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Pain and dermal reaction caused by injected glycerin in immunotherapy solutions

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Background: Fifty percent glycerin preserves immunotherapy solution potency for at least 3 years but must be diluted before injection to reduce glycerin-induced pain and inflammation. We studied pain, erythema, induration, and bruises caused by glycerin (0% to 30%).

Methods: In 15 healthy subjects we compared, in double-blind fashion, pain scores, injection site erythema, induration, and bruising caused by subcutaneous injections in randomized order of 0.1, 0.5, and 1 ml of glycerin 0%, 10%, 20%, and 30%.

Results: Injection volume did not significantly influence pain scores from diluent alone (0% glycerin) ($p > 0.1$). Pain scores of subjects given glycerin (0.1 to 1 ml, 10% to 30%) increased significantly as both injection volume ($p < 0.001$) and glycerin concentration ($p < 0.001$) increased. Pain scores correlated with total glycerin dose administered (volume $\times$ concentration) ($r_s = 0.67$, $p < 0.0005$) but varied widely, from minimal to severe, in those given the same dose. Injection site erythema, induration, and bruising occurred in some subjects with significant positive correlations between total glycerin dose and both frequency and diameters of erythema and induration. However, these dermal reactions were of trivial clinical importance.

Conclusion: Injected glycerin produces pain that is proportional to total injected dose of glycerin, but individual variation in perceived discomfort is substantial. Total glycerin doses of less than 0.05 ml rarely produce clinically important pain. (*J Allergy Clin Immunol* 1996;97:1033-9.)

Key words: Glycerin, human serum albumin saline, immunotherapy solution, potency, dose, pain, erythema, induration, bruising

Stock concentrated allergenic extracts preserved in 50% glycerin retain their potency for at least 2 to 3 years under standard conditions of storage, ship-

Abbreviation used

HSA: Human serum albumin saline

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ping, and administration.¹⁻⁵ Therefore extracts in 50% glycerin are frequently used as sources for dilutions prepared for diagnosis and immunotherapy.³⁻⁵ However, because 50% glycerin is a recog-

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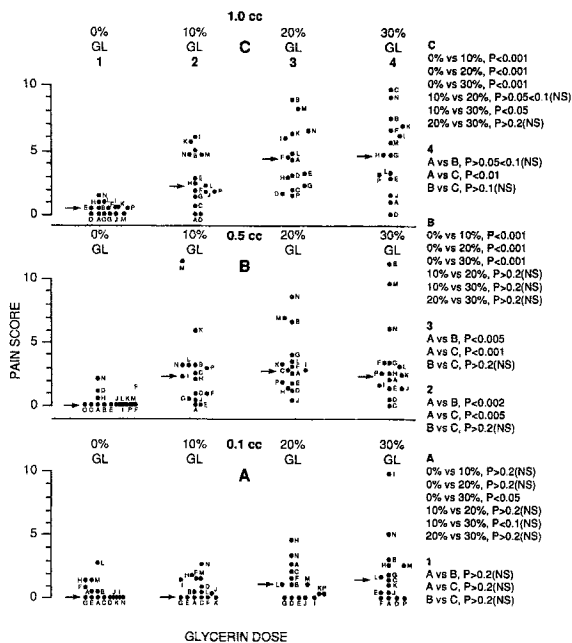


FIG. 1. Pain scores produced by injected glycerin concentrations and volumes in 15 subjects in 12 dose groups. Arrows indicate medians. Probability (*p*) values were derived by Mann-Whitney U Test.^{7,8} A two-factor (volume, percent glycerin) analysis of variance⁹ was also used to confirm that both volume and percent glycerin were related to pain score ($p < 0.005$) and that volume $\times$ percent glycerin interaction was also highly significant ($p < 0.001$). GL, Glycerin; NS, not significant.

nized irritant that produces pain and inflammation when injected into skin or subcutaneous tissue,^{3,5} it must be diluted before injection. Information on the irritative effects of these dilutions is needed. Therefore we studied the relative amount of pain, erythema, induration, and bruising produced by four concentrations of glycerin (0%, 10%, 20%, and 30%) commonly used in immunotherapy solutions, when given in the customary volumes of 0.1 to 1 ml.

