A NEW TECHNIQUE FOR PERFORMING QUANTITATIVE CONTACT (PATCH) SKIN TESTS

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INTRODUCTION

The patch test has become a firmly established diagnostic procedure for determining the etiologic agent in allergic contact dermatitis. The procedure, when used for this purpose, is relatively simple in concept and consists essentially of exposing the skin sufficiently to contact with the suspected substance to elicit the reaction of hypersensitivity. The technique has been adequately described (1, 2, 3, 4, 5) and consists essentially of placing the material to be tested, or a solution of it in suitable concentration in a proper vehicle, on a small square of several thicknesses of gauze and anchoring it on the skin to be tested with a piece of cellophane lined adhesive tape.

The usual patch test procedure, while qualitatively adequate for diagnostic work, is not adapted to exact quantitative determinations of the degree of cutaneous hypersensitivity to a specific substance. Differences in the degree of reaction can be shown by varying the concentration of the test substance, but these variations need to be fairly large. The resulting reactions vary relative to each other, but the amount of the test substance responsible for the degree of reaction in each case in unknown. The most obvious defects of this procedure for quantitative testing are: first, the skin and vehicular solvent are in competition for the solute (test substance); and second, the period of exposure is terminated after an arbitrary period of time and after an unknown amount of test substance has been absorbed. The purpose of this paper is to describe a technique for performing quantitative contact (patch) tests by applying a known dosage of test substance uniformly over an area of skin the size of which is accurately controlled.

There have been attempts made to modify the usual patch test procedure, particularly in experimental work where quantitative information on the degree of hypersensitivity has been desired. One such modification is the use of a volatile organic solvent as a vehicle for the test substance. This modification has the advantage of subjecting the skin to the entire test dose at once and of eliminating the need for the gauze carrier and adhesive plaster covering. Some workers have applied such solutions to the skin with a cotton applicator (6, 7) or camel's hair

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brush (8) and attempted to keep the stripe painted of fairly uniform size. The obvious disadvantages of this method are that the exact dosage applied to the skin is not known and the size of the skin area subjected to the test is only roughly controlled. Other workers have applied such solutions in a measured volume without controlling the area of spread (9) or have used a ring of vaseline on the skin as a method of limiting the area of spread (10). The former method has the advantage of exact dosage, and the latter the additional improvement of controlling the size of the skin area subjected to test. But, as will be shown later, both methods have faults which limit their usefulness as quantitative procedures.

DEVELOPMENT OF QUANTITATIVE CONTACT SKIN TEST TECHNIQUE

The reaction of cutaneous hypersensitivity (allergic contact dermatitis) may be considered a chemical reaction with one of the reagents—a component of the skin—measurable in units of skin area, the test substance measurable in some unit of weight, and the amount of reaction resulting measurable in terms of intensity of visible reaction. By controlling the area of skin subjected to testing, the intensity of the reaction will be some function of the amount of specific test substance applied. The visible reaction with its elements of color intensity, edema and vesiculation cannot be quantitatively measured satisfactorily, but by using graded doses of test substance, the threshold dose that is required to elicit any visible reaction has been found to be a suitably objective end point.

Accordingly, the desirable attributes of a quantitative contact test are: 1) the exact amount of test substance used should be known and should be completely available for absorption from the time it is placed on the skin; 2) the distribution of the test substance over the area of skin being tested should be uniform; 3) the area of skin being tested should be controlled; and 4) the skin being subjected to test should be uniform. The skin of the ventrum of the forearm exclusive of the wrist and cubital area in humans and the abdominal skin in guinea pigs have been found suitably uniform.

Acetone was chosen as the solvent to be used because it has good solvent properties, it spreads readily on the skin, evaporates quickly, and its volatility is not so great that it cannot be handled quantitatively in small volumes under the conditions of the technique described here. Poison ivy extract and compounds related chemically to urushiol and known to give sensitivity reactions were used as test materials because of the availability of human subjects already sensitized to these substances, and because it is well known that cutaneous hypersensitivity to these materials can be induced in guinea pigs (11).

The behavior of a known volume of acetone when placed on the skin of the forearm was first investigated by placing 0.01 ml. of an 0.15 per cent solution of methyl violet in acetone at various points on several individuals. The dye solution was found to spread rapidly over a large area which was usually oval in shape. The size of the area over which the acetone spread varied somewhat with the location on the ventrum of the forearm to which the solution was applied, and also between different individuals. A dye solution in alcohol when similarly tested spread over a larger area than the acetone, presumably because it evaporated more slowly and had a longer time interval in which to spread. The residual

stain with either solvent showed a concentration of the dye at the periphery with a fairly uniform, lighter color over the remainder of the stained area. It is obvious that a procedure such as this gives no control over the skin area subjected to the test dose, nor is the test dose spread uniformly over the skin being tested.

