TABLE IX—RECOVERY OF VITAMIN $D_2$ FROM THE THIN LAYER

| | Day of Expt. 1 | | | 2 | | |
|---|---|---|---|---|---|---|
| Repetition → | 1 | 2 | 3 | 1 | 2 | 3 |
| No. 1[a] | | 0.2495 | | | 0.280 | |
| $V_B$ - - - $A_B$ No. 2[a] | | 0.320 | | | 0.350 | |
| No. 3[a] | | 0.432 | | | 0.462 | |
| $V_A$ - - - $A_A$ | 0.356 | 0.210 | 0.3275 | 0.365 | 0.327 | 0.420 |
| $V$ (ml.) | 0.8875 | 1.3438 | 0.9766 | 0.9571 | 1.0657 | 0.8000 |
| $V_C$ - - - $A_C$ | 0.280 | 0.425 | 0.310 | 0.332 | 0.378 | 0.281 |
| Recovery, % | 98.8 | 99.0 | 99.4 | 99.1 | 101.3 | 100.4 |
| Av. % | | 99.07 | | | 100.27 | |
| Range | | 0.6 | | | 2.2 | |

[a] Vitamin $D_2$ concentration of developed color solutions No. 1, 2, and 3 was 2, 2.5, and 3.3 mcg./ml., respectively. Absorbance data were represented as the value of $(A_{600} - A_{560})$.

TABLE X—RECOVERIES OF VITAMIN $D_2$ FROM A-$D_2$ EMULSION

| | Day of Expt. 1 | | | 2 | | |
|---|---|---|---|---|---|---|
| Repetition→ | 1 | 2 | 3 | 1 | 2 | 3 |
| Std. No. 1[a] | | 0.187 | | | 0.183 | |
| $A_S$ No. 2[a] | | 0.321 | | | 0.242 | |
| No. 3[a] | | 0.461 | | | 0.326 | |
| $A_C$ | 0.234 | 0.362 | 0.362 | 0.1745 | 0.236 | 0.333 |
| $V_A$ - - - $A_A$ | 0.332 | 0.2735 | 0.325 | 0.319 | 0.3125 | 0.292 |
| $V_B$ - - - $A_B$ | 0.318 | 0.312 | 0.3055 | 0.3115 | 0.3145 | 0.307 |
| $V$ (ml.) | 0.9560 | 1.1234 | 0.9362 | 0.9759 | 1.0064 | 1.0489 |
| Recovery, % | 101.7 | 100.4 | 96.4 | 97.5 | 96.9 | 97.5 |
| Av. | | 99.50 | | | 97.30 | |
| Range | | 5.3 | | | 0.6 | |

[a] In the first day of experiment, vitamin $D_2$ concentration of developed color solution No. 1, 2, and 3 was 1.5, 2.5, and 3.5 mcg./ml., respectively, and in the second day, that of No. 1, 2, and 3 was 1.5, 2.0, and 2.5 mcg./ml., respectively.

## REFERENCES

(1) Barua, R. K., and Rao, M. V. K., *Analyst*, **89**, 534 (1964).
(2) *Ibid.*, **90**, 571(1965).
(3) Bolliger, H. R., and König, A., *Z. Anal. Chem.*, **214**, 1(1965).
(4) Hirayama, K., *J. Chromatog.*, to be published.
(5) Ball, B., Goodwin, T. W., and Morton, R. A., *Biochem. J.*, **42**, 516(1948).
(6) Henbest, H. B., Jones, E. R. H., and Owen, T. C., *J. Chem. Soc.*, **1957**, 4909.
(7) Peifer, J. J., *Mikrochim. Acta*, **1962/3**, 529.

# Method for the Evaluation of Antihidrotic Substances in the Anesthetized Cat

By R. S. ALPHIN, DALE SAUNDERS, and JOHN W. WARD

**An improved method using newly designed equipment is described for the evaluation of antihidrotic substances in the anesthetized cat. The substances to be tested were applied locally on the foot pads. The effect on the sweating induced by the administration of a pharmacological agent such as pilocarpine was then observed by recording changes in the moisture content of air passed at a constant flow over the foot pads. The method as described gave consistent and reproducible results with a substance known to produce antihidrotic effects.**

FROM A SEARCH of the literature, there appear to be few methods available for investigating the pharmacological activity of substances on sweating in laboratory animals. Kuno (1) was among the first to use the moisture produced by the sweat glands as a measurement of sweating in man. The method had limitations since changes in sweat secretion could only be observed for a period of 5 min. Adams *et al.* (2) have

Received November 4, 1966, from the Department of Pharmacology, A. H. Robins Research Laboratories, Richmond, VA 23220
Accepted for publication January 9, 1967.
The authors thank Mr. Robert Eagles, Mr. Joseph P. Green, and Mr. Ronald Southward for assistance in the construction of the apparatus used in these experiments.