METHODS

Subjects

By advertisement, we recruited eight healthy volunteers from atopic patients currently receiving maintenance doses of immunotherapy solution (immunotherapy recipient group: Subjects f, g, j, k, l, m, n, and p) and seven healthy volunteers who had not received immunotherapy injections for at least 3 years (immunotherapy nonrecipient group: Subjects a, b, c, d, e, h, and i). All subjects gave written informed consent to the protocol. All procedures were approved by the Johns Hopkins University School of Medicine Joint Council on Human Investigation.

Materials

Sterile undiluted glycerin lot no. D0020, expiration date 1/6/96, (glycerin 100%) was obtained from Miles Hollister Stier (Spokane, Wash.). Human Serum Albumin Saline lot no. SH060, expiration date 9/95 (HSA) (normal saline solution with 0.03% human serum albumin) was obtained from Greer Laboratories (Lenoir, N.C.). Glycerin (100%) was diluted with HSA to make 30%, 20%, 10%, and other glycerin dilutions in 10 ml vials with thorough mixing to avoid layering of low-density HSA over high-density glycerin.

Double-blind technique

The various concentrations of glycerin and HSA used in the tested solutions were colorless and could not be identified by appearance. However, their differences in viscosity were obvious to the worker giving the injections, who therefore could not be blinded. Unblinded workers randomized the doses, loaded the syringes, and gave the injections.

Each subject and the workers who assisted the subject in making observations were blinded and made judgments about the pain, erythema, induration, and bruising produced by the injections without knowing their contents.

Randomization of injections

Contents of the glycerin injections for each subject were determined by random selection of one of four different sequences. Each sequence tested all four glycerin concentrations once on each test day at constant volume. Each of the three volumes was tested once in the sequence. Four injection sites on the upper arm were used on all 3 injection days: 1, right upper; 2, right lower; 3, left upper; 4, left lower. On the day on which the tested volume was closest to the volume of an immunotherapy recipient group subject's maintenance immunotherapy injection, the subject received as a fifth injection his or her customary dose of immunotherapy solution. This was given by random selection in either the right or left upper arm between and at a distance of 6 cm from the two glycerin injection sites. A control injection of glycerin of equal volume and concentration to that contained in the maintenance dose of the immunotherapy solution was given in the same location in the opposite arm.

Injection technique

Glycerin injections were given in the subcutaneous tissue of the upper arm, by the same person (with one exception, patient b), using the same technique. To duplicate the storage temperature recommended for immunotherapy solutions, each loaded syringe was kept in a refrigerator at 2 to 6°C until immediately before the injection. Injections were given subcutaneously in the lateral surface of the upper arm, and care was taken to avoid intravenous, intramuscular, and intradermal injections. The injection sites on the same arm were separated by at least 6 cm. Injections were given swiftly and smoothly. One hand grasped and steadied the skin site.

The other hand was in charge of the syringe. The thumb and second and third fingers were used to guide and steady the syringe. The fourth and fifth fingers were used first to pull back on the plunger (to rule out intravenous position) and then to push the plunger forward to inject the contents of the syringe.

Each injection was separated from the previous injection by an interval of at least 10 minutes, during which the subject communicated to the blinded observers the degree and duration of the pain.

Pain scores

The blinded patient, assisted by a blinded worker, measured pain at the injection site by use of an ordinal (rank) scale. They recorded:

(A) A number to represent the degree of pain (grade 1 = minimal, barely perceptible; grade 2 = mild, definitely present, but not annoying; grade 3 = moderate, tolerable, but definitely annoying; grade 4 = severe, intolerable, must be relieved)

(B) The duration in minutes of each grade of pain

(C) The pain score for each injection was calculated by determining the sum of the products obtained by multiplying each pain grade number by its duration in minutes.

(D) Clinically important pain was defined as grade 3-4 pain.

Measurements of erythema, induration, and bruises

Thirty minutes after the injection, the diameters of any wheal, induration, erythema, or bruise at the injection site were outlined with a ballpoint pen with black ink. Hypoallergenic Micropore Surgical Tape (3M No. 1535; 3M Health Care, St. Paul, Minn.) was applied to the tracing to transfer the results of the test and produce a permanent record. Both the greatest diameter and the orthogonal diameter (the diameter perpendicular to the midpoint of the greatest diameter⁶) were measured, and the arithmetic mean of these two values was recorded. Tenderness at injection sites and pain during arm motion were also recorded.