A first attempt to control the spread of the known volume of acetone on the skin was made by using a lanolin ring. A stamp pad containing lanolin impregnated filter paper was warmed on a steam bath until the lanolin liquified. The open end of an ordinary test tube having an inside cross-sectional area of 1.3 square centimeters was tapped on the pad until it had a thin coating of liquid lanolin and then placed on the skin of the forearm. The slight excess of lanolin was carefully blotted off with lens paper leaving a thin film on the skin. When 0.01 ml. of the dye solution in acetone was placed in the center of this ring, it was found to spread over the encircled area and to be held adequately by the lanolin barrier. It was noted, however, that the dye still tended to be concentrated somewhat at the periphery.

Preliminary tests on individuals sensitive to poison ivy were made with this method using various dilutions of a commercial poison ivy extract[4], hydrourushiol[5], and anacardol[6] in acetone (technical grade). It was found that if a number of tests with one of these materials were placed at various points on the ventrum of the forearm of a subject, using the same dosage at each site, reactions of comparable intensity resulted. An exception to this occurred on two occasions with one subject when a test on the ventrum of the wrist was included with those on the ventrum of the forearm. The dosage in both instances was less than that necessary to produce a reaction on the forearm and yet reactions occurred at the wrist. As a result of this experience we have avoided the latter site for quantitative testing. When 1 to 2 serial dilutions of these materials were applied to the subjects, there were definite gradations in the intensity of the resulting reaction paralleling the amount of test substance used.

In the course of this work it became evident that the technique being used, while it gave fairly satisfactory results, had certain disadvantages. When dosages were used that did not produce reactions of the whole test area, it was noted that reactions tended to appear at the periphery, as could be anticipated from the preliminary dye experiments. Occasionally it was found that the acetone broke through the lanolin barrier. In addition, the film of lanolin used for the ring could not be removed after the test dose was applied, and the clothing smeared it to some extent on the surrounding skin. On one occasion when a fairly large test dose was used, it was thought that this may have been the means of spreading the poison ivy extract around the test area. Finally, the technique was not well suited to animal work.[7]

[4] Kindly furnished by the Lederle Laboratories, Inc. (13 per cent solids in acetone).

[5] A synethetic compound. See Mason, Howard S.: Jour. Amer. Chem. Soc. (in press).

[6] Kindly supplied by Dr. C. R. Dawson, Department of Chemistry, Columbia University, New York.

[7] This preliminary work was done while the senior author was attached to the Dermatoses Investigation Section, Medical Director Louis Schwartz, Chief; Division of Industrial Hygiene, Bureau of States Relations.

Various modifications of the technique were attempted to eliminate the disadvantages mentioned. It was thought that if a smaller area were used, the volume of acetone would flow over the whole area at the time of application rather than spread from the center of the area to the periphery by creeping, and the tendency for peripheral concentration could be eliminated. This necessitated using a barrier with more depth to hold the acetone. The difficulty of making such a barrier with lanolin or any other similar material was such that this method had to be abandoned.

Metal rings were then tried as barriers, but acetone readily crept under the rings along the natural grooves in the skin. A water soluble preparation described for use as an ether insoluble stop-cock grease[8] (12) was tried as a seal between the skin and the ring and found to be satisfactory. This material has the distinct advantage of being removable from the skin by cold running water after testing without disturbing the water insoluble, active principle of poison ivy.

Brass cylinders were made having the following measurements: bore, 8 mm.; wall, 2 mm.; and height, 4 mm. Brass wire loops were attached to each side of the ring in a groove cut in the outer cylinder wall 1 mm. from one edge. This placed the loops off center on the cylinder to make the ring set more stably on the skin. A rubber band with two brass wire hooks was used to encircle the arm and hold the ring securely on the forearm. Eight millimeters was chosen as the diameter of the cylinders because it provides a skin area of 0.5 square centimeters for testing. Also the skin exposed in a ring of this size remains relatively flat in spite of the ring pressure; larger rings will produce bulging of the test area, and the test solution will gravitate peripherally.

Tests with these brass rings using 0.01 ml. doses of the dye solution produced fairly uniform coloring of the skin without peripheral concentration. Preliminary testing with poison ivy solutions on sensitive individuals gave satisfactory results with the advantage that minimal reactions did not tend to appear at the periphery of the test area as with the original technique.

There is always some dosage loss when pipetting small volumes of acetone or similar solvents because of their tendency to creep up the glass tip of the pipette and not discharge completely. In addition, using the brass rings as a means of limiting the spread of acetone on the skin resulted in the deposition of solute, as could be readily seen using dye solution, on the portion of the inside wall adjacent to the skin. It was important to determine the amount and variability of this dosage loss in order to define the quantitative limits of the method.

Before attempting such measurements, however, an effort was made to reduce the dosage loss to a minimum. By reducing the dosage volume from 0.01 ml. to 0.005 ml. it was found that the dye solution in acetone would still spread over the skin satisfactorily but would not ascend the inner wall of the brass ring as high. This volume has the advantage of evaporating more quickly, and as will be pointed out below, it was necessary to use this volume with the small tip pipette

[8] Soluble starch 9 grams; glycerol 22 grams. Heat to 140°C. with stirring and cool. Not all soluble starchs are satisfactory.

finally selected. Acetone does not creep on clean brass and no practicable coating was found which appreciably improved this physical characteristic with the possible exception of ordinary bar soap. After considerable trial and error the most satisfactory preparation of the rings for use was found to be as follows: the rings are rinsed with acetone to remove any material adhering from prior testing; they are scrubbed with an abrasive soap cleanser, rinsed twice with water and dried. No attempt is made to rinse the rings individually since any remaining soap from the cleanser will improve the wetting characteristics of the brass rings.

