THE INTRADERMAL MECHOLYL TEST FOR ANIDROSIS; A DIAGNOSTIC AID IN LEPROSY [1]

HARRY L. ARNOLD, JR. [2]
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Honolulu, T. H.

It has long been known that leprosy interferes with the sweating mechanism. Duhring (8) remarked in 1877 that "in anesthetic leprosy...the skin...is dry"; Father Joseph Damien de Veuster stated in 1889 (15) that on the early macules of leprosy which appeared on his own skin in 1876, "perspiration did not appear as elsewhere"; and many students of leprosy have made use of this fact, either for the early diagnosis of leprosy (7) or for its differentiation from syringomyelia (12). Pilocarpine has ordinarily been employed for this purpose; some workers have also used heat, or physical exertion, as a stimulant to the sweat glands.

The purpose of this paper is to review briefly the physiologic, pharmacologic, and pathologic-physiologic principles involved in testing for leprotic anidrosis, and to describe a simple and easily performed method of demonstrating it.

PHYSIOLOGY OF SWEATING

The physiology of sweat secretion is a complex matter, not as yet perfectly understood. It is known that the sweat glands are innervated by the sympathetic nervous system, by post-ganglionic fibers from the thoraco-lumbar sympathetic plexus. Unlike other structures with this innervation, however, they are not "adrenergic" (i.e., activated by adrenalin or sympathin) but are "cholinergic" (i.e., activated by acetylcholine). This activation can be accomplished either by stimulation of the nerves themselves—which causes acetylcholine to be liberated at the nerve ends—or by direct stimulation of the gland by liberation of acetylcholine around it.

This latter mode of stimulation, by direct action of the "chemical mediator" on the secretory cells, has long been supposed to be effective independently of the nerve supply. Indeed, Cannon's law states that sympathetically innervated structures

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[2] Consulting Dermatologist, Board of Hospitals and Settlement, Territory of Hawaii.

which have been deprived of their nerve supply will thereafter respond with increased vigor to their chemical mediator. Gask and Ross (10) cite Adson and Brown, and Lewis, to the effect that pilocarpine will act in this way, and will elicit sweating in an area deprived of its sympathetic nerve supply. Cushny (6) cites Burn as his authority for the statement that section of the sympathetic nerves does not prevent pilocarpine from inducing sweating in the affected area, though section of peripheral nerves does do so; and Best and Taylor (2) state that denervated sweat glands, though they do not respond to heat, do respond to pilocarpine. Fulton (9) actually states that "denervated sweat glands become sensitized to acetylcholine." These statements, in view of the observations of DeGotte (7), Jeanselme and Giraudeau (12), and of Rothman and his associates (5), seem open to question to say the least.

Myerson and his associates (16) reported in 1937 that a 1:1000 solution of mecholyl (brand of metacholine or acetyl beta-methyl choline) chloride, a relatively stable synthetic compound which pharmacologically is virtually identical with acetylcholine, would elicit sweating upon being injected intradermally into normal human skin. In cold weather it seemed less effective, so that a 1:200 dilution was sometimes required. This response was enhanced by prostigmin and abolished by either atropine or locally injected adrenalin, indicating that it was a typical cholinergic response. Coon and Rothman (3) later extended these observations by showing that on intradermal injection metacholine elicited sweating in two quite different ways: (*a*) in high concentrations (1 or more parts per 1000) it acted directly, like muscarine, on sweat gland cells, while (*b*) in low concentrations (1 part per 10,000 or more) it acted indirectly, like nicotine, on the post-ganglionic nerve fibers.

In 1941 Coon and Rothman (5) made the remarkable observation, in 5 patients who had recently undergone thoracolumbar sympathectomy, that the denervated sweat glands failed to respond to metacholine injected intradermally. This observation was extended by Kahn and Rothman (13) in a series of experiments with both metacholine and acetylcholine, in which they showed that the sweat response to the intradermal injection of either drug became diminished in from 3½ to 48 hours following pre-and post-ganglionic sympathectomy, and became consistently negative or nearly so within from 2 to 62 days after operation. They cited Gurney and Bunnell (11), who had shown that denervated sweat glands remain histologically normal, to prove

that this failure of response was not due to degeneration of the glands following sympathectomy. Kahn and Rothman postulated a diminished permeability of the sweat gland cells to the drugs as a possible explanation of the diminished response. However an ingenious attempt to demonstrate this condition by intravital staining gave inconclusive results.

