THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 46, No. 2

Printed in U.S.A

TECHNIC OF DERMAL PERFUSION*

R. K. WINKELMANN, M.D.

There are few ways of assessing the dermis. Organ culture, wound healing experiments, and biopsy technics have provided what few facts are available about this fibrocellular gel and its responses. A new method of study of the living dermis therefore would be of great interest, and dermal perfusion seems to be just such a method.

The dermal perfusion method was first used by Fox and Hilton (1) in their experiment on reflex vasodilation in the skin. As a consequence of their work, it was proposed that the vasodilation occurred secondary to the activity of the eccrine glands which released a vasoactive peptide, a kinin, into the dermis. Chapman, Goodell and Wolff (2) described the production of neurokinin in the axon flare zone of the skin following intradermal injection of histamine. These results have not been unchallenged and this emphasizes that a new and interesting technic usually leads to unique results which must be interpreted cautiously. Winkelmann and Wilhelmj (3) studied vasodilation induced, by a rubefacient (Trafuril) and by methacholine (Mecholyl), in the skin of man and the question of whether slow-reacting polypeptides were producing the vasodilation was raised. Dermal perfusion was selected as the method which could provide sampling of the dermis for study of kinins. Now, after 2 years of trial with different experimental approaches, I believe that dermal perfusion is a satisfactory procedure for study of selected problems of dermal physiology and biochemistry.

METHOD

Materials.—Two kinds of 20-gauge needles are used. They have four 0.013-inch diameter, equally spaced holes bored in the 1½-inch shaft beginning ¾ inch from the hub. In one type of needle, the hole is bored through both sides of the shaft and, in the second type of needle, the holes penetrate only one side (Fig. 1). The first type is used for collection of samples and the second type is used for delivery of perfusate into the dermis.

Disposable intravenous kits and 250-ml bottles of sterile isotonic saline are used. Disposable plastic tuberculin syringes are used for collection.

Technic.—The skin is prepared by gently wiping it with a gauze soaked in medicated alcohol. The site is selected so that no prominent blood vessels are in the field—usually the extensor surface of the forearm or the scapular region of the back. The delivery needle is inserted superficially into the skin and a slow saline drip is established. The collecting needle is then placed beside the delivery needle along the side which is perforated (Fig. 1). The needles are placed ¼ inch apart and taped to the skin surface (Fig. 2). A flow of clear saline is usually established in the first 5 minutes by gentle traction on the plunger of the collecting syringe. The rate of flow of saline is usually 15 to 30 drops per minute and, during a perfusion of 30 to 40 minutes, 20 to 40 ml of saline are utilized; 0.1 to 0.3-ml samples are available at 3-minute intervals and a total collection of 2 to 5 ml is usual. The bottle of saline should be kept at a constant height to maintain a standard pressure. A blood pressure cuff may be used to reduce diffusion.

Complications.—An inconstant and unpredictable flow of saline was the most disturbing characteristic of early perfusion attempts. In order to do any physiologic experimentation, it was absolutely necessary to have a constant sampling rate. The difficulty was finally interpreted as due to the pressure of the saline forcing the connective tissue and dermal structures into the holes of the collecting needle. The flow problem was solved by routinely establishing a small wheal of saline around the delivery needle before placing the collecting needle in the skin.

On rare occasions, one of the needles will damage a small cutaneous vessel and the resulting hemorrhage may necessitate discontinuing the experiment. Usually, bleeding is slight and can be washed away in a few minutes by the saline flow. One of the surprises of the technic was how seldom bleeding of any consequence occurred.

No infection or dermal change of consequence has been observed in over 300 perfusions. The needles are sterilized by autoclaving and, since sterile syringes and saline delivery tubing are used, there is little opportunity for contamination.

Early in the experiments a needle broke in the skin and was recovered surgically. With the holes ¾ inch from the hub of the needle and with care not to exert vertical pressure on the needle, no similar accident has occurred in 2 years. Occasionally, the needles will bend and must be discarded. They must be sharpened regularly.

DISCUSSION

More than 300 dermal perfusions have been done without incident and with interesting

This investigation was supported in part by Research Grant AM-5299 from the National Institutes of Health, Public Health Service.
Received for publication February 20, 1965.
* From the Section of Dermatology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

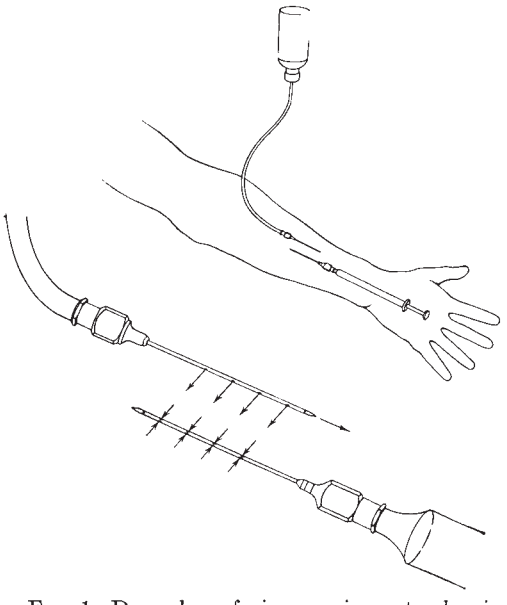

FIG. 1. Dermal perfusion equipment, showing the delivery and collecting needles.