Various types of pipette tips were tried in order to reduce this source of dosage loss to a minimum. By collecting glass at the tip of a pipette and then pulling this tip to a fine capillary of 1 to 1.5 mm. outside diameter and a bore of 0.4 to 0.6 mm., and by coating the tip with potassium oleate before discharging a dose, the dosage loss proved to be minimal. It was necessary to use 0.005 ml. dosage volume rather than 0.01 ml. to prevent the drop from falling off this size tip.

To determine dosage loss with the technique evolved, a solution of methyl violet in acetone comparable to the one previously mentioned was used. Since dosage loss was compared to total dose by spectrographic observation, it was not necessary to know the exact concentration. Doses of 0.005 ml. of this dye solution were applied to human subjects and to guinea pigs in the usual manner of testing. After the dose had been discharged and the remaining dye solution pulled up in the pipette, the tip was immersed in a weighing bottle containing 5 ml. of absolute alcohol. The brass ring was removed from the skin when the acetone had evaporated and also was placed in the weighing bottle. Total doses were taken by discharging the dose on the pipette tip and then immersing it in a weighing bottle containing 5 ml. of absolute alcohol. Density measurements were made on a Beckman spectrophotometer at a wave length of 572 mμ (peak of the absorption band), and the density ratio of dosage loss on the ring and tip to total dose taken as a measure of the per cent dosage loss. The starch-glycerine compound present on the rings was found to have no absorption at this wave length nor did 0.005 ml. of acetone in this volume of alcohol have any effect.

As a result of 64 determinations on six human subjects the dosage loss was found to be 20.8 per cent with a standard deviation of 3.4 per cent. A similar series of 59 tests on guinea pigs showed a dosage loss of 22.3 per cent with a standard deviation of 2.9 per cent. The average dosage loss on guinea pigs was slightly but significantly higher than on human subjects.

To determine whether dosage loss would be affected by concentration, the dye solution was diluted with an equal volume of acetone and a small series of tests run. The per cent dosage loss was not different from that with the more concentrated dye solution.

It still remained to determine whether the dosage loss of the dye solution represented the dosage loss of the test substances. A series of tests was run on guinea pigs with a 5 per cent poison oak extract[9] in absolute alcohol. Unfortunately the total dose at the absorption peak of 274 mμ gave a fairly low reading, and it was

[9] Kindly supplied by Cutter Laboratories, Inc., Berkeley, California.

not possible to measure the absorption of the material removed from the ring and pipette tip. Also the starch-glycerine compound altered the absorption of absolute alcohol at this wave length. A greenish pigment in the solution of poison oak (probably chlorophyll) showed a good absorption peak at 412 mμ. Measurements made at this wave length gave dosage loss percentages comparable to those obtained with methyl violet. This indicated that the test results with alcohol as a solvent were comparable to those with acetone and that the dosage loss using this extract material did not differ from that of the dye.

Another series of tests was run with an acetone solution of poison ivy extract. Since acetone also absorbs at 274 mμ, it was necessary to eliminate this substance for purposes of spectrographic measurement. The total dose was run into a small amount of acetone in a weighing bottle, the acetone then evaporated in an air stream, and the solid material redissolved in 5 ml. of absolute alcohol. Before placing the rings in absolute alcohol the starch-glycerine compound was washed from them to eliminate the absorption effect of this material. The dosage loss from these determinations was comparable to that obtained with the dye solution.

In applying these dosage loss measurements to the actual conditions of serial dilution testing, it is necessary to remember that dosage losses at the opposite extremes of the range of these measurements are important only when they occur at the end point of the series of dilutions (minimum reacting dose). For example, from the dye measurements we might expect in $\frac{2}{3}$ of the tests on humans that dosage losses would vary as much as seven per cent, and with guinea pigs slightly less. The chance of a dosage loss occurring more than one sigma away from the mean in either direction is $\frac{1}{3}$, and for it to occur in one direction or the other is $\frac{1}{6}$. The chance of these two events occurring successively and in opposite directions is once in 18 times. The possibility of this event happening at the end-point (MRD) in a series of tests is much less.

This would indicate that it might be feasible to work with serial dilutions varying by as little as 10 per cent. The ability to evaluate reaction intensities visually imposes a limitation, however, and we have found it most practicable to use dilutions varying in concentration by about 20 per cent. Our procedure is to make serial dilutions (using technical grade acetone) in which each dilution is 90 per cent of the concentration of the preceding dilution. This furnishes an adequate range of dilutions, and by using every other dilution or every third dilution, the difference between any dilution and the preceding one will be 81.0 per cent or 72.9 per cent, respectively.