Of particular interest was their observation that 4 hypertensive women, and 37 of 41 normal female controls, gave weak or negative responses to intradermally injected acetylcholine or metacholine in a concentration of 1:1000. They did not try, as Myerson and his associates had done, the 1:200 concentration. Their tests were done for the most part, on the forearm or thigh, but they believed that the distribution of sweat glands made it likely that the site of the test was of no great importance.

LEPROTIC ANIDROSIS

The importance of the foregoing material is predicated largely on the supposition that the anidrosis observed in skin lesions of leprosy is produced by denervation of the sweat glands, that condition resulting from leprous neuritis of either whole nerve trunks or peripheral twigs of post-ganglionic nerve fibers. This supposition is arrived at largely in a negative way. The only apparent alternative to it is the supposition that the glands themselves are atrophied or destroyed by the disease process, and the evidence for that is extremely scanty. Gurney and Bunnell (11) have shown that denervation alone does not result in atrophy of the glands; and although Sato (19) described both atrophy of them (in simple macules and in skin which is merely anesthetic) and infiltrative destruction of them (in elevated tuberculoid lesions), there is little or nothing in either the literature or our own experience in Hawaii to confirm his observations. Indeed, we have made a special effort to compare the appearance of the sweat glands within and outside of lesions of tuberculoid leprosy; and visible damage to the glands has only occasionally been seen in elevated tuberculoid lesions, and rarely in simple macules—including those in which anidrosis has been demonstrated prior to biopsy. It seems quite unlikely that so inconstant a phenomenon as sweat gland atrophy or destruction could be the only reason, or even the chief reason, for so constant and so early-appearing a phenomenon as leprotic anidrosis appears to be.

If, then, we may accept the postulate that denervation of sweat glands by leprous neuritis is the cause of the anidrosis,

we may further postulate that it is usually the most distal portions of the post-ganglionic nerve fibers that are the seat of the damage. It is commonplace to see circumscribed macular skin lesions in which sweating does not occur, while it is relatively less common to see an entire leg or forearm which does not sweat. Any portion of the post-ganglionic fiber may be the site of the damage, however, and the result will be the same as if the nerve lesion were situated in the affected skin itself.

The association of analgesia and thermal anesthesia with normal tactile perception is produced in syringomyelia by a lesion—in the spinal cord—which does not affect the post-ganglionic nerve fibers; and thus it should not interfere with the cutaneous sweat response to intradermally injected pilocarpine or mecholyl. The same sensory dissociation may be produced in leprosy by a lesion—in the peripheral nerve trunk or in the skin itself—which does affect the post-ganglionic nerve fibers, and should interfere with the sweat response to such injections. Work already referred to has shown this to be the case.

DeGotte's reports of the use of pilocarpine to induce sweating in normal skin showed that failure of this response was observed in leprous macules more regularly than any other sign of nerve damage. It appears likely from his studies that the earliest evidence of such damage may be this interference with the sweat response, unless perhaps hypopigmentation results from interference with the sympathetic nerve supply. It seems reasonable to suppose that the intracutaneous damage to sudomotor nerve fibers may occur at about the same time as the nerve destruction which is responsible for the positive histamine test. [Rodriguez and Plantilla (18) and Pardo-Castello and Tiant (17)]. Indeed, since Rothman and his associates showed that the sweat response to metacholine occurred, like the histamine response, as a result of an axon reflex mechanism, it is possible that the same nerve fibers are involved.

TECHNIQUE OF THE METACHOLINE SWEATING TEST

The method of performing this test (1) does not differ in any essential respect from that employed by earlier workers, except that it involves (*a*) the use of metacholine, which is a relatively stable and readily available substance, and (*b*) the demonstration of the sweat response in control areas outside the lesion by Minor's method (19).

