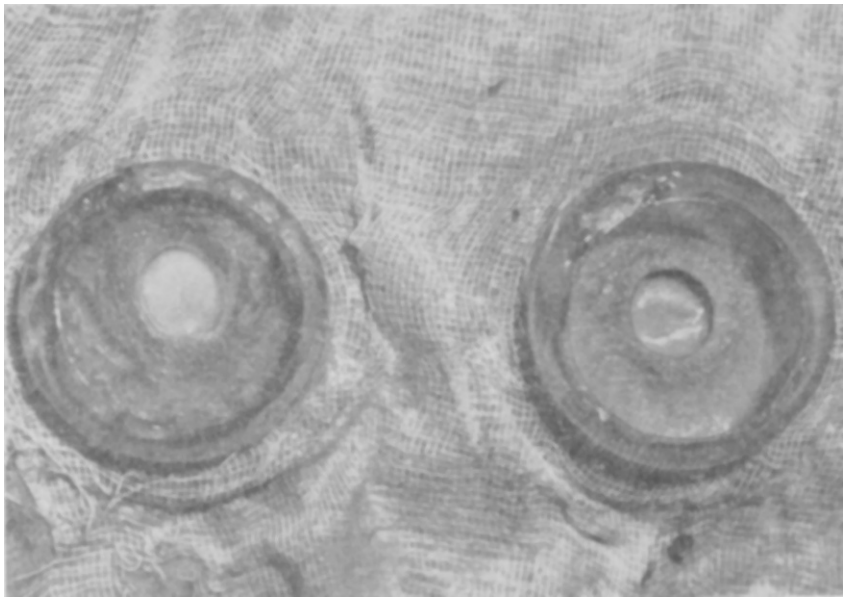

FIG. 2A.
Photograph of 2 wounds, 24 hours after inoculation with staphylococci.

to be the best instruments for producing wounds of uniform size in the collars, although several types of rotating cylindrical cutting tools were constructed and tried but were unsatisfactory due to the mobility of rabbits' skin. Usually within 6 days such wounds, if kept moist, stretched to approximately 1 cm or more in diameter, after which they began to epithelize and close. In the rabbit, clean skin wounds always close much more rapidly when they are dry, but it was necessary to keep them wet for these first studies, hence the method was standardized for moist wounds. After the stretching period, all the wounds were usually uniform in size, as illustrated in Fig. 2A, but practice and care were necessary before uniform wounds could be reproduced. Four such wounds usually were produced on each rabbit, in the skin over the large back muscle bundles of the multifidus and sacrospinalis, 2 wounds on each side of the animal, as close to the mid-dorsal line as possible without overlapping the dressings on the other side of the animal.

An inoculum of 10 cc of a 24-hour culture of *Staphylococcus aureus* in peptone water was poured into 1000 cc prescription bottles containing 100 cc of sterile nutrient agar which had been allowed to solidify on the broad flat surface of the bottle, thus affording a maximum of surface for bacterial growth. After the solution was uniformly spread over the agar, the bottle was incubated for 24 hours at 37°C. At this time 50 cc of sterile saline was used to wash off the growing organisms. To this wash fluid, 50 cc of a sterile 4% aqueous "methocel" gel was added. The methocel was a pharmaceutical grade of synthetic methyl cellulose ether,§ essentially inert, non-toxic, neutral, and of a specific gravity of 4000 centipoises for a 2% solution at 20°C. After thorough stirring, this suspension of the organisms in the gel was used for inoculation. Approximately 10 cc of the suspension was introduced into each celluloid collar and on the freshly made wound, and the celluloid caps replaced firmly. Each inoculum contained approximately 20 billion organisms as determined by duplicate plate counts.

After such an inoculation, the wounds were not touched for about 4 days, at which time they were uniformly infected. The infections were allowed to develop, and then treated with various forms of therapy, two serving as experimental and 2 as control wounds. The therapeutic agents were dissolved in and made

§ The Dow Chemical Co., Midland, Mich.

up to 2% sterile methocel. The gels were changed every other day and the dimensions of the wounds measured at this time with sterile protractors. Both the X and Y axes were measured, if the wounds were not perfect circles, and the areas usually calculated. Treatment was continued until the wounds healed so that the time to healing by secondary intention could be determined.

Ointments of various kinds, and other types of gels such as gum karaya or tragacanth, whether glycerated or not, were not as satisfactory as methocel for inoculation or treatment because many of them cause skin maceration. Maceration due to the aqueous gel being confined under fairly air tight conditions was not a major consideration. All types of methocel were not satisfactory because of more or less maceration. Pure pharmaceutical grades should be used.

The problem of attempting to devise a method of standardly infecting wounds was twofold, first to secure a localized ulcer which would not spontaneously regress, and second to fix the organisms in the skin lesion so that they would not invade the blood stream to produce a fatal bacteremia before termination of the chemotherapy studies. Occasional strains of staphylococci isolated from the human skin ulcers, when inoculated directly into rabbit skin wounds, satisfied the first condition described above, but fatal bacteremia frequently resulted, making these strains unsuitable for our purpose. At first, attempts were made to increase the virulence of the organisms for the type of wound described, by repeated intravenous passage through rabbits. This gave little improvement over the virulence of strains directly inoculated after isolation from human sources. Then various combinations of mucin, methocel, and staphylococci as the inocula were tried, because of the striking action of mucin in enhancing the virulence of various bacteria.[2] Mucin‖ did not seem to satisfy the first and second requisites described above. It has been long known that filtrates of staphylococcus cultures may contain a dermotoxin which produces skin ulcers.[3] Therefore it was thought that repeated passage through skin might increase the affinity of the organisms for that tissue so that they would produce localized, ulcerating lesions of the type desired. This proved to be the case.