DESCRIPTION OF PRESENT TECHNIQUE

The test doses are applied from an 0.1 ml. pipette with a capillary tip as previously described, graduated in 0.001 ml. This is mounted in a Guthrie pipette controller (Eimer and Amend) which is held in a rack and pinion. The rubber tubing attached to the top of the pipette is connected to a suction bottle for cleaning between test doses; a screw clamp just above the pipette controller serves to close the tubing while the test dose is being taken up and applied (fig. 1).

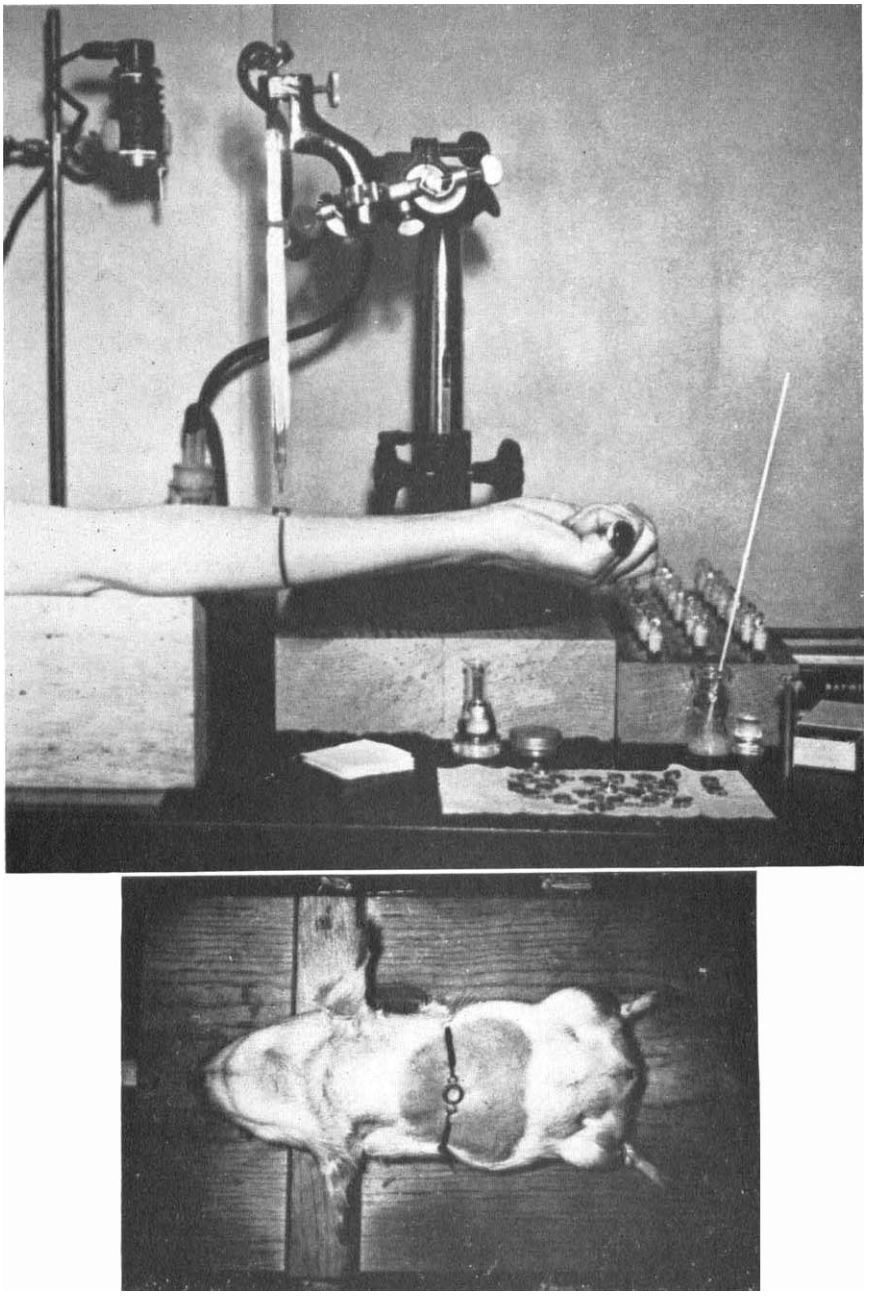

FIG. 1. APPARATUS USED FOR APPLYING QUANTITATIVE CONTACT TESTS SHOWING A FOREARM IN POSITION TO RECEIVE A TEST DOSE AND A GUINEA PIG WITH A TEST RING IN PLACE

No preparation is made of the skin before testing except to clip the hair from the ventrum of the forearm, if necessary, or in the case of guinea pigs to clip the hair from their abdomen. It is often necessary to clean cage soil from the skin of guinea pigs that have been used previously. Water without soap has proved most satisfactory for this.

Occasionally with guinea pigs the acetone of the test dose will not spread over the whole skin area exposed in the ring. This happens infrequently and for some unknown reason; it may be troublesome in a particular animal, although the same animal may present no difficulty at another time. By placing small drops of approximately 0.005 ml. of acetone on the skin with a fine tip eye-dropper prior to testing, it is usually possible to determine whether this difficulty will be encountered. Occasionally washing the skin with water or dropping acetone on the area to be tested will overcome this condition. By observing each test site in a vertical beam of light immediately after applying a test dose, it is possible to see whether the acetone has spread over the entire test area.

