TABLE IV.
Failure of Sodium Salicylate to Alter Action of Hyaluronidase on Increased Erythrocyte Sedimentation Rate and Plasma Turbidity Following Intravenous Injection of Sodium Hyaluronate.

Rabbit	Sodium hyaluronate, mg/kg	Time after injection	Salicylate level, mg %	Erythrocyte sedimentation rate after incubation with		Plasma turbidity after incubation with	
				PS.S.,* mm/hr	Hyaluronidase,* mm/hr	P.S.S., % transmission	Hyaluronidase, % transmission
1	91.7	30 min.	37.5	1	1	37	100
		5 hr	16.7	2	2	90	100
2	66.6	1 "	5.6	38	1	48	100
		2 "	4.6	10	1	66	100
3	62.5	1 "	52.8	15	2	52	100
		5 "	33.4	12	2	66	100
4	82.3	1 "	73.8	1	1	53	100
		5 "	43.0	12	1	63	100

* See text.

Discussion. The data presented in this report fail to substantiate the possibility that hyaluronic acid, at least in a polymerized form, is present in the blood of patients with rheumatic fever, rheumatoid arthritis, and several other diseases (Table III) as has been suggested by Meyer.[4,5] The polysaccharide could not be demonstrated in the blood of normal individuals.[25] Depolymerized hyaluronic acid would not be demonstrated by the methods employed here, but previous work[1] and our own indicate that the depolymerized acid will not increase E.S.R. to a marked degree. In a personal communication, Dr. Karl Meyer has stated that in studies with his preparations of hyaluronidase, spreading activity and E.S.R. inhibition did not run parallel. He was able to separate the factor that decreased E.S.R. from hyaluronidase activity by adsorption on lead sulfide.

Summary. 1. Hyaluronic acid is apparently not responsible for the increased erythrocyte sedimentation rate of blood from patients with rheumatic fever and rheumatoid arthritis. 2. Sodium salicylate failed to inhibit the effect of hyaluronidase on elevated E.S.R. produced in rabbits by the intravenous injection of sodium hyaluronate.

25 Duran-Reynals, F., *Bact. Rev.*, 1942, **6**, 197.

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Immunologic Reactions Following the Intradermal Inoculation of Influenza A and B Vaccine.*

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It has been shown[1] that the intradermal injection of heat-inactivated mumps virus into human beings may exert a pronounced antigenic effect. Data are[2] available which sug-

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1 Enders, J. F., Kane, L. W., Maris, E. P., and Stokes, J., *J. Exp. Med.*, 1946, **84**, 341.

2 Stokes, J., and Enders, J. F., 1947, unpublished data.

gest that this procedure may also induce active immunity. More recently, Van Gelder and associates[3] compared the rise in antihemagglutinins following intradermal and subcutaneous inoculation of concentrated vaccine prepared from strains of A and B influenza viruses. Although the intradermal dose was only one-tenth as large as the subcutaneous dose, the smaller dose induced a greater mean antibody response. The incidence of generalized reactions was lower in the group which received intradermal inoculations. Localized reactions characterized by redness and swelling were observed in 90% of the subjects on the day following the intradermal injection. Previously, Beveridge and Burnet[4] had noted immediate (10-20 minutes) and delayed (24 hours) dermal responses in certain individuals to intracutaneous injection of inactivated influenza virus. Although in adults they found no correlation between the antibody titer and the delayed reaction, in children the occurrence of the latter appeared to be associated with the presence of antibody.

In the early spring of 1947, an epidemic of influenza appeared imminent in Boston. Intradermal vaccination was then carried out in a group of adult hospital personnel. Information was obtained in respect to the antibody response following vaccination. The occurrence of local and generalized reactions was recorded as well as the incidence of upper respiratory infection following vaccination. These data are presented below.

Materials and Methods. The group consisted of persons working in the Children's Hospital, Boston. On March 19 and 20, 1947, 495 adults were interviewed and a history taken regarding allergic manifestations and previous influenza vaccinations; 24 individuals were eliminated because of a history of severe allergy and 4 because of a history of reaction to previous influenza vaccination. The remainder were given intracutaneously 0.1 cc of a 1:5 dilution in 0.85% salt solution of mixed A and B influenza vaccine (prepared of Lederle Laboratories, Lot No. 2032-34A). Twenty minutes after vaccination, each person was examined and the extent of any erythema and induration at the site of inoculation recorded. Between 44 and 52 hours after vaccination, 449 individuals were re-interviewed, a history obtained of any systemic reaction, and the local reaction was measured. Before vaccination venous blood specimens were obtained from 110 individuals selected at random; 3 weeks after vaccination 88 of these persons were re-bled and questioned as to the occurrence in the interim of attacks of respiratory disease.