The materials required for the test are as follows: (1) a bottle of Minor's solution (crystalline iodine 2 grams, castor oil

10 cc., absolute alcohol to make 100 cc.); (2) ordinary cotton applicators for applying it to the skin; (3) metacholine chloride in 1 per cent aqueous solution (conveniently prepared from a 25 mg. ampule of mecholyl chloride by dissolving it in 2.5 cc. of sterile saline in a rubber-capped vaccine bottle); (4) a hypodermic syringe graduated in 0.01 cc.; (5) a 26 gauge, one-half inch hypodermic needle; (6) dry gauze for blotting off the drop of solution that back-leaks from the injection-site; (7) powdered starch (ordinary cornstarch seems to be quite as satisfactory as the rice starch powder recommended by Minor); and (8) a powder-blower type of atomizer for application of the starch.

The lesion or area to be tested, plus a roughly equal area of adjacent normal skin, is first painted with Minor's solution; this will dry rapidly, but it is not necessary to wait for it to do so. Approximately 0.05 to 0.1 cc. of the metacholine chloride solution is then injected intradermally at the border of the lesion, so that the elevated wheal will be partly inside and partly outside the involved area. In larger lesions two additional injections may be made, one entirely within, the other entirely without, the lesion. These merely make the demonstration more dramatic and in general add little to the ease of interpreting the test. The droplet of solution that leaks back from the injection-site should be gently blotted (not wiped) off, and the whole area quickly and lightly dusted with powdered starch, blown from the atomizer.

Within a few seconds, in some patients, gooseflesh (cutis anserinus) will appear around the injection site in an area 3 to 5 cm. in diameter; it disappears within a minute or two. This nicotine-like pilomotor effect, described by Coon and Rothman (4), seems to be abolished along with sweat reaction; but it is not a very useful test because it is such a fleeting reaction and because it is not elicited with certainty even with higher dilutions of the drug. It has perhaps some value as providing collateral evidence that the loss of the sweat response is due to nerve damage rather than sweat gland destruction, at least in areas not actually scarred.

Within a few seconds, also, sweat droplets will begin to appear at the mouths of those sweat glands which are still functionally intact. They moisten the dry white-over-tan iodine-starch combination, which immediately turns deep blue-black and remains so. This clearly visible sweat secretion spreads rapidly over an area concentric with the intradermal wheal, to

a radial distance of about 1 to 3 cm. The response reaches its maximum within two or three minutes.

If more than three injections of 0.1 cc. each are made, the patient may experience transient systemic discomfort from the absorption of metacholine. If the dose does not exceed, say, double that amount, the reaction is almost always limited to generalized sweating, flushing of the face, some salivation, and slight malaise. Urination and defecation may be stimulated by larger doses, or in the rarely-encountered hypersensitive individual. Atropine, in the usual therapeutic doses given by hypodermic injection, is the precise pharmacologic antidote.

RESULTS OF THE METACHOLINE SWEATING TEST

Responses to the test may be recorded as negative, doubtful, or positive. The division of a doubtful result into "doubtful" and "slight" and of a positive result into 2-plus, 3-plus and 4-plus, as suggested by Kahn and Rothman (13) is unnecessarily precise so far as our present purposes are concerned; as the German proverb has it, *"sogenau schüssen die Preussen doch nicht."* The response is negative when no sweat droplets can be visualized within the area tested; it is read as doubtful when no more than 6 or 8 pinpoint-sized droplets appear; otherwise it is read as positive. Experience suggests that a doubtful response is exceptional in the sort of cases with which we are concerned; and in any event, just as with testing for sensory changes, we are interested not so much in the absolute response as in the difference between the response in the involved area and the response outside.

A preliminary report (1) gave the results obtained in 12 consecutively-tested cases of tuberculoid and presumably tuberculoid leprosy, and in 6 presumably nonleprous controls with skin lesions. The sweat response inside the lesion was recorded as negative in 1 case and doubtful ($\pm$ or $+$) in 11 cases; in the adjacent normal skin it was doubtful in 1 case, in which no skin lesion was present and hence no margin to define the correct site for testing, and it was 2-plus in 1, 3-plus in 3, and 4-plus in 7 cases. In 3 cases of vitiligo and 1 of tinea circinata 4-plus responses occurred in both the lesions and in the control sites. On the other hand, 2 cases of so-called achromia parasitica (also called impetigo sicca and erythema streptogenes) showed failure of the response within the oval, hypopigmented facial macules, and positive responses outside them.