With humans we have had difficulty in getting the acetone to spread well only when the skin was continually moist from sweating or when the test area was washed with soap and water immediately before testing. The salt content of the sweat and residual soap seem to be the cause of the difficulty under these two conditions.

If more than a few tests are to be applied, guinea pigs are held on their backs on an animal board so constructed that their heads are held down with a wire loop over their upper jaws, which can be tightened with a wedge, and their legs held down with cord loops. A strip of wood under the shoulders facilitates placing the rubber band around their trunk (see fig. 1). Test sites on guinea pigs are marked with a moistened, indelible pencil.

The brass rings are prepared as previously described by rinsing with acetone, scrubbing with an abrasive cleanser, rinsing in water and drying. A square inch of lens paper is placed over the end of a brass rod 8 cm. long and about 8 mm. in diameter which fits the rings snugly. A ring is placed over the end of this rod on top of the lens paper until the bottom of the ring is flush with the end of the rod. The starch-glycerine sealing compound is then applied to the bottom of the ring in a thin even coating with a wooden applicator and the ring slid off the length of the rod. The lens paper serves to protect the inside of the ring[10]. A rubber band of suitable length with brass hooks attached is placed around the forearm or around the guinea pig, hooked to the prepared ring, adjusted to equalize the tension, and the ring set on the skin (fig. 1).

When an amount of acetone solution somewhat in excess of the amount needed for the test dose has been taken up in the pipette, the solution is pulled up in the pipette away from the tip and the latter wiped immediately. Just before apply-

[10] The consistency of the starch-glycerine compound for use on guinea pig skin should be such that it can be spread on the rings evenly without lumpiness. The rings should be coated just before use because thin preparations of this material have a tendency to change from a film to droplets after standing a time on the brass surface. For humans a more viscous preparation can be used, and the rings may be prepared in advance.

ing the test dose, the pipette tip is wiped with gauze impregnated with potassium oleate, and the column of solution is brought down to the pipette tip. The forearm with the ring in place, in the case of human subjects, or the guinea pig on an animal board with the test ring on the abdomen is placed under the pipette and held with the ring in a horizontal position. The dose is run out into a drop on the tip, the pipette lowered into the ring until it touches the skin, allowed to remain there momentarily until the dose has run onto the skin and then withdrawn. The remaining test material is drawn out of the pipette by opening the screw clamp, and sufficient acetone drawn through the pipette to thoroughly cleanse it. The pipette is refilled for each successive dose in the manner described. When using alcohol as a solvent, potassium oleate will not prevent creeping, but a silicone stopcock lubricant will.[11]

There is a slight over-dosage from the pipette amounting to an estimated 0.0003 to 0.0005 ml. This is the result of two factors: the drag due to capillary attraction at the time the pipette tip touches the skin and the gradual falling of the column of acetone until the air in the pipette above the solution becomes saturated with acetone. It takes several minutes for this latter factor to become stabilized although it can be eliminated by filling the pipette with acetone saturated air before taking up the dose. However, we have not complicated the procedure with this refinement because the variability from these sources of error was a component of the variability of the dosage loss measurements. The latter have been shown to be much less than is necessary for the dilution differences that are practicable to use. By applying only one dose from each pipette filling we have considered this a fairly consistent error for all tests.

After the test dose is applied and inspection shows that the acetone has evaporated (a few seconds), the ring is lifted from the skin and removed. The site of the test is easily discernible from the ring of starch-glycerine remaining on the skin and aids in choosing sites for successive applications. Four rows of tests can be applied to the ventrum of the forearm, and twenty tests can readily be placed on an area $2\frac{1}{2}$ by 3 inches.

After the desired number of tests have been applied, the test area (forearm or guinea pig abdomen) is placed under running tap water for a few seconds to flood off the starch-glycerine mixture. The tested area is gently overlaid with a sheet of cellulose tissue to absorb excess water. No evidence of the test procedure remains, and the subject observes no precuations other than refraining from washing the test area until the next day.

Difficulty was encountered in marking the test sites satisfactorily on the forearm. To obviate this a wooden trough was devised with a hinged frame cover containing a glass grid marked at 1 cm. intervals. The edges of the sides of the trough were cut at a slope to correspond to the change in thickness of the forearm from elbow to wrist, and the bottom of the trough was lined with graph paper. A wooden dowel was placed in a slot at the lower end at right angles to the trough. After tests are applied, and before the starch-glycerine rings are washed off, the subject places his forearm in the trough with the wooden dowel between his

[11] Dow Corning Stopcock Grease used.