results. The technic makes it possible to obtain samples for analysis directly from the dermis during its normal physiologic processes or during its reaction to experimental conditions. It has been possible to measure histamines and kinins in such dermal perfusates by bioassay (Fig. 3). These materials are not normally in a saline perfusate of the dermis but may be released into it by the injection of 48:80 into the dermis overlying the needles. The tracings shown in Figure 4 indicate kinin activity eluted from the skin of a patient with atopic dermatitis. Rocha e Silva and Antonio (4) demonstrated kinin but no histamine in edema fluid from rat limb heated to 46° C. Studies of this kind have been done in normal skin, in atopic skin, and in skin with urticarial dermographism, experimental (Trafuril) dermographism, and urticaria pigmentosa; the findings will be reported shortly.

The needles are not truly in the dermis in the sense of an intradermal injection but are super-

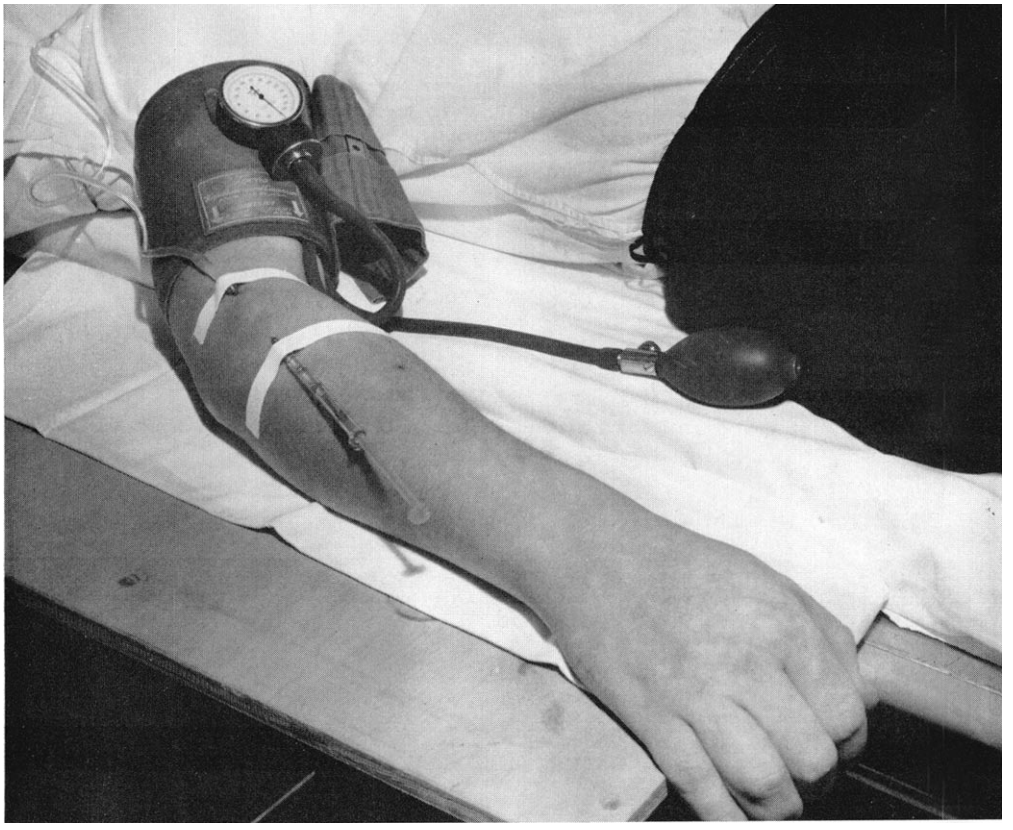

FIG. 2. Dermal perfusion of the skin of a human subject.

FIG. 3. Equipment for bioassay of histamine and kinins.