Each subject was telephoned on the day after the test to check for induration, erythema, tenderness, and bruises at injection sites and pain during arm motion. This information was recorded.

Statistical analysis

The ranked pain scores of each dose group were compared with those of the appropriate other dose groups as shown in Fig. 1. Because the pain scores were ordinal (rank) numbers that did not follow a normal distribution, the nonparametric Mann-Whitney U test^{7,8} was used to test the hypotheses that: (1) higher glycerin concentrations produced higher pain scores, and (2) higher injection volumes produced higher pain scores. We used a two-tailed test with a significance level of 0.05 to reject the null hypothesis. A two-factor

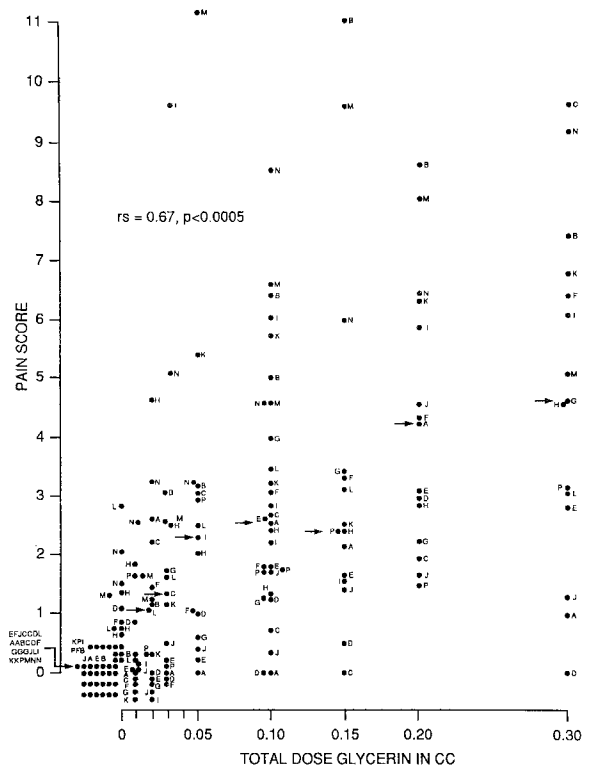


FIG. 2. Correlation between pain scores and the responsible injected total dose (volume $\times$ concentration) of glycerin. Arrows indicate medians.

(volume, percent glycerin) analysis of variance⁹ was also used to confirm that both volume and percent glycerin were related to pain score and that volume $\times$ percent glycerin interaction was significant.

To test the combined effect on the pain score of both volume and percent glycerin in a specific injected dose, we calculated the injected total dose of glycerin by multiplying glycerin concentration by volume. We used nonparametric Spearman's rank correlation coefficient (r_s)^{7,8} to measure the correlation of responsible total dose of glycerin with specific pain scores and the correlation with frequency and mean diameters, for each glycerin dose group, of injection site erythema areas, induration areas, and bruising. We used a two-tailed test with a significance level of 0.05 to reject the null hypothesis.

In the immunotherapy recipient group, we compared the effects of the usual maintenance dose of immunotherapy solution with those of the same volume and concentration of glycerin solution and with those of the nearest volume of HSA diluent (0% glycerin) (injected doses shown in Table I). For each glycerin dose group, pain scores, and frequency, and mean diameters of injection site areas of erythema, induration, and bruising were compared. Pain scores were analyzed with the Mann-Whitney U test. Because erythema, induration, and bruise measurements were made with normally distributed numbers in a ratio scale, frequencies were analyzed in 2×2 contingency tables with chi square test,

TABLE I. Content of antigen, glycerin, and HSA in the usual maintenance dose of immunotherapy solution, in the same dose of glycerin, and in the nearest dose of HSA given to the eight subjects in the immunotherapy recipient group