Between April 25 and May 5, 1947, questionnaires were filled out by 375 persons concerning the nature of any respiratory disease which occurred subsequent to vaccination. Data were obtained regarding the date of onset, duration, severity, and the presence or absence of the following symptoms: headache, fever, chills, sweating, cough, running nose, pain in eyes, muscle aches, sore throat, and general weakness. During the same period similar questionnaires were filled out by another group of 405 hospital workers who had not received influenza vaccine in March, 1947.

The paired specimens of serum were examined by a modification, developed at the Army Medical School,[5] of the Hirst agglutination-inhibition test using the PR8 strain of influenza A virus and the Lee strain of influenza B virus. Instead of human type "O" cells 0.5% chicken cells were employed.

(1) *Local and Systemic Reactions.* The immediate and delayed cutaneous responses were similar to those described by Beveridge and Burnet.[4] Twenty minutes after inoculation, in those who reacted, a moderate erythema of varying extent appeared around the site with occasionally a small central papule or wheal. The majority also showed after 48 hours a delayed reaction which consisted of a diffusely erythematous and slightly indurated area with a fairly well defined margin; at this time, however, in many persons the reaction had started to subside. The maximum diameters of the local reaction are classified in Table I.

Seventy-five or 16.7% of 449 individuals

[3] Van Gelder, D. W., Greenspan, F. S., and Dufresne, N. E., *U. S. Naval Med. Bull.*, 1947, **47**, 197.

[4] Beveridge, W. I. B., and Burnet, F. M., *Med. J. Australia*, 1944, **1**, 85.

[5] *Bull. U. S. Army Med. Dept.*, 1946, **6**, 777.

TABLE I.
Maximum Diameter of the Immediate and Delayed Cutaneous Responses in 449 Adults Following Intracutaneous Vaccination.

Diameter of erythema, mm	Reaction after 20 min		Reaction after approx. 48 hr	
	No.	%	No.	%
0-4	33	7.3	26	5.8
5-9	43	9.6	40	8.9
10-14	68	15.2	30	6.7
15-19	58	12.9	31	6.9
20-24	64	14.2	35	7.8
25-29	79	17.6	51	11.4
30-34	63	14.1	76	16.9
35-39	22	4.9	43	9.6
40-44	11	2.5	60	13.2
45-49	4	0.9	18	4.0
50-54	2	0.4	22	4.9
55-59	0	0	6	1.3
60-64	1	0.2	3	0.7
65-69	0	0	3	0.7
70 or over	1	0.2	5	1.1
Total reactions > 10 mm	373	83.1	383	85.3

TABLE II.
Frequency of Symptoms Reported by 75 Individuals Who Complained of a Systemic Reaction Within 48 Hours After Intracutaneous Vaccination.

Symptoms	No. of times
Malaise	31
Headache	28
Fatigue	24
Nausea	14
Chills	9
Fever	6
Muscle pain	4
Sweating	1
Urticaria	1

reported that they had experienced a systemic reaction within 48 hours after vaccination. With one exception, the symptoms which are summarized in Table II were very mild. One person requested medical attention because of chills, malaise, and a temperature of 100.3°F.

(2) *Antibody Response Following Intracutaneous Vaccination.* Specimens of serum were obtained immediately before intracutaneous vaccination and again 3 weeks thereafter from 39 individuals who had not been previously vaccinated against influenza and who were free from respiratory disease during this 3-week period. The differences between the pre- and post-vaccination titers are recorded in Table III. From these data it is apparent that the geometric mean of the antihemagglutinin titer rose 3.6 × for Influenza A and 2.9 × for Influenza B following vaccination. If those individuals whose initial titers were over 1:64 are excluded, it has been calculated that the geometric mean titer of 27 persons rose 5 × for Influenza A (1:38 to 1:193) and that of 29 persons 3.2 × for Influenza B (1:28 to 1:89).

Sixteen additional individuals who had reported having a respiratory infection during the period between bleedings showed a rise of the geometric mean titer against Influenza B from 1:38 to 1:134. This group is considered separately because of the presence of respiratory infection in the community, as noted below. However, since there was no evidence of infection with type B virus, there can be little doubt that the rise in titer occurred as a result of vaccination.