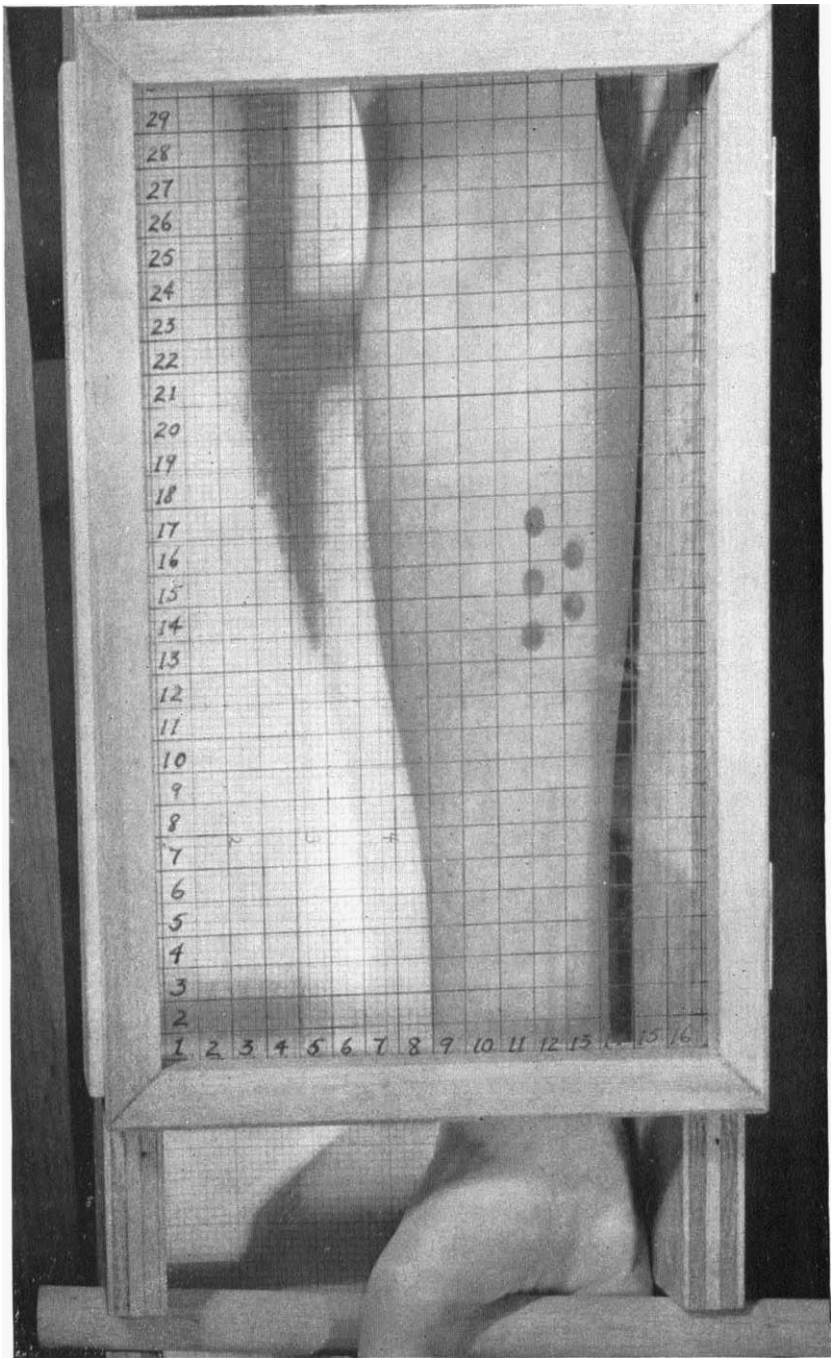

FIG. 2. APPARATUS USED FOR LOCALIZING THE SITES OF SKIN TESTS ON THE FOREARM, DYE SOLUTION HAVING BEEN USED FOR PURPOSES OF ILLUSTRATION

thumb and index finger. Firm pressure is made against the dowel by the subject, and the whole forearm is placed firmly against one side of the trough with the ventrum of the forearm upward. The hinged glass grid is then swung into place. The point at which the forearm bulges most is noted on the underlying graph

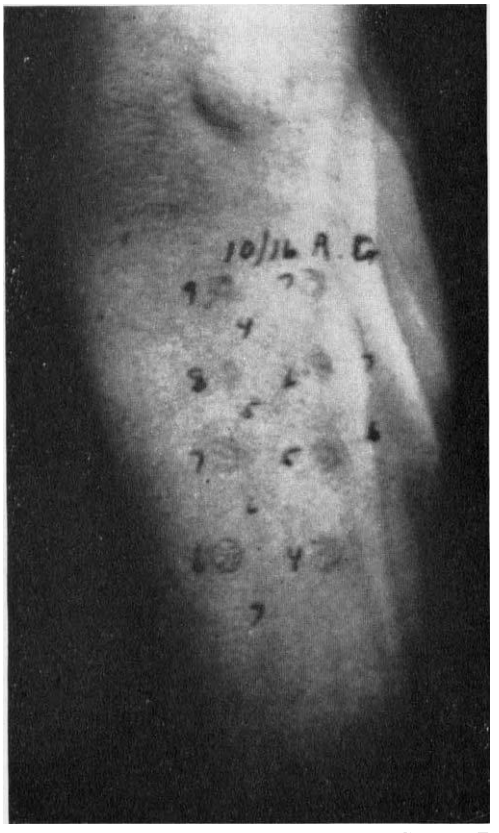

FIG. 3. APPEARANCE OF TEST SITES ON THE SIXTH DAY FROM SERIAL DILUTIONS OF POISON IVY EXTRACT, POISON OAK EXTRACT, ANACARDOL AND HYDROURUSHIOL

