were used to identify the cells which were preparing for division and colloidal saccharated iron oxide was used to identify the active phagocytic cells. In livers of mice whose reticulo-endothelial system was stimulated by estradiol, it was established that the cells preparing for division and those which had recently divided were actively phagocytic. In livers of mice whose reticulo-endothelial system had been "blockaded" with saccharated iron oxide, it was established that the cells which had phagocytized colloid were able to divide in the process of recovery from "blockade." No evidence was found for a stem cell which proliferates and differentiates to provide the active phagocytic population.

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Origin of the Galvanic Skin Response.* (27579)

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If a metal plate electrode is placed on the skin surface of the human palm, or on the foot or toe pads of the cat, and the body of the experimental subject grounded by means of another metal plate electrode at some point on the body at a distance from the first electrode, a change in electrical potential will occur between the 2 electrodes in response to a stimulus transmitted to the skin by the sympathetic nervous system. There is a simultaneous decrease in the skin's resistance to the passage of an electric current. When the potential change is elicited by any painful, startling, or threatening event in the environment of the experimental subject, it is referred to as the galvanic skin *reflex*. When the stimulus is applied peripherally as when a peripheral sudomotor nerve, or the sympathetic trunk to the hind extremity is stimulated, the potential change is called the galvanic skin *response*. The wave forms of the galvanic skin reflex and the galvanic skin response, when recorded from metal plate electrodes (hereafter referred to as "macroelectrodes") covering a portion of the skin's surface, are identical. Their latent periods differ as would be expected from the difference in the path length and conduction time of the nerve fibers carrying the stimulus to the skin(1).

Various theories of origin of the galvanic skin reflex and the galvanic skin response have been advanced at one time or another since their discovery by Fere(2) and Tarchanoff(3), respectively, over 70 years ago. Veraguth(4), by implanting the grounded, or indifferent, electrode beneath the skin surface, demonstrated that the potential change originated within the skin, rather than from muscle and other underlying tissues. The controversy primarily centers around the question of whether this phenomenon is caused by sweating, the widely accepted view. These theories and the experimental evidence supporting them have been reviewed by Wang (1).

Richter(5) pointed out that the skin po-

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tential tracings obtained from the palm of the human subject contain 2 components: a fast component, negative in direction, and a slow component, positive in direction. He recorded the slow positive component from the skin of a patient with congenital absence of the sweat glands. The fast negative component was absent in this patient. He concluded that the fast negative component was related to sweating, but that the slow positive component was not, and had its origin in capillary or epithelial cells of the skin.

Lloyd(6), studying the galvanic skin response of the skin of the cat's paw pad, recently discovered that Richter's observation (5) of 2 components was valid in that species also. However, Lloyd interpreted his data to indicate that the slow component was a sweat gland "secretory potential" that was related to reabsorption, and to the amount of moisture in the sweat gland ducts.

The present study was undertaken to determine which of these conflicting interpretations is valid. Cats were employed as the experimental subjects. The use of micropipette electrodes permitted direct measurement of the electrical potential from the lumen of individual sweat glands and from the epidermal and dermal tissues surrounding them. Simultaneously, the galvanic skin response was recorded from a macroelectrode covering the surface of a paw pad of the same extremity.

Methods. In our experiments, the galvanic skin response was studied by measuring the electrical potential arising in the skin of the paw pad of anesthetized mongrel cats in response to electrical stimulation of the lumbar sympathetic trunk supplying the homolateral hind extremity. A nerve preparation similar to that of Dale and Feldberg(7) was used. The apparatus and technics employed are similar to those used in the microelectrode impalement of single nerve fibers. Glass capillary tubes were drawn out into micropipettes with a pipette puller similar to the one described by Alexander and Nastuk(8). The electrodes were filled with methanol by boiling under vacuum, and subsequently with 3-Normal potassium chloride by diffusion replacement of the methanol. Electrical contact with the electrolyte solution within the pipette was provided for by inclusion of a silver-silver chloride electrode as an integral part of the electrode holder.

Indifferent, or ground return, electrodes of several kinds, placed in a variety of locations were used with uniform results. Electrode materials included platinum, steel, stainless steel, and zinc. Locations included the abdominal cavity of the laparotomized animal, skin of the thigh, muscle of the thigh, the tongue, and the external ear. The recorded responses were not affected by the nature or location of the indifferent electrode, unless the latter was placed near the stimulating electrode within the abdominal cavity. In the latter case, as would be expected, considerable stimulus artifact was introduced into the tracings.

The signal from the recording micropipette electrode was fed into a coupling amplifier employing a single-ended CK-5889 electrometer tube. The measured electrometer tube grid current was 10^{-14} amperes. The signal was further amplified by Offner transistorized voltage and power amplifiers.