(3) *Incidence of Respiratory Disease in the Vaccinated and Unvaccinated Groups.* From the second to the fifth weeks following vaccination there was an increased incidence of respiratory disease in the community. The increase, in part at least, could be attributed to a virus resembling Influenza A as indicated by a significant rise in agglutinin-inhibition titer against the PR-8 strain in the sera of a few unvaccinated patients that were studied and by the isolation of atypical strains of Influenza A virus by other workers in Boston.[6] But analysis of the questionnaires revealed very few cases in which a diagnosis of influenza could be made on the basis of the symptoms reported. Therefore the incidence of all acute upper respiratory infection was determined in the vaccinated and in the control groups. Of 316 individuals who received intracutaneous vaccine and who had not been previously vaccinated against influenza, 109 or 34% reported symptoms of an acute upper respiratory disease; in a comparable unvaccinated control group, 85 or 28% of 329 individuals had an acute respiratory disease.

(4) *Antibody Titer and Local and Systemic Responses to Intracutaneous Vaccination.* No correlation could be discerned between the antibody titer at the time of vaccination

[6] Finland, M., and Morgan, H. R., 1947, personal communication.

TABLE III.

Changes in the Titer of Antihemagglutinins Against Influenza A and B Virus During a 3-Week Period Following Intracutaneous Vaccination.*

Antigen	Reciprocal of agglutinin-inhibition titer	No. of individuals before vaccination	No. of individuals after vaccination	No. showing change in titer						
				Fall	No change	Rise in titer				
						2x	4x	8x	16x	32x
PR-8	<16	0	0	0	0	0	0	0	0	0
	16	7	0	0	0	0	0	3	3	1
	32	6	0	0	0	2	2	1	1	0
	64	14	2	0	0	6	6	2	0	0
	128	8	13	0	2	5	1	0	0	0
	256	2	15	0	0	2	0	0	0	0
	512	2	8	0	1	1	0	0	0	0
	1024	0	1	0	0	0	0	0	0	0
		Total 39								
		Geom. mean titer *1:62*	Geom. mean titer *1:226*							
Lee	<16	6	0	0	0	3	0	2	1	0
	16	8	3	0	0	0	5	3	0	0
	32	7	3	0	2	2	2	1	0	0
	64	8	8	0	2	4	1	1	0	0
	128	8	16	0	4	4	0	0	0	0
	256	2	7	1	0	1	0	0	0	0
	512	0	2	0	0	0	0	0	0	0
		Total 39								
		Geom. mean titer *1:35*	Geom.mean titer *1:104*							

* Previously vaccinated individuals and those with respiratory infection during the serological period were excluded.

and the size of either the immediate or delayed skin reaction. Thus, in 15 previously unvaccinated subjects in whom the agglutinin-inhibition titer against both PR-8 and Lee antigens was 1:32 or less, the average maximum diameter of the delayed skin reaction was 34 mm with extremes of 8 and 50 mm. In 16 similar persons with titers against both PR-8 and Lee of 1:64 or higher, the average maximum diameter was 36 mm with extremes of 10 mm and 60 mm.

Fifty-four of the 449 persons who were inoculated intradermally had previously received influenza vaccine; 39, or 72%, exhibited immediate skin reactions of 20 mm or greater. In contrast, comparable immediate reactions were observed in 205, or 52%, of those vaccinated for the first time. No relationship was noted between the size of the delayed skin reaction and previous vaccination.

No correlation was found between the extent of the delayed skin reaction and the antibody response following vaccination. The average size of the immediate and of the delayed skin reactions did not differ significantly in the group that reported a systemic reaction and in the group that had no systemic response.

Discussion. The results of the tests for changes in antihemagglutinin against Influenza B following intradermal vaccination with inactivated virus showed that when the initial titer of the antibody was low (*i.e.* 1:64 or less), the antigenic effect was appreciable. When the initial titer was moderate or high the response was slight or absent. Inactivated Influenza A vaccine introduced intracutaneously also appeared to exert a comparable, if not a greater, antigenic effect in persons with initial low titers. The fact, however, that an epidemic caused by an atypical Influenza A virus was current at the time this study was carried out prevents a categorical statement in respect to the effect of this antigen since the possible occurrence of inapparent infection cannot be eliminated. Failure of influenza vaccine to stimulate large increases in the antihemagglutinin titer when the initial titer is high has been noted by others after subcutaneous inoculation.[7]

The serologic data indicate that one-fiftieth of the dose of vaccine usually administered by the subcutaneous route when injected into the skin may give rise to antibody levels which are only slightly lower than those reported for the standard subcutaneous dose.[8,9,10,11] In respect to the effectiveness of this route of inoculation our findings are in general in agreement with the observations made by Van Gelder and his associates[3] on influenza vaccine and with those of Enders and his co-workers[1] on inactivated mumps virus. Quantitatively, though, Van Gelder and his associates obtained larger mean increases in titer for both A and B antigens. This difference may possibly be attributed to their employment of undiluted vaccine whereas in our experiments the vaccine was diluted 1:5. Furthermore, they noted that the antibody concentration continued to increase during the period from 2 to 4 weeks after vaccination. Our final determinations were made 3 weeks after inoculation.