Subject	Immunotherapy solution			Glycerin solution			HSA diluent		
	Antigen No.	Glycerin (%)	HSA (ml)	Antigen No.	Glycerin (%)	HSA (ml)	Antigen No.	Glycerin (%)	HSA (ml)
F	14	10.0	0.9	0	10.0	0.9	0	0	1.0
G	7	0.8	0.1	0	0.8	0.1	0	0	0.1
J	9	8.0	0.9	0	8.0	0.9	0	0	1.0
L	8	7.5	0.9	0	7.5	0.9	0	0	1.0
K	14	10.0	0.1	0	10.0	0.1	0	0	0.1
P	6	5.5	0.7	0	5.5	0.7	0	0	0.5
M	9	9.5	0.9	0	9.5	0.9	0	0	1.0
N	14	10.0	0.5	0	10.0	0.5	0	0	0.5

and diameters were analyzed with Student's *t* test for difference between two means.⁸ We tested the hypothesis that the complex immunotherapy solution produced more pain, erythema, induration, and bruising than either its glycerin content or its HSA content by using a two-tailed test with a significance level of 0.05 to reject the null hypothesis.

RESULTS

Pain scores

Pain scores (Fig. 1) were not influenced by volume of injection when diluent alone (0% glycerin) was given ($p > 0.01$). Pain scores of patients given glycerin increased significantly as both injection volume ($p < 0.001$) and glycerin concentration ($p < 0.001$) increased but varied considerably in individual patients.

There was a highly significant positive correlation ($r_s = 0.67$, $p < 0.0005$) between pain scores and injected total doses (volume $\times$ concentration) of glycerin (Fig. 2). In the majority of subjects, most pain scores increased progressively as the total injected dose of glycerin increased. Nevertheless, many subjects had outlying scores that were lower or higher than expected.

There was also significant positive correlation between both frequency (Fig. 3) and duration of clinically important pain (grades 3 and 4) and the responsible injected total dose of glycerin (grade 4 [intolerable] pain: frequency $r_s = 0.55$, $p < 0.05$; duration $r_s = 0.56$, $p < 0.05$; grade 3 [annoying] pain: frequency $r_s = 0.96$, $p < 0.0005$; duration $r_s = 0.89$, $p < 0.0005$).

Skin erythema at injection site

An area of skin erythema was present at the injection site 30 minutes after the injection in a few subjects. The frequency (i.e., number of sub-

jects with skin erythema in each glycerin dose group of 15 patients) was low (range, 0 to 5; median, 1 per glycerin dose group). The clinical importance was trivial, because the areas of erythema produced little or no discomfort. However, we found highly significant positive correlations between the responsible injected total doses of glycerin and both their frequency ($r_s = 0.86$, $p < 0.001$) and mean diameters in each glycerin dose group, 30 minutes after the injection ($r_s = 0.90$, $p < 0.001$).

Skin erythema areas were infrequently associated with the subcutaneous areas of induration described in the next section. Of the 15 occurrences of skin erythema and 40 occurrences of subcutaneous induration in the 12 glycerin dose groups, coexisting erythema and induration were present in seven instances only.

Circumscribed induration area at injection site

A circumscribed induration area, the "glycerin button," was found in the subcutaneous tissue at the site of some injections. These induration areas usually became detectable after injection pain had subsided at about 2 to 10 minutes after the injection, reached greatest diameter about 30 to 40 minutes after the injection, and slowly disappeared over the next 20 to 30 minutes (Fig. 4). Usually, such areas were not associated with pain, tenderness, or skin erythema and were considered brief phenomena, interesting to observe, but without clinical importance. However, highly significant positive correlations were found between the responsible injected total doses of glycerin and both their frequency (number of subjects with a subcutaneous induration area in each glycerin dose group of 15 subjects) ($r_s = 0.88$, $p < 0.001$) and their mean diameters in each

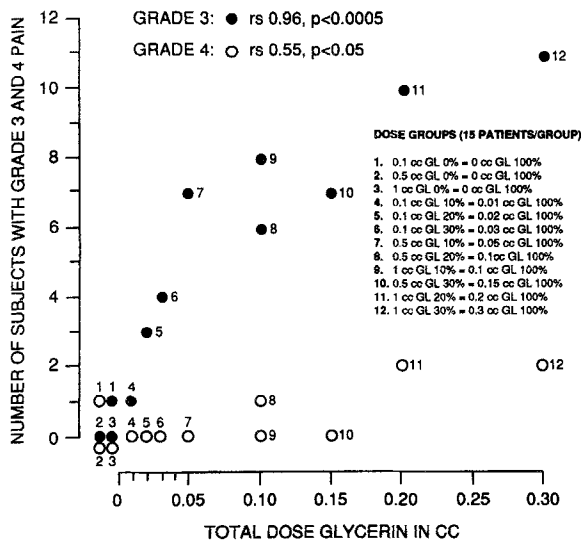


FIG. 3. Clinically important pain frequency (number of patients in each dose group with grade 4 [intolerable] and grade 3 [annoying] pain): correlation with the responsible injected total dose (volume $\times$ concentration) of glycerin. GL, Glycerin.

glycerin dose group 30 minutes after the injection ($r_s = 0.85$, $p < 0.001$).