Further experience has tended to confirm the tentative con-

TABLE 1.—*Sweat response to acetylcholine and metacholine in 1:100 and 1:20,000 dilutions in leprous lesions and control areas, and associated sensory changes.*

Case	Sex	Type *	1:100		1:20,000		Anesthesia	
			Lesion	Control	Lesion	Control	Thermal	Tactile
ACETYLCHOLINE								
SA	F	NaNt	—	+	±	+	Yes	Yes
WK	F	Na	—	+	+	+	Yes	Yes
GM	F	NaNt	±	+	—	+	Yes	Yes
HH	M	NaNt	±	+	±	+	?	No
RT	M	NaNs	—	+	—	+	Yes	Yes
JH	M	NaNt	—	+	—	+	No	No
AM	F	Na	—	+	—	+	Yes	Yes
METACHOLINE								
AP	M	Na3Nt3	—	+	±	+	Yes	Yes
MK	F	Na2Nt1	—	+	±	+	S1t	No
AJ	M	Na1Nt3	—	+	±	+	S1t	No
EC	M	Na1Nt1 †	±	+	±	+	Yes	Yes
JM	M	Na1Nt1 †	±	±	....	....	Yes	Yes
FM	F	Na1Nt1 †	±	+	±	+	Yes	Yes
KT	F	Na2Nt2	—	+	—	+	Yes	Yes
RK	F	Na2	+	+	+	+	Yes	Yes
SS	M	Na1Nt3	±	+	±	+	Yes	Yes
WK	M	Na3	±	+	±	+	Yes	Yes
BC	F	Na1	—	±	—	+	Yes	Yes
KT	M	Na2Nt2	—	+	—	+	Yes	Yes
RK	F	Na2 Nt §	+ —	+	+ —	+	Yes	Yes
S.S.	M	NtsNa1	±	+	±	+	Yes	Yes
WK	M	Na2 Nt §	+ —	+	+ —	+	Yes	Yes
BC	F	Na1Nt1 †	—	+	—	+	Yes	Yes
FM	M	Na2Nt1	—	+	±	+	No	No

*The type symbols employed are those adopted by the Cairo Congress. All cases were of the tuberculoid type or probably tuberculoid.

†Healed.

§Healed?

clusions reached in the preliminary report. From Table 1 it will be seen that the results obtained with acetylcholine parallel closely those with metacholine, and that there is no great difference between the 1:100 dilution (which acts, like nicotine, on the nerve fibers) and the 1:20,000 dilution (which acts, like muscarine, on the sweat-gland cells themselves). No evidence is here offered that the 1:100 dilution is preferable, but it is recommended for routine testing because of the observation of Myerson and associates (16) that the stronger solution is occasionally required to elicit a response in normal skin in cool weather, and the finding of Rothman and Coon (4) that women tend to react less vigorously than men.

Table 1 includes 5 cases in which the sweating test indicated nerve damage despite the absence of sensory changes (in 2 cases) and the presence of only doubtful thermal anesthesia (in 3 cases). In 2 cases (RK and WK) the sweat response was positive just inside an anesthetic area on an extremity, but negative at a point lower down, which suggests that in cases of nerve-trunk damage the loss of sweat response occurs over a less wide area than does the anesthesia. All of the cases in which macular or tuberculoid annular skin lesions were tested, however, showed that the area of anidrosis corresponded exactly to the area of visible involvement or demonstrable anesthesia, or both.

It is noteworthy that the results of this test may be observed and recorded with assurance in every case. This is in sharp contrast to the histamine test in our experience; in dark-skinned patients the results of that test are so often equivocal that it is almost entirely useless. Moreover, the result can be photographed with ease for a permanent record.