In view of the small quantity of virus inoculated the high incidence of systemic reactions which were reported was unexpected. With one exception, however, these reactions caused no loss of working time. It is possible that a large number of very mild reactions were revealed by the method of personal interview which was employed.

Immediate and delayed local erythematous reactions of 10 mm or greater in diameter were encountered in over 80% of the subjects. The immunologic mechanisms underlying these reactions remain obscure. They may be either manifestations of hypersensitivity established by previous experience with the viruses or the effect of a specific toxic action of the vaccines. An unknown proportion of the immediate type might be occasioned by a

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7 Hirst, G. K., Rickard, E. R., Whitman, L., and Horsfall, F. L., *J. Exp. Med.*, 1942, **75**, 495.

8 Salk, J. E., Menke, W. J., and Francis, T., Jr., *Am. J. Hygiene*, 1945, **42**, 57.

9 Rickard, E. R., Thigpen, M., and Crowley, J. H., *Am. J. Hygiene*, 1945, **42**, 12.

10 Eaton, M. D., and Meiklejohn, G., *Am. J. Hygiene*, 1945, **42**, 28.

11 Hirst, G. K., Plummer, N., and Friedewald, W. F., *Am. J. Hygiene*, 1945, **42**, 45.

non-specific irritative effect of the inoculum.

No evidence for an increased resistance attributable to the vaccination was obtained. The incidence of all types of upper respiratory disease during the 2-month period succeeding vaccination in both inoculated and uninoculated groups did not differ significantly. Several explanations may be offered for the ineffectiveness of the vaccine. The strains of Influenza A may have differed in antigenic composition from the prevailing epidemic strain. This possibility has been invoked to explain the relative ineffectiveness of vaccination in other parts of the country during the epidemic of last spring.[12,13,14] The quantity of vaccine administered may have been too small to establish resistance. Vaccination may have been undertaken too late, since serologic evidence was obtained that Influenza A was present among the hospital personnel within ten days following the administration of the vaccine.

Although the prophylactic value of intradermal vaccination has not been demonstrated, additional experiments would seem desirable in view of the results of Van Gelder and his co-workers and those reported in this communication.

12 Francis, T., Jr., Salk, J. E., and Quilligan, J. J., *Am. J. Public Health*, 1947, **37**, 1013.

13 Sigel, M. M., Shaffer, F. W., and Henle, W., *J. Bact.*, 1947, **54**, 277.

14 Smadel, J. E., *Bull. U. S. Army Med. Dept.*, 1947, **7**, 795.

Summary. 1. Intradermal inoculation into 39 adults of 0.02 ml of concentrated Influenza A and B vaccine gave rise to a mean antibody response (antihemagglutinin) against Influenza B virus which approached that obtained by others following the subcutaneous injection of 1.0 ml of undiluted vaccine. A greater mean increase in antibody against Influenza A was observed in the same group, although this increase could not be attributed conclusively to the effect of the vaccine.

2. The intradermal injection of 0.02 ml of concentrated vaccine into 449 adults was followed within 20 minutes by a local erythematous reaction exceeding 10 mm in diameter in 373 or 83.1%. In 383 or 85.3% of the same group delayed local erythematous reactions exceeding 10 mm in diameter were present after approximately 48 hours accompanied in many cases by slight induration.

3. No correlation could be established between the intensity of the immediate or delayed dermal reaction and the level of antihemagglutinin in the blood.

4. Mild systemic reactions were reported by 75 (16.7%) of 449 vaccinated persons.

5. Although cases of Influenza A infection were demonstrated in the community shortly after vaccination, the rate of all upper respiratory disease during the succeeding 2-month period in a group of 316 vaccinated persons was not significantly different from the rate among 329 unvaccinated persons working in the same institution.

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Use of the Spectrophotometer for Measuring Melanin Dispersion in the Frog.

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It is well established that melanophore changes in the frog are related to environmental conditions as well as endocrine factors within the animal. However, there apparently is no satisfactory data on how completely a dispersion or concentration of melanin in the skin will interfere with the reflectance of light by the other pigments present.

A review of the literature regarding methods for measuring melanin dispersion or concentration in the skin indicates that most of the observations made were patterned after