Bruises at injection site

Bruises appeared at some injection sites within 1 day after the injection in 11 of 15 subjects. Bruise frequency (number of subjects with a bruise in each glycerin dose group of 15 patients) showed an insignificant correlation with the responsible injected total doses of glycerin ($r_s = 0.35$, $p > 0.1$). However, mean bruise diameters in each glycerin dose group showed a significant, positive correlation with the responsible total doses of glycerin ($r_s = 0.67$, $p < 0.05$). Bruises occurred more often in specific subjects; the 12 injections given to each of the 15 subjects produced 7 to 8 bruises in three subjects, 1 to 3 bruises in eight subjects, and no bruises in four subjects. All three subjects who had 7 to 8 bruises had bruises not only after glycerin injections but also after some injections containing no glycerin and had bruised easily after minor trauma and needle sticks in the past. However, these three subjects had no diagnosed bleeding disorder, had tolerated major surgery in the past, and were not receiving anticoagulants.

Comparison of effects of injections of immunotherapy solution, glycerin, and HSA diluent in the immunotherapy recipient group

In the eight subjects in the immunotherapy recipient group, the ranks of the pain scores pro-

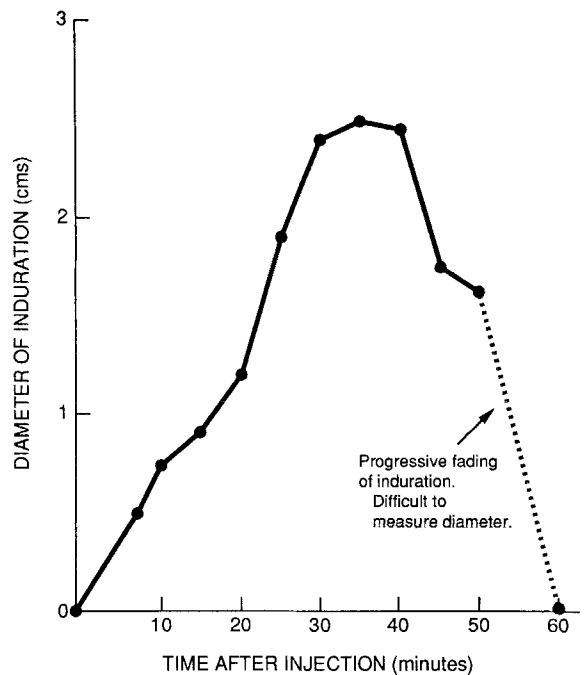


FIG. 4. Expansion and decline of a typical subcutaneous area of induration, "glycerin button," at the site of an injection of glycerin (Subject d, 0.5 ml of 20% glycerin, left upper arm low).

duced by immunotherapy solution were not significantly different from the ranks of the pain scores produced by glycerin solution (Mann-Whitney $U = 27$, $p = 0.646$) but were significantly greater than those produced by HSA solution (Mann-Whitney $U = 12.5$, $p = 0.044$ (Fig. 5).

A measurable area of skin erythema was present at the injection site in seven of eight subjects 30 minutes after the injection of immunotherapy solution but was not present in any of these subjects after injection of glycerin solution or HSA diluent. Erythema area frequency and mean diameter after injection of immunotherapy solution were significantly greater than after injection of glycerin solution or HSA diluent. After injection of immunotherapy solution, erythema frequency was 0.88 (7 of 8), and mean diameter was 2.7 ± 1.47 cm. After injection of glycerin solution, erythema frequency was 0 ($\chi^2 = 12.44$, $p < 0.002$), and mean diameter was 0 ± 0 cm ($t = 3.66$, $p < 0.01$). After injection of HSA diluent, erythema frequency was 0 ($\chi^2 = 12.44$, $p < 0.002$), and mean diameter was 0 ± 0 cm ($t = 3.66$, $p < 0.01$).