The electrical resistance of the micropipette electrodes used in these experiments ranged from 20 to 60 megohms. This resistance was continuously monitored by means of the pulse injection technic, and did not significantly change during the experiments reported here. The usual precautions in selecting micropipette electrodes with low tip potentials were observed. Negative feedback was used to cancel out input capacitance.

The microelectrode implantations were made under direct vision with the aid of a dissecting microscope and a Zeiss micromanipulator. To obtain the galvanic skin response in the same animal, a small stainless steel cup electrode, 1.5 cm in diameter, covered another toe pad of the same paw. A layer of electrolyte-containing paste was interposed between the toe pad and the cup electrode. The latter electrode was connected to an independent direct-current differential amplifier.

Because of its ready availability and convenience, the electrolyte paste used in most of these experiments was that made by the Sanborn Co. to be used between the skin and an electrocardiograph electrode. To exclude

polarization phenomena at the skin and indifferent electrodes, the results obtained when using the Sanborn paste and a stainless steel macroelectrode were compared with results obtained when using a zinc macroelectrode covered with a paste composed of kaolin and a saturated aqueous solution of zinc sulphate. The zinc-zinc sulphate macroelectrode was used in Richter's experiments(5,10), and is a combination in which polarization over the voltage range in these experiments is negligible. Under the conditions of the experiments reported here, the results obtained with the 2 electrode and paste combinations were identical.

The output signals from both the amplifier connected to the microelectrode, and the amplifier connected to the macroelectrode covering the toe pad, were fed into a 2-channel Offner recording galvanometer by means of which the 2 signals could be recorded simultaneously.

A Grass stimulator supplied the stimulating pulses to a platinum boot electrode placed at laparotomy around the lumbar sympathetic trunk supplying the hind extremity of the animal. Squarewave pulses, varying from threshold, usually 2 to 3 volts, to 7 to 10 volts were employed. Pulse duration was 1 to 5 microseconds.

Results. Single pulse stimulation. In tracings recorded simultaneously from the macroelectrode covering a toe pad and from a microelectrode with the duct of a single sweat gland of the adjacent toe pad, the initial fast negative (downward) component of the two curves ran an almost identical course. The exponential decay of the fast component in returning toward the base line proceeded more rapidly in the case of the recording made from the macroelectrode (galvanic skin response) and became positive with respect to the resting potential, whereas the tracing from the lumen of a single sweat gland did not (Fig. 1). The electrical response from over a thousand individual sweat glands in 20 cats always returned to the base line after a decay period of from 4 to 6 seconds following the initial negative deflection. In no instance did the tracing from a microelectrode in the lumen of a single sweat gland (Fig. 1A) show this slow positive component, but the simultaneously recorded galvanic skin response (Fig. 1B) frequently did. All of the microelectrode responses obtained from individual sweat glands were identical in waveform to that shown in Fig. 1A. The latent period varied from 0.6 to 0.8 second, the mean and median values being 0.7 second. The magnitude of the peak negative response varied with stimulus intensity, the more electronegative responses being obtained with the higher voltage stimuli. The positive component of the galvanic skin response (Fig. 1B) was not always present in the tracings obtained from the macroelectrode. It appeared most consistently in response to higher voltage stimuli. Its presence or absence appeared to be related to the intensity of the stimulus, discussed below.

Repetitive squarewave stimulation. In the experiments performed with repetitive stimulation, a stimulating frequency of 10 per second was employed. In response to repetitive stimulation (Fig. 2), the microelectrode within a sweat gland duct lumen held a uniform negativity for the first half minute and thereafter began to decay toward the base line, and finally rose to lumen positivity by the end of the third minute of repetitive stimulation, whereas the galvanic skin response recorded from the macroelectrode covering a toe pad became positive early in the first minute of stimulation after an initial negative deflection.

Effect of stimulus voltage on fast and slow components of galvanic skin response. The voltage threshold necessary to elicit the slow positive component was higher than that required to elicit the fast negative component. In one experiment, the latter was obtained from the macroelectrode with a squarewave pulse of 2 to 3 volts in a fresh preparation. Increasing the stimulus to 4 volts, the fast negative and the slow positive components were obtained in the same preparation. The voltage threshold for the slow positive component was more variable than that required to elicit the fast negative response and became lower with the application of frequent stimuli. If the preparation was rested, this effect was reversed. The same threshold effect was

