500 p.p.m. acriflavine solution at pH 7.7.

To test the action of the disinfectant in the presence of trout eggs, ova were aseptically taken from ripe rainbow *(Salmo irideus)* and brown trout *(S. fario)*. They were charged with *Bact. salmonicida*, by placing 100 eggs in 100 ml of saline solution containing approximately 10,000,000 bacteria per milliliter. The number of bacteria adsorbed on an egg is not known, but they clouded broth medium more rapidly than eggs removed from contaminated trout abdomens. This is suggestive but is not clear cut evidence that the laboratory contaminated eggs were more heavily charged than naturally contaminated ova. Eggs were then treated for 20 min at 10° in the alkaline acriflavine solution, and removed to semi-solid, carp-infusion agar. After one week's incubation all tubes were negative for *Bact. salmonicida*. Eggs that had been exposed to the disinfectant for shorter periods carried viable *Bact. salmonicida* to the culture medium.

Next, it was desirable to know the effect of 500 p.p.m. of acriflavine at pH 7.7 on trout eggs under actual treatment conditions. Therefore, 7,500,000 ova from rainbow, brown, and brook trout *(Salvelinus fontinalis)* were treated for 30 min with the disinfectant with a total loss of only 3.65% of these eggs in the period between treatment and hatching. As a control, the records of 10,900,000 untreated eggs of the same species at the same hatcheries showed a loss of 7.92% for a similar period. Obviously, the treatment is not harmful; rather, it seems actually to benefit the eggs.

From these data, it is concluded that *Bact. salmonicida* on the shell of trout eggs is killed by a 500 p.p.m. acriflavine solution at pH 7.7 in 20 min and that the treatment of eggs for one-half hour with this solution is harmless to the eggs. Several hundred thousand trout from disinfected eggs were kept under observation for more than one year. No abnormalities in the trout were observed.

The efficacy of the disinfection in practical use cannot yet be judged because it is only one of several quarantine measures which are necessary to protect a disease-free stock. However, in one trout hatchery where egg disinfection has been combined with other quarantine measures, there has been no recurrence of trout furunculosis for 2 years.

13959

Assay of the Immunizing Effect of Scarlet Fever Toxin in the Rabbit.

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Evaluation of agents for immunization against scarlet fever has been restricted to the direct experience in human beings, the criteria of success being (1) the Dick test, (2) antitoxin determination in the serum, (3) clinical protection.[1-3] The latter will, of course, always remain the ultimate criterion of success. However, it was felt that for the development of an optimal immunizing agent for scarlet fever it would be of considerable help if a method could be found that would allow an experimental evaluation in the animal.

The assay of the scarlet fever toxin and its antitoxin in the skin of the rabbit has been successfully employed for some time.[4,5]

[1] Toomey, J. A., *Ann. Int. Med.*, 1941, **15**, 959.

[2] Kolchin, B. S., and Klein, J. F., *J. Immunol.*, 1941, **41**, 429.

[3] Veldee, M. V., Peck, E. C., Franklin, J. P., and DuPuy, H. R., *Public Health Reports*, 1941, **56**, 957.

[4] Buttle, G. A. H., and Lowdon, A. S. R., *J. Path. and Bact.*, 1935, **41**, 107.

[5] Plummer, H., *Br. J. Exp. Path.*, 1934, **15**, 80.

TABLE I.
Exemplifying Dermal Resistance to Dick Toxin of Rabbits One Week After End of Immunization

Immunizing agent*	No. of rabbits treated	Pos. 1 STD	Neg. 1 STD Pos. 4 STD	Neg. 4 STD Pos. 16 STD	Neg. 16 STD Pos. 64 STD	Neg. 64 STD Pos. 264 STD	Neg. 264 STD
Scarlet Fever Toxin	13	1	0	2	3	4	3
Formalized Scarlet Fever Toxin	7	0	4	3	0	0	0
Broth	3	3	0	0	0	0	0
Not treated	5	5	0	0	0	0	0

*5 injections as described in text.

TABLE II.
Dermal Resistance to Dick Toxin of Rabbits 24 Hours After Intravenous Injection with Antitoxin.

Antitoxin	No. of rabbits injected	Pos. 1 STD	Neg. 1 STD Pos. 4 STD	Neg. 4 STD Pos. 16 STD	Neg. 16 STD Pos. 64 STD	Neg. 64 STD Pos. 256 STD	Neg. 256 STD Pos. 1064 STD	Neg. 1024 STD
Scarlatinal	10	0	0	0	4	3	2	1
Diphtheric	5	5	0	0	0	0	0	0

The following experiments will show that a dermal test in the rabbit will give an opportunity for a quantitative evaluation of both active and passive antitoxic protection.