Immunotherapy solution produced subcutaneous induration areas and bruises that were not significantly different in frequency or mean diameter from those produced by glycerin solution or

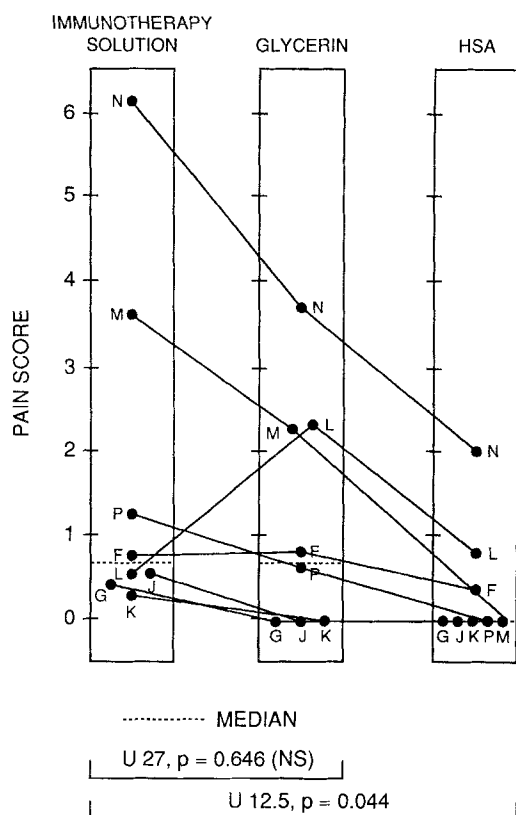


FIG. 5. Comparison of pain scores produced by the usual dose of immunotherapy solution with those produced by the same dose of glycerin solution and the nearest volume of HSA diluent in the eight subjects in the immunotherapy recipient group. NS, Not significant.

HSA diluent. After injection of immunotherapy solution, induration frequency was 0.38 (3 of 8), and mean diameter was 1.02 ± 1.85 cm. After injection of glycerin solution, induration frequency was 0 ($\chi^2 = 3.69$, $p = 0.057$), and mean diameter was 0 ± 0 cm ($t = 1.10$, $p > 0.2$). After injection of HSA diluent, induration frequency was 0 ($\chi^2 = 3.69$, $p < 0.057$), and mean diameter was 0 ± 0 cm ($t = 1.10$, $p > 0.2$). After injection of immunotherapy solution, bruise frequency was 0.13 (1 of 8), and mean diameter was 0.48 ± 1.34 cm. After injection of glycerin solution, bruise frequency was 0.38 (3 of 8; $\chi^2 = 1.33$, $p > 0.4$), and mean diameter was 0.43 ± 0.90 cm ($t = 0.06$, $p > 0.5$). After injection of HSA diluent, bruise frequency was 0.13 (1 of 8; $\chi^2 = 0$, $p > 0.99$), and mean diameter was 0.48 ± 0.42 cm ($t = 0.46$, $p > 0.5$).

DISCUSSION

Our results indicate that for the studied injected glycerin concentrations (0%, 10%, 20%, and 30%) and injected volumes (0.1, 0.5, and 1.0 ml), injec-

tion pain intensity as measured by pain scores increased significantly ($p < 0.001$) as both injected glycerin concentrations and volumes increased (Fig. 1). When injected glycerin concentration $\times$ volume was considered as one variable, the total dose of glycerin, a highly significant positive correlation ($r_s = 0.67$, $p < 0.0005$) was demonstrated between pain scores and the total injected dose of glycerin (Fig. 2). Clinically important pain (grade 4 [intolerable] and grade 3 [annoying]) was unusual when the injected total dose of glycerin was less than 0.05 ml (0.5 ml of 10% glycerin) but progressively more frequent as the total glycerin dose increased, with an almost perfect positive correlation between the frequency of annoying pain in each dose group and the responsible total dose of glycerin ($r_s = 0.96$, $p < 0.0005$) (Fig. 3).

Pain scores of subjects receiving diluent alone (0% glycerin) were usually (with one exception) low and clinically unimportant in the dose groups receiving injected volumes of 0.1, 0.5, and 1.0 ml.