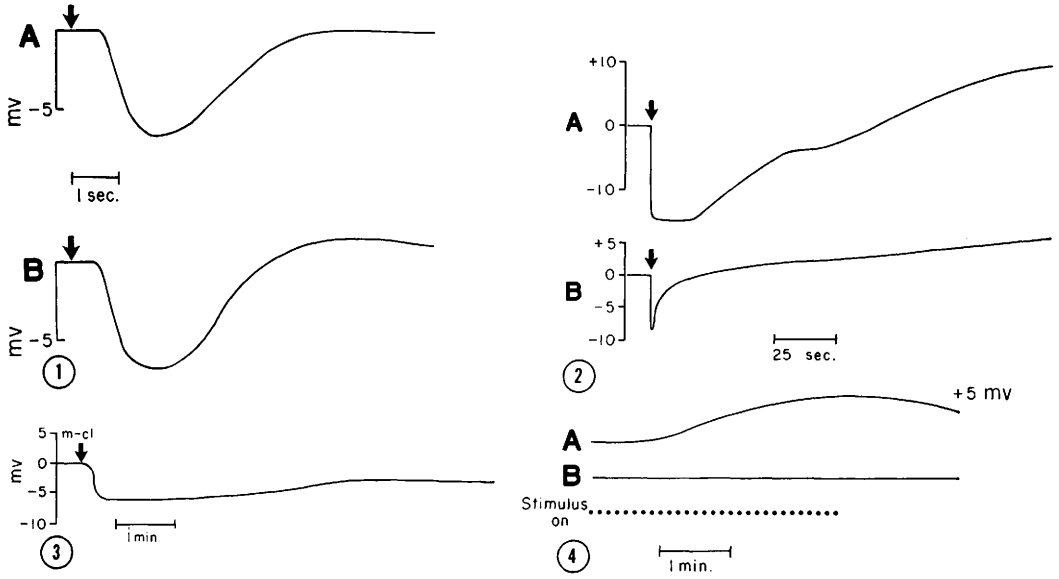

FIG. 1. (A) Microelectrode response from a single sweat gland. (B) Galvanic skin response from a macroelectrode covering toe pad of same extremity, recorded simultaneously with (A). A single monophasic squarewave pulse stimulus was applied at the arrow. Negative deflection is downward in this and succeeding figures.

FIG. 2. (A) Microelectrode response from lumen of a single sweat gland. (B) Galvanic skin response from a macroelectrode covering toe pad of same extremity. Repetitive stimulation, 10 per sec., begins at arrow and continues to end of tracing. (A) and (B) recorded simultaneously.

FIG. 3. Microelectrode response from lumen of a single sweat gland to intra-arterial inj. of methacholine chloride.

FIG. 4. (A) Microelectrode response from epidermal cells of skin of cat's paw pad obtained during the life of the animal. A similar tracing could be obtained for nearly one hr post-mortem, long after sweating had ceased. (B) Microelectrode response from dermis of skin of living animal. Repetitive stimulation of 10 per sec. was used in obtaining both tracings.

noted in the experiments in which repetitive stimulation was used. Using low voltage stimuli in a fresh, or rested, preparation, the tracing from the macroelectrode became negative and remained so for several minutes, as reported by Richter and Wheelan(10). At a slightly higher stimulus voltage, often a differential of as little as 1 volt, the positive component appeared (*cf.* Fig. 2A, and Lloyd (6)). Stimulus pulse duration over 1 to 25 microseconds had no appreciable effect on the responses obtained.

Pharmacologic stimulation. The microelectrode tracing from a sweat gland of a cat's toe pad in response to the intra-arterial injection of methacholine chloride (*via* the abdominal aorta) is shown in Fig. 3, which is included to show the similarity of the response obtained by repetitive stimulation to that produced by a parasympathomimetic drug (compare Fig. 2 and 3), and to indicate that electrical responses from the lumen of the individual sweat glands and from the skin surface are not artifacts related to the electric shock stimulus.

Localization of the slow component of galvanic skin response. The superficial cornified layer of the epidermis was dissected away so that the papillary layer of the epithelium was exposed. A microelectrode was touched to the surface of the exposed epithelium, but not in proximity to a sweat gland duct orifice, and a single pulse was applied. No negative deflection was obtained with a stimulus which readily elicited the fast negative component when the microelectrode was subsequently inserted into the lumen of a sweat gland in the same prepared area. With the microelectrode in contact with the epidermal layer of the skin but not near a sweat gland, repetitive stimulation identical to that used in Fig. 2 was applied. There was observed a slow posi-

tive component of the galvanic skin response from the macroelectrode. If the animal was killed, visible sweating soon ceased, and the fast component of the galvanic skin response could no longer be detected. However, the slow component could be obtained from the epithelium with the microelectrode for nearly an hour post-mortem (Fig. 4A), long after the sweat glands had ceased to function.

To exclude the possibility that the slow positive component originates from the skin structures below the epidermis, a strip of epidermis was dissected away leaving the dermis exposed. Sweat glands in this area poured forth sweat when stimulated repetitively, but the positive component was not recorded when the microelectrode was in contact with the exposed dermis (Fig. 4 B).