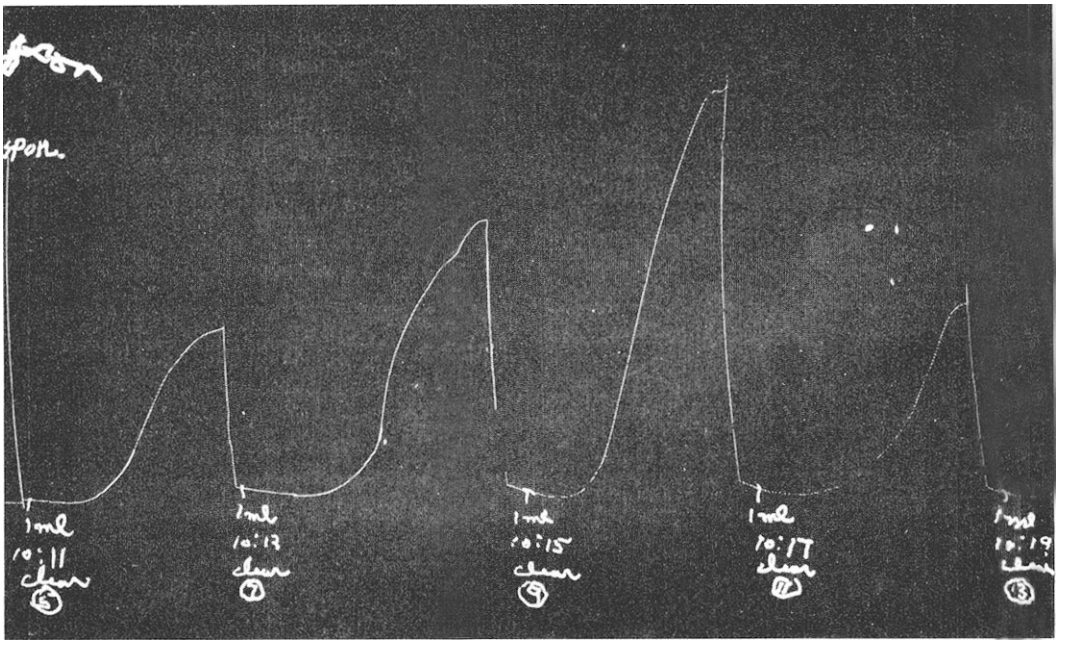

FIG. 4. Kymograph tracing from kinin bioassay (rat uterus) of 1-ml. samples of perfusate of skin of a patient with atopic dermatitis. Samples were collected at 2-minute intervals.

ficially located in the lower dermis and subcutaneous tissue. The saline pool diffuses into the dermis and samples of the saline contain some of the materials which can be extracted from living dermis. When a histamine-releasing agent is injected intradermally over these needles, the intervening dermis would be expected to release its histamine into the saline pool which could then be sampled.

The problems of dilution can be dealt with only by studying a fixed area of dermis and by using a fixed volume of fluid. The addition of known quantities of materials to the skin for assay by dermal perfusion can give a relative measure of the dilution factor. In the histamine studies, it seems that a dilution of approximately 1:100 occurs. Diffusion may be reduced by external pressure or by a tourniquet, but no technic completely solves this problem.

There is no appreciable difference between the volumes of 15 and of 30 ml of saline used for individual dermal perfusions. Only isotonic saline has been used to date. It may be that another fluid would be less disturbing to the dermal milieu. Either a smaller amount of saline or a different type of fluid might be used in future experimentation to produce minimal effect on the normal physiologic processes of the dermis.

The opportunities that such a technic provides for examining cutaneous reactions and disease are almost limitless. For example, it is possible to foresee a study of ultraviolet erythema and its products or a study of the photoreactivity of the porphyric patient and possible elution of the material which produces the photosensitivity. It would be possible to study the extracellular fluid in a number of states, to measure the extracellular glucose in patients with diabetes, to estimate the amount of bilirubin in the skin of a patient with jaundice, or to detect changes in electrolyte distribution in the skin in various conditions of acidosis and alkalosis. It might be possible to utilize a technic of this kind in a study of wound healing by the extraction of salt-soluble collagen or of water-soluble polysaccharides from the dermis. It may be possible to study the consequences of antigen-antibody interaction in the skin and determine which chemical mediators are important.

As another aspect, the technic might be used for placing therapeutic materials, such as alkylating agents or steroids, into the dermis. It appears to be a technic of considerable potential.

SUMMARY

The technic of dermal perfusion has been described; the problems of trauma, hemorrhage, and infection have been noted to be minor. Various uses for the technic have been suggested.

REFERENCES

1. Fox, R. H. and Hilton, S. M.: Bradykinin formation in human skin as a factor in heat vasodilatation. J. Physiol., *142:* 219, 1958.
2. Chapman, L. F., Goodell, Helen and Wolff, H. G.: Structures and processes involved in the sensation of itch. In Montagna, William: Advances in Biology of Skin: Cutaneous Innervation, vol. 1, pp. 161–188. New York, Pergamon Press, 1960.
3. Winkelmann, R. K. and Wilhelmj, C. M., Jr.: Variations of skin reaction to vasodilators: Metacholine (Mecholyl) and Trafuril. J. Invest. Derm., *41:* 313, 1963.
4. Rocha e Silva, M. and Antonio, A.: Release of bradykinin and the mechanism of production of a "thermic edema (45° C)" in the rat's paw. Med. Exp., *3:* 371, 1960.