In this experiment the dilutions were made with each 70 per cent of the next stronger concentration. The dilution numbers are at the left of the test sites. The first row is poison ivy extract with the following dosages, dil. 9: 0.61γ; dil. 8: 0.87γ; dil. 7: 1.24γ; dil. 6: 1.77γ. The second row is poison oak extract with the following dosages, dil. 4: 1.71γ; dil. 5: 1.20γ; dil. 6: 0.84γ; dil. 7: 0.59γ. The third row is anacardol with the following dosages, dil. 7: 10.1γ; dil. 6: 14.5γ; dil. 5: 20.7γ and dil. 4: 29.6γ. The last row is hydrourushiol with the following dosages, dil. 7: 1.16γ and dil. 6: 1.66γ. The dosages given are the measured dose and not the corrected applied dose.

paper so that the forearm can again be placed in the same position for future observations. The location of the test sites is determined by measurements made by means of the glass grid cover (fig. 2).

In testing human subjects considerable variation in the length of time necessary for reactions to appear has been observed. This is partially dependent on dosage since any individual will show some delay in the appearance of reactions to higher dilutions, particularly when his minimal reacting dose is being approached. In figure 3 are shown reactions on the forearm of one subject tested

simultaneously with poison ivy extract[12], poison oak extract[13], anacardol and hydrourushiol. The picture was taken on the sixth day after application of the tests at which time he was reacting to all the dilutions of poison ivy extract and to anacardol; the reactions were well graded. In the evolution of these reactions the strongest concentration of poison ivy extract and of anacardol showed a beginning visible reaction at 48 hours; the remainder reacted the following day except

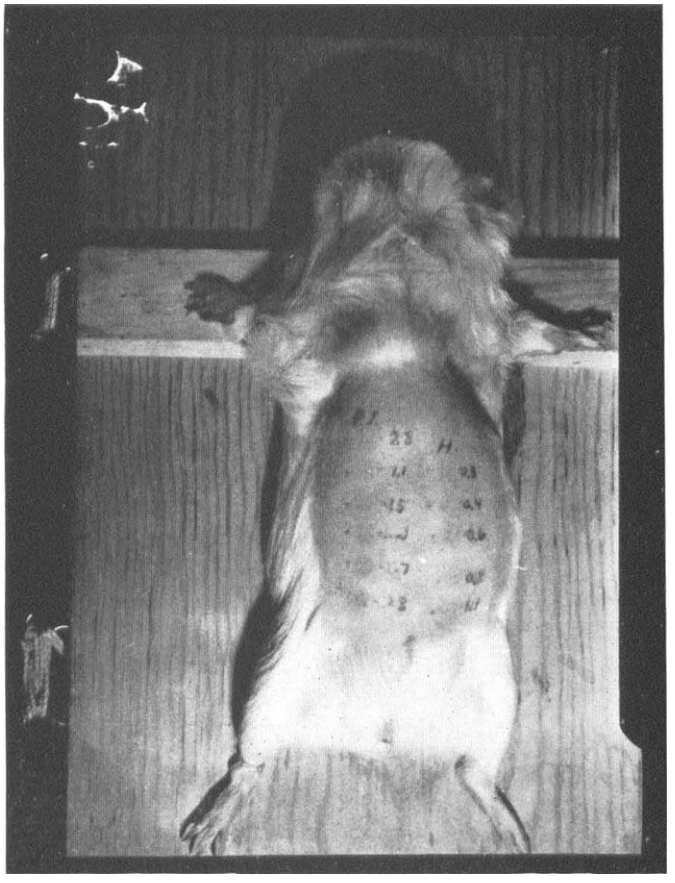

FIG. 4. REACTIONS TO SERIAL DILUTIONS OF POISON IVY EXTRACT AND HYDROURUSHIOL
Each dilution was 81 per cent of the next stronger. The dosage in gammas (rounded to the first decimal for convenience) is indicated. Beginning anteriorly these are, P.I.: 0.8, 1.1, 1.5, 2.0, 2.7 and 2.8 gamma; H.: 0.3, 0.4, 0.6, 0.8 and 1.1 gamma. Dosages given are the measured and not the corrected applied dosage.

the weakest anacardol dilution. The latter did not appear until the fifth day. On the seventh day reactions began to appear to the poison oak dilutions and to the strongest concentration of hydrourushiol. The subject eventually reacted

[12] Kindly supplied by Lederle Laboratories, Inc. (containing 16.5 per cent solids in acetone).

[13] Kindly supplied by Cutter Laboratories, Inc. (containing 5 per cent solids in absolute alcohol).

to all tests except the weakest dilution of hydrourushiol. It is obvious from these results that it is difficult to quantify reactions that are evolving over a period of several days and reaching their maximum intensity at different times. The minimal reacting dose provides the most objective end point.