Procedure. White rabbits were tested for sensitivity to 1 STD* of scarlet fever toxin and only such animals were employed that gave a distinct reaction. Such animals were injected with the immunizing agent, for example, scarlet fever toxin, and at the end of a period of immunization tested against the toxin. This testing was done with 1, 4, 16, 64, and 256 STD, occasionally also with 1024 STD. Appropriate controls were added. It was ascertained that healthy rabbits can be expected to stay Dick sensitive for the time period corresponding to that of immunization. In a control series, rabbits were injected with the double amount of the same nutrient broth that had been used for the preparation of the toxin. Prior experience on the Dick reaction in rabbits that had been injected with suspensions of toxigenic strains of streptococci has shown that antibacterial antibodies would not interfere.

In the first series, parallel dermal tests were made both on the ears and on the backs of each animal. The results were identical for both sites within the limit of the experimental error. In later experiments tests were made on the backs only.

Readings were made 24 and 48 hr after injection. As both readings gave similar results, only the readings after 24 hr are given in the tables.

For immunization, an erythrogenic toxin was used that was obtained according to standard methods for the preparation of toxins for human immunization. It contained 300,000 STD per ml.

A standardized toxin from the National Institute of Health was used for the dermal tests.

All dilutions were made in M/20 phosphate buffer of pH 7.2.

The animals were immunized by subcutaneous injection. We immunized several series of animals with toxin in the same 5 doses that are used for human immunization, namely: 650, 2,500, 10,000, 30,000, and 120,000 STD, the injections being spaced at 3-day intervals. One week after the last injection, the animals were tested. Typical results of such tests can be seen from Table I. The individual response varies considerably.

* STD — Skin Test Dose. For definition see f. i.: A. B. Wadsworth, *Standard Methods of the Division of Laboratories and Research of the New York State Department of Health*, Second Edition, Baltimore, 1939, Williams & Wilkins Co., p. 382.

"Failure to respond to 100 STD" would indicate the order of average resistance acquired under the conditions of the procedure of immunization just described.

Parallel with some of these series, animals were injected with the same toxin treated with 0.3% formol until well over 90% of its toxicity as measured in the rabbit skin had disappeared. Some immunizing effect was observed (Table I), but markedly less than with the original toxin. It is irrelevant for the moment whether this remaining effect was due to the traces of unmodified toxin still present. The purpose of reporting these experiments here is to show that quantitative differences in immunizing effect can be detected by the intradermal test.

We recommend to test the acquired resistance one week after completion of immunization. At this time, dermal resistance is at its high point. Animals tested 2 weeks after completion of immunization already showed signs of lessening of resistance.

As a preliminary standard for comparative experiments the immunizing effect of scarlet fever toxin in amounts as used for human immunization might form a useful basis. It is obvious from the tables that 6 to 10 animals should be employed in each series to guard against deception by individual variation.

Dermal resistance to toxin can also be obtained by passive immunization. Two groups of rabbits were injected with commercial scarlatinal antitoxin (Globulin Modified, Lederle), in a dosage of 1 U.S.P.H.S. unit per g body weight. At the same time, rabbits were injected with the same amount (measured as serum protein) of commercial diphtheric antitoxin (Globulin Modified). Twenty-four hr later, all animals were tested with graded amounts of toxin in the skin. Results can be seen from Table II. It will be seen that a dermal resistance similar in degree to that obtained with active immunization with scarlet fever toxin was obtained after passive immunization with scarlatinal antitoxin, whereas injection with diphtheric antitoxin would not change Dick sensitivity.

Summary. Dermal resistance to graded amounts of Dick toxin can be used as a method for evaluation of agents for scarlet fever immunization.

13960

Simplified Technic for Inoculating into Amniotic Sac of Chick Embryos.*

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Burnet[1] has reported the isolation of influenza virus direct from throat washings, by inoculation into the amniotic sac of chick embryos and subsequent passages with a suspension of the ground tracheas removed from the embryos following a suitable period of incubation.

The technic employed by Burnet involves cutting a window in the egg shell over the embryo, puncturing the shell membrane and transferring the air sac to between the shell and the chorioallantoic membranes beneath the opening. The cut portion of the shell, including the attached shell membrane, is then removed exposing the chorioallantois beneath the window in the shell. A small slit is made in the chorioallantois, and by means of a fine forceps the underlying amniotic membrane is grasped and drawn through the opening in the chorioallantois. Inoculation is made into the amniotic sac by puncturing the exposed membrane with a capillary pipette. The membrane is then released and allowed to withdraw through the opening in the chorioallantois. Finally, the window in

* These studies were supported in part by a grant from the International Health Division of The Rockefeller Foundation.

1 Burnet, F. M., *The British J. Exp. Path.*, 1940, **21**, 147.