Discussion. It is unlikely that curves such as Fig. 2A would be seen under physiological conditions. If a flat electrode is placed on the skin surface of the palm, and another on the back of the hand of a human subject (the same obtains for the paw of the cat), spontaneously arising negative waves will be recorded which are identical to the microelectrode responses from the lumen of a single sweat gland of the cat. These responses occur in random fashion in the unstimulated subject and by their identity with the microelectrode responses (Fig. 1A), are presumably the electrical potentials generated by the individual sweat glands or groups of sweat glands covered by the skin electrode. In a resting patient with extreme hyperhydrosis of the palms of the hands from whom such tracings were taken, the sweat gland potential changes recorded from a skin electrode with an area of 6 sq cm occurred as often as 100 per minute. These responses often were superimposed on each other because of their frequency, but in no case was fusion observed such as occurs with strong repetitive stimulation of the lumbar sympathetic trunk or a peripheral sudomotor nerve(6,10). On tracings obtained from the resting, unstimulated cat or human subject, only the fast negative component was seen. It appears that under the enormous stress upon the sweat glands when repetitive stimulation is used (Fig. 2A), the sweat glands become fatigued and are unable to maintain their lumen negativity. That the site of this fatigue is the sweat glands and not the sudomotor nerve, is supported by the experiments in which pharmacologic stimulation was used (Fig. 4) (See Thaysen and Schwartz(9)).

We can account for the data presented in Fig. 1 and 2 if, as Richter(5) contended, the positive component is not related to the sweat glands, but originates in other skin structures. Our data substantiate Richter's view and are consistent with the following mechanism. With single shock stimulus, the fast negative component originates in the sweat glands. With repetitive stimulation, the fast components fuse to become a sustained negative potential as has been shown by Richter and Wheelan(10) and by Lloyd(6). This would also appear to be the case with a pharmacologic stimulus of long duration of action as methacholine chloride (Fig. 3). In response to an enormous stress such as repetitive stimulation or with intra-arterial methacholine chloride, the sweat gland begins to fatigue (after approximately 600 shocks in Fig. 2) and is unable to maintain its lumen negativity. The skin surface has simultaneously become positive as the epidermal cells have become positive (Fig. 4A). When the sweat gland is no longer able to maintain its lumen negativity (after approximately 1400 shocks in Fig. 2A), the microelectrode within the sweat gland duct lumen records the positive potential of the surrounding epidermal cells.

We agree with Lloyd(6) that the slow positive component is obtained in response to a single pulse stimulus only after a period of rest following repetitive stimulation. The slow positive component in response to repetitive stimulation of a given intensity reaches a maximum or ceiling for that stimulus strength and frequency. Since the positive component runs an exceedingly long time course(6), subsequent stimulation, either single shock or repetitive, would not elicit the slow positive component of the galvanic skin response until a sufficient period of time had elapsed for the decay of the slow positive component. During this period of rest, reabsorption of water may be taking place in the sweat gland ducts as Lloyd(6) has suggested.

However, this hypothesis does not appear to be supported by Lloyd's data, since, as we have shown here, the slow positive component is unrelated to sweat gland activity. If one must assign the term "secretory potential" to either of the 2 components of the galvanic skin response, it should be applied only to the initial fast negative component since the slow positive component originates in the cells of the epidermis. The physiological significance of these potentials recorded from within the lumen of single sweat gland ducts will be reported later.

In addition to the Emf generated by sweat glands and epidermal cells described above, another component of the galvanic skin response is the simultaneous fall in skin resistance. Although resistance measurements were not made during these experiments, this report would not be complete without relating skin resistance to the present work.

According to Lloyd(6), the slow phase of the galvanic skin response and the impedance (alternating current resistance) change in response to repetitive stimulation "show an identical course and are, therefore, considered as signs of the same fundamental process at work." Considering the data presented above, it appears that the skin resistance changes recorded by Lloyd originate in the epidermal cells and not in the sweat glands. Further evidence to support this view is given by Edelberg(11) who employed a microsurgical technic to isolate physically and electrically a slab of epidermis from surrounding sweat ducts. By measurement of resistance on the surface of such a preparation with microelectrodes, he demonstrated that the epidermis contributes significantly to the resistance changes of the skin in the galvanic skin response. Therefore, interpretations relating to the physiology of sweating which have been based on the slow phase of the galvanic skin response(6) and on skin impedance changes (12,13,14) should be reevaluated in the light of our report.

Summary. The galvanic skin response from a macroelectrode covering the toe pad of the cat was compared with simultaneously recorded potentials arising from individual sweat glands and cells of the surrounding epidermis and dermis of the toe pad skin of the same animal. By direct measurements employing microelectrode technics, the fast negative component of the galvanic skin response was shown to originate in the sweat glands, and to be related to sweat gland activity. The slow positive component of the galvanic skin response was shown to originate in the cells of the epidermal layer of the skin and is therefore unrelated to sweating.

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