The objection may be raised that the delayed, minimal reactions to these antigens in human subjects are the result of increased sensitivity stimulated by earlier reactions in the same group of tests. On the basis of analogy from other immunological reactions such a result might be expected. This question will be investigated further, but from present data it seems that there is considerable delay in absorption of these extremely low dosages in humans and this is an important, although possibly not the only factor, in these delayed reactions. For example, one subject known to be extremely sensitive to poison ivy was tested with 0.25γ of poison ivy extract. A reaction began on the sixth day. On the day preceding this he was given tests of 1.0γ and 0.5γ. In spite of the reaction from the first dose, the latter two stronger doses took four days to produce a reaction.

Guinea pigs are much more regular in the evolution of their reactions to poison ivy extract and to compounds related to its active principle. As a rule their their reactions are maximal in 48 hours, with our test procedure, and are fading in 72 hours. With the technique described here it is possible to work with dosages in sensitized animals below the level that produces primary irritation in normal animals (varying between 10γ and 20γ with the poison ivy extract used).

In figure 4 is shown a guinea pig tested simultaneously with serial dilutions of poison ivy extract and hydrourushiol. The reactions were well graded over the series. Later testing showed the minimal reacting dose of the animal to be 0.19γ of poison ivy extract and 0.10γ of hydrourushiol. It has been our experience that guinea pigs which are good reactors (easily sensitized and reacting to high dilutions) show approximately the same level of sensitivity upon repeated testing.

DISCUSSION

The technique as described is designed for quantitative skin testing with those sensitizing compounds that are soluble in volatile solvents. While acetone was used in the development of the testing technique, a few tests were run with alcohol with similar results, and no doubt any other volatile solvent or combination of solvents can be used. If the test substance were water soluble, flushing off the starch-glycerine mixture with water would need to be omitted and the excess sealing compound blotted off with absorbent paper. Our only experience in testing with such materials has been some preliminary work using an acid-alcohol solution of tuberculin.

The quantitative contact skin test technique described should be useful in the study of cross sensitivity of chemically related compounds and to measure accurately the effectiveness of immunological procedures directed toward reducing or eliminating a specific cutaneous hypersensitivity. Work is in progress using the technique described to assay poison ivy and poison oak extracts against pure, immunologically related compounds such as hydrourushiol and anacardol. This work will be reported later.

SUMMARY

A technique has been described for performing quantitative contact (patch) skin tests. Measurements of dosage loss with this technique indicate that the applied dose is about one-fifth less than the measured dose. The range of dosage loss variability was determined, and under the conditions of serial dilution testing the experimental error was less than the concentration differences that can be quantitatively graded by observation of reaction intensity. Use of this method for testing humans and guinea pigs with poison ivy extract and compounds related to its active principle are discussed.

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REFERENCES

(1) SCHWARTZ, L., AND TULIPAN, L.: Occupational diseases of the skin, Lea and Febiger, Philadelphia, 1943.
(2) SULZBERGER, M. B.: Dermatologic allergy, Charles C. Thomas, Springfield, 1940.
(3) URBACH, ERICH: Allergy, Grune and Stratton, New York, 1943.
(4) SULZBERGER, M. B., AND BAER, R. L.: Year book of dermatology and syphilology, The Year Book Publishers, Chicago, 1943.
(5) SCHWARTZ, L., AND PECK, S. M.: Patch test in contact dermatitis, Public Health Reports, **59:** 546, 1944.
(6) STRATTON, E. K.: Poison oak and poison ivy, dermatitant units as standard measure of potency for extracts from these plants, J. Allergy, **11:** 591–599, 1940.
(7) SIMON, F. A., AND LOTSPEICH, E.: Observations on sensitivity to poison ivy, J. Invest. Dermat., **2:** 143–150, 1939.
(8) GINSBERG, J. E., STEWART, C. D., AND BECKER, S. W.: Cutaneous sensitization studies; gross and microscopic changes in ragweed and 2-4 dinitrochlorobenzene sensitization of guinea pigs, and in poison ivy sensitization of human beings, J. Invest. Dermat., **2:** 81–92, 1939.
(9) WEDROFF, N.: Die Tropfmethode zur Prüfung der überempfindlichkeit der Haut gegen chemische Stoffe, Arch. f. Dermatologie u. Syph., **167:** 225–232, 1933.
(10) SULZBERGER, M. B., AND ROSTENBERG, A., JR.: Acquired specific supersensitivity (allergy) to simple chemicals; method of experimental sensitization; and demonstration of increased susceptibility in individuals with eczematous dermatitis of contact type, J. Immunology, **36:** 17–27, 1939.
(11) RACKEMANN, F. M., AND SIMON, F. A.: The sensitization of guinea pigs to poison ivy, Science, **79:** 344, 1934.
(12) HERRINGTON, B. L., AND STARR, M. P.: An ether-insoluble stopcock lubricant, Ind. Eng. Chem., (Analytical Edition) **14:** 62, 1942.