Table 2 indicates the results of the test in a few presumably nonleprous controls. It suggests that a negative response is not to be expected in at least four or five of the commoner conditions for which tuberculoid leprosy is apt to be mistaken. More important, it indicates that the sweat response is liable to be absent in the oval, hypopigmented, facial macules of so-called achromia parasitica (which might better be called "hypochromia aparasitica"). These observations have recently been extended to cover some 15 or 20 cases, 4 of which have been subjected to biopsy. Histologically they show only scanty banal perivascular round-cell infiltration, with nothing to suggest leprosy. The great majority of them appear to heal within a few weeks under treatment with either ammoniated mercury ointment or White's

TABLE 2.—*Sweat response to metacholine in 1:100 and 1:20,000 dilutions in a variety of presumably nonleprous conditions.*

Case	Sex	Diagnosis	1:100		1:20,000	
			Lesion	Control	Lesion	Control
CL	M	Tinea circinata	+	+	....	....
HK	M	Tinea circinata	+	+	+	+
LM	F	Pityriasis rosea	+	+	+	+
NH	F	Vitiligo	+	+	+	+
LH	F	Scar (burn)	—	+	....	....
HA	M	Meralgia paresthetica	+	+	+	+
WL	F	Nevus anemicus	+	+	+	+
TN	M	2d cervical ganglionectomy 6 months previously	±	+	±	±
TO	M	Traumatic transverse myelitis 4 days before	±	+	—	+
		Same a week later	+	+	±	±
JS	M	Cordotomy for pain	+	+	±	+
JK	M	Achromia parasitica	—	+	—	+
JG	F	Achromia parasitica	—	+	—	±
MT	F	Achromia parasitica	—	+	—	+
JH	M	Achromia parasitica	—	±	—	±
MK	F	Normal volunteer	....	+	....	+
GS	F	Normal volunteer	....	+	....	+

crude coal tar paste. No case so diagnosed has as yet developed leprosy, though the possibility that these lesions may represent a *forme fruste* of leprosy has been considered; indeed, both their appearance and their racial distribution, at least in Hawaii, correspond exactly to the latter condition. Possibly the sweating test should not be relied upon in any facial lesion.

SUMMARY

Sweat glands, though of sympathetic innervation, have acetylcholine as their chemical mediator, and normally they respond

directly to that substance upon its injection. Metacholine (acetyl beta-methyl choline) or its chloride has the same effect on sweat glands as acetylcholine or its chloride.

Removal or destruction of the post-ganglionic sympathetic nerves, resulting in denervation of the sweat glands, prevents them from responding to acetylcholine or metacholine. The reason for this is not known.

It seems probable that one of the earliest consequences of tuberculoid leprosy in the skin is the denervation of sweat glands, and that this is an eventual consequence of leprosy of either type involving nerve trunks. The intradermal injection of metacholine chloride, therefore, frequently gives evidence of peripheral sympathetic nerve damage by showing loss of the normal sweat response.

This manifestation may be interpreted as indicative of leprosy in cases in which no equally cogent evidence to the contrary can be adduced, and may further be interpreted as indicative of nerve-damaging (i.e. tuberculoid) leprosy when it shows anidrosis which is coextensive with the visible skin lesion.

Failure or marked diminution of the sweat response is frequently observed in facial lesions of so-called achromia parasitica, and occasionally in the normal skin of the face as well.

The greatest advantage of the sweating test over the histamine test is that its results may be read with ease even in dark-skinned patients.

CONCLUSIONS

The intradermal injection of metacholine chloride solution and evaluation of the resulting sweat response constitutes a useful method of demonstrating the presence and the extent of peripheral sympathetic nerve damage in the skin or nerve trunks.

It is suggested that a normal sweat response is rarely met with even in very early lesions of tuberculoid or indeterminate macular leprosy. The abnormal response probably precedes the onset of demonstrable sensory changes.

The test is offered primarily as a reasonably simple and easily interpreted substitute for the histamine test, for use as an adjunct to the diagnosis and classification of skin lesions or neurological changes which suggest, or which are actually due to, leprosy.

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DESCRIPTION OF PLATE

PLATE 9.

Fig. 1. Materials assembled for test. The first step is to paint the lesion (or area) to be tested, and the adjacent normal skin, with Minor's iodine solution. As soon as this has been done, approximately 0.1 cc. of 1 per cent solution of metacholine chloride (Mecholyl) is injected intradermally in each area. (If the lesion is small, a single injection may be made at the junction of the lesion with normal skin.) The entire area is then dusted with powdered starch from a powder-blower.