Several strains of hemolytic, coagulase-positive staphylococci were obtained from various types of clinical skin infections in the University Hospital,¶ and one from Doctor T. F. Nicholson, of the University of Toronto.** The latter strain had been used for studies of skin infection in rabbits.[4] The organisms were inoculated intradermally into shaved areas on the backs of 6- to 8-week-old rabbits, and after 24 to 48 hours, the organisms were recovered from the resulting abscess, or if no abscess developed, from the general area inoculated. They were then isolated in pure culture and kept on blood agar for no longer than 2 weeks, before wounds were inoculated with the staphylococcic suspensions in methocel, as previously described. Thereafter the virulence and skin affinity of the strain was maintained merely by isolation of the organisms from the open experimentally infected wounds every two weeks.

It was observed that older rabbits were less susceptible to the infection than younger ones. Fairly young animals, 12 to 16 weeks of age, were always used.

Fig. 2A shows a photograph of 2 wounds on one side of a rabbit which had been inoculated 3 days before. Thirty such wounds had an average diameter of 9.5 ± 0.8 mm, and healed in 17-20 days when treated with 5% sulfathiazole in methocel. The infections became much more involved with time and gradually

[2] Nungester, W. J., Jourdonais, L. F., and Wolf, A. A., *J. Infect. Dis.*, 1936, **59**, 11.

‖ We wish to thank Doctor David Klein of the Wilson Laboratories, Chicago, Ill., for the sample of granular mucin "Type 1701-W" which was supplied.

[3] Parker, J. T., *J. Exp. Med.*, 1924, **40**, 761.

¶ We wish to acknowledge the cooperation of our colleague, Dr. E. A. Strakosch, Div. of Dermatol. and Syphilol., University of Minnesota, for supplying these strains, one of which proved to be sulfonamide-resistant.

** We wish to thank Dr. Nicholson for his kindness in sending us this strain of staphylococcus.

[4] Jackson, S. H., Nicholson, T. F., and Holman, W. L., *J. Path. Bact.*, 1940, **50**, 1.

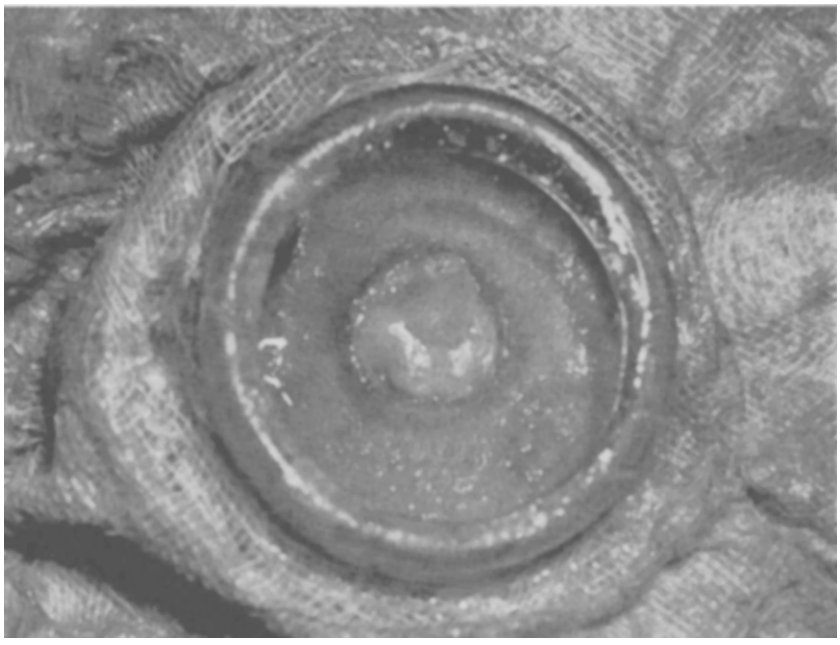

FIG. 2B.
Photograph of infected wound, 7 days after inoculation.

became more undermining and ulcerative after a week or more. Fig. 2A is shown to illustrate the appearance and uniformity of the wound dimensions and infection, and Fig. 2B represents an infected wound after 7 days duration.

Smears and gram strains were made from the wounds each time they were dressed in order to ascertain whether the infection was still associated with the causative organism, and broth cultures were made once weekly for a qualitative bacteriological test. In all cases, the wounds yielded pure cultures of *Staphylococcus aureus*.

Jensen[5] has suggested that the virulence of staphylococci could be maintained constant by lyophilizing them on glass beads or in vials to be used for subcultures for the wound inocula as desired. This probably would be as satisfactory, if not better, than the method of repeated passage from wound to wound. The lyophilization method is being investigated at present.

Summary. This report describes a method of producing and dressing open skin wounds so that a uniform standardly infected wound can be made for controlled topical chemotherapy studies, with healing time by secondary intention used as the main criterion of the effectiveness of the therapy. If desired, as described in the previous paper,[1] histological studies of epithelization and granulation can be made on biopsied wounds, in addition to the determination of healing time.

[5] Jensen, N. K., personal communication.