The data are consistent with the hypothesis that injected glycerin in the subcutaneous tissue is an irritant that produces pain, which is usually trivial when small amounts are injected but of increasing clinical importance when larger amounts are injected.

There were many examples of exceptions to the general rule discussed previously. Some subjects had lower pain scores, and others had higher pain scores than those usually found after a given dose of glycerin. These exceptions indicate that some subjects tolerated, without clinically important pain (grade 3 or 4), injections of as much as 1 ml of 30% glycerin. Others had clinically important (grade 3 or 4) pain after injections of glycerin in the currently recommended and here tested dose range between 0.1 and 1 ml of 10% glycerin. These observations confirm previous statements, which were based on uncontrolled clinical observations.^{3,5}

Skin erythema was present at the injection site 30 minutes after the injection in a few subjects. This was an unusual occurrence (range, 0 to 5; median, 1 subject with erythema in each dose group of 15 patients) that produced little or no discomfort and was considered of no clinical importance. However, we found highly significant positive correlations between the responsible total dose of glycerin and both erythema area frequency ($r_s = 0.86$, $p < 0.001$) and mean diameter in each glycerin dose group ($r_s = 0.9$, $p < 0.001$).

An interesting observation related to subcutaneous injections of glycerin was the appearance of a circumscribed area of induration in the subcutane-

ous tissue after some injections, particularly the larger doses (0.5 and 1 ml) of the higher (20% and 30%) concentrations. These subcutaneous "glycerin buttons" were essentially brief, painless phenomena, of no bother to the patient. Nevertheless, highly significant positive correlations were found between the total injected dose of glycerin and both their frequency ($r_s = 0.88, p < 0.001$) and their mean diameters in each glycerin dose group ($r_s = 0.85, p < 0.001$). We hypothesized that these transient subcutaneous indurated areas might be due to the known hygroscopic effect of the injected glycerin.¹⁰

Another observation was the occurrence of a bruise at the injection site within 24 hours after injection in many subjects. Bruise frequency after the 12 injections appeared to be related to that person's susceptibility to bruising caused by trauma. Of 15 subjects, the three with the most (7 to 8) bruises reported bruising not only after glycerin injections but also after the studied injections containing no glycerin and after minor trauma and hypodermic injections in the past. Nevertheless, they had tolerated major surgery, were not receiving anticoagulants, and did not have a diagnosed bleeding disorder.

Comparison among the eight patients in the immunotherapy recipient group of the effects of the usual maintenance dose of their immunotherapy solution with those of a control injection of glycerin solution of the same volume and concentration and with those of the nearest injected volume of HSA diluent revealed two pertinent findings.

First, the immunotherapy solution pain scores were low because the responsible total glycerin doses were low but were higher than those produced by HSA diluent ($p = 0.04$) and were not significantly different ($p = 0.646$) from those produced by glycerin solution. Taken together, these findings strongly suggest that in the dose range of these immunotherapy solutions (0.1 ml 0.8% glycerin to 0.9 ml 10% glycerin), glycerin is the major cause of injection pain.

Second, local skin erythema was present at the injection site 30 minutes after injection significantly more often in these patients after injection of their customary dose of immunotherapy solution (7 of 8) than after injection of either glycerin solution (0 of 8) or HSA diluent (0 of 8) ($\chi^2 = 12.44, p < 0.002$). These findings strongly suggest that in the dose range used (0.1 ml 0.8% glycerin to 0.9 ml 10% glycerin), allergens in the immunother-

apy solution, not glycerin, were the main cause of the skin erythema.

We conclude that injected glycerin produces pain that is proportional to the total dose of glycerin, but individual variation in perceived discomfort is substantial. Some patients tolerate, without clinically important pain (grade 3 or 4), the highest tested dose, 1 ml of 30% glycerin; a very few have clinically important pain caused by the lowest doses tested here, doses that are in the currently recommended tolerated range.^{3,5} When desirable, the pain tolerance of a patient to a proposed maintenance dose injection volume and percent glycerin can be measured before the immunotherapy solution is prepared by use of the techniques described in this study. If the proposed volume and percent glycerin of the injected solution are well tolerated, they may be used to deliver the proposed dose of immunotherapy solution. If not, the proposed dose can be given in two or more injections, each of smaller total glycerin content.

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