Fig. 2. Thirty seconds after the injections were completed, numerous blue-black dots indicate the secretion of sweat in the vicinity of the injection in the normal skin. The wheal inside the tuberculoid annule shows only the black mark made by the back-leaking of the injected solution through the needle puncture wound.

Fig. 3. Three minutes later, the wheal inside the lesion shows only one or two black specks indicative of sweat secretion. The normal control injection outside the lesion is profusely stippled with black specks. This is a "positive" sweating test, and may be found in many lesions which show no anesthesia of any sort, even thermal.

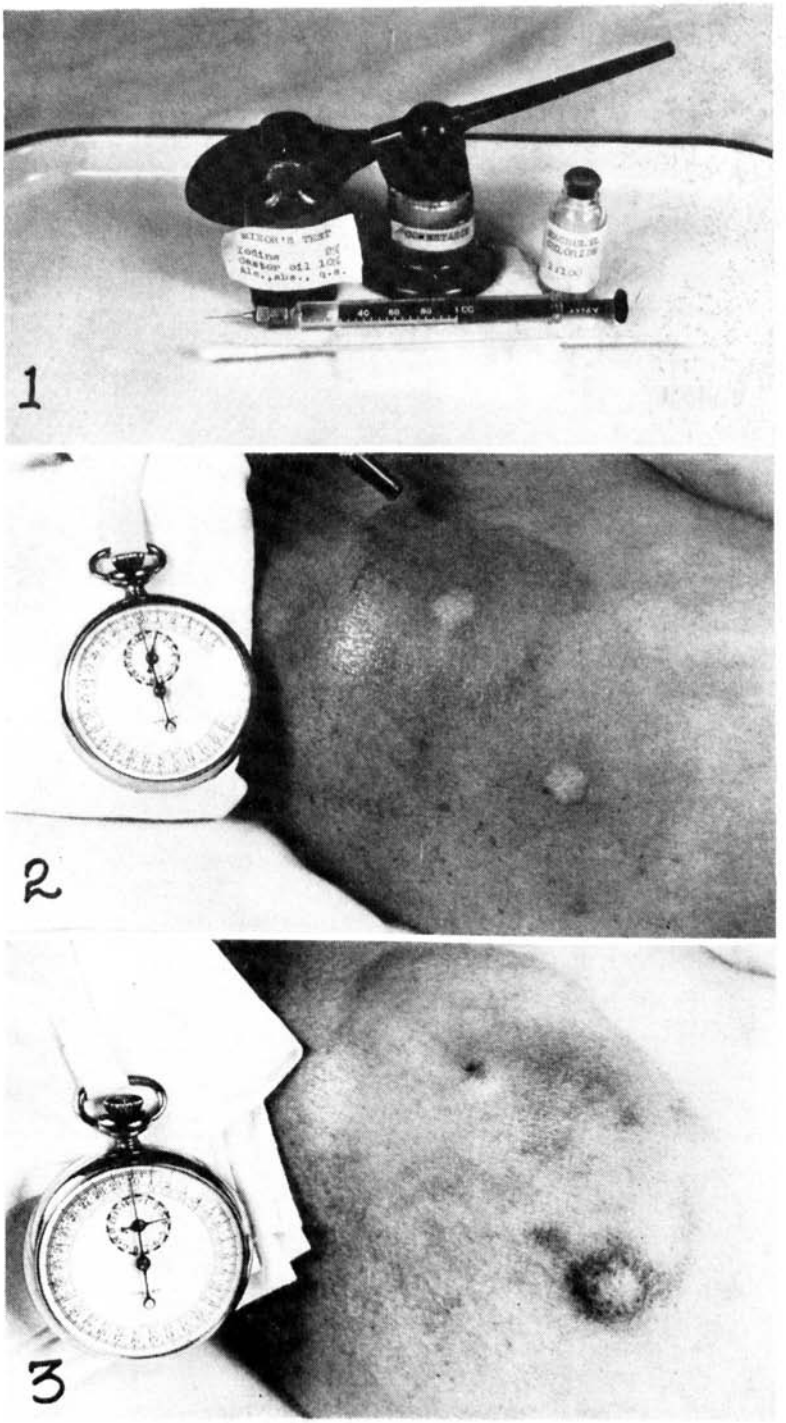

PLATE 9