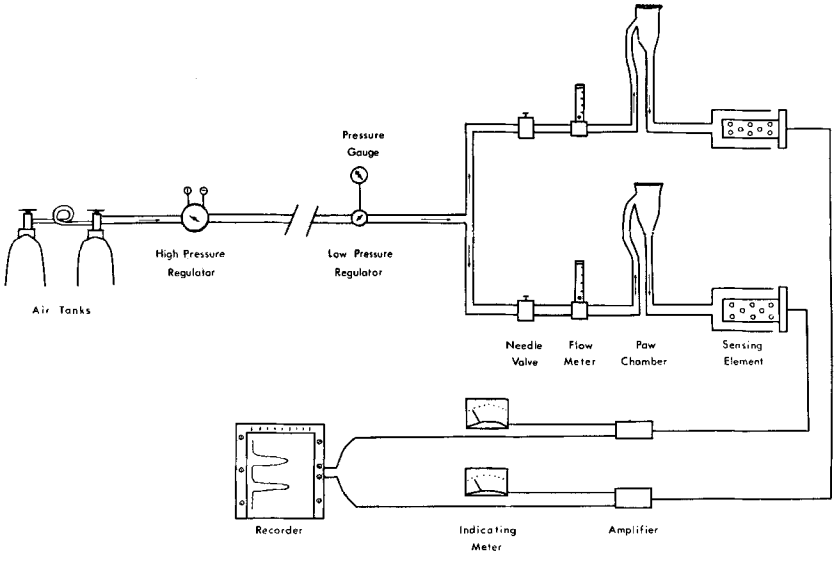


*Fig. 1—Diagram illustrating the sweating rate measuring system. The sensing elements were partially enclosed to prevent accumulation of dust and other foreign material in the room air.*

described the measurement of evaporative water loss by a thermal conductivity cell. Nakayama and Takagi (3) used, instead of the absorption of water as Kuno had done previously, a sensitive resistance hygrometer which enabled them to make a continuous recording of sweating. Bullard (4) later described a modification of the method used by Nakayama and Takagi for studies in man. The purpose of this report is to describe a method for the study of sweating, especially in cats. The method is a modification of the one described by Bullard (4) and which has been adapted for animal use. In principle, when air of any known low relative humidity is passed at a fixed and appropriate rate over the foot pads of cats, the change in the water content of the air is dependent upon the sweating rate.

## MATERIALS AND METHODS

The sweating rate measuring system is shown diagrammatically in Fig. 1. The air (less than 5% relative humidity) supplied by storage tanks was reduced in pressure by high and low pressure regulators and fed into two separate flowmeters. By the use of needle valves at the flowmeters, the air flow could be adjusted and maintained at any desired rate. The air flow selected to be used in all of the experiments was 0.5 L./min. The air passed into a specially designed glass chamber which contained one of the cat's paws. The small chambers were not only designed to provide minimal air volume, but also large enough to permit the use of larger cats. The chamber dimensions were approximately 7 cm. in length and had an inside diameter of 4 cm. The air inlet and exit openings were 6 mm. inside diameter. The position of the paw was always such that the air inlet opening was opposite the foot pad surface. The cat's paw entered into the chamber through a small opening made in a rubber dam which was used to close the open end of the chamber. It was imperative that the position of the paw did not interfere with its blood circulation. This was accomplished by using a very flexible rubber dam with various size holes for entrance of the paw and to avoid inhibiting or occluding the local circulation. To insure that there was uniformity in distribution of air in the "paw chamber," the position of the paws inside the chambers was as nearly the same as possible. Figure 2 shows a close-up view of the cat's paw inside the chamber. After the air had passed over the foot pads at a known flow rate, it then passed into the humidity sensing elements (Hygrodynamics, Inc., Silver Spring, Md.). The principle of the sensing element is based on the ability of a hygroscopic film to change its

*Fig. 2—The position of the cat's paws within the chambers. The small polyethylene cannula in the lower right was used for the intravenous injections via jugular vein.*

*Fig. 3—The entire system showing the cat; paws enclosed in the small glass containers.*

electrical resistance instantly with microchanges in the relative humidity. The resistance change is then measured in terms of electric current flowing through the element and is indicated and recorded by suitable instruments. The hygroscopic film used in the sensing element is lithium chloride. The wide-range sensing element used is capable of detecting humidity changes in the range of 5 to 40%. The Hygrodynamics humidity indicator (catalog No. 15-3000) serves several purposes, that of an a.c. voltage source to the sensing elements, a meter showing the electrical current changes, and a d.c. signal source for recording purposes. The humidity changes may be visually recorded from the indicator meter. However, it is much simpler and less time consuming to use a suitable recording instrument. A Moseley (model 7100A) two pen strip chart recorder was used to record the results reported here. Each humidity sensing element contained a thermistor for temperature measurement. The elements were supplied with a graph of correction factors to be used between −40° and 120°F. The correction factors were added to or subtracted from the humidities read from the calibration curves depending upon the temperature at the level of the sensor. As the air flow rates are known, it may also be desirable to express the data as the amount of water vapor evaporated from a measured paw surface area per unit time. Lawton *et al.* (5) have used the following expression to determine the sweating rates:

$$\text{sweating rate (mg./min.)} = \text{air flow (L./min.)} \times \frac{\Delta RH}{100} \times \text{density of sat. steam (mg./L.)}$$

The entire system used for measuring the sweating response is shown in Fig. 3. The housing for the humidity sensing elements, flow and pressure regulators, amplifiers and meters was designed and made in the machine shop of these research laboratories. A heat lamp was installed, since it has been our experience that a more pronounced sweating response is obtained when heat is applied. The heat lamp consisted of a 125 w. infrared bulb contained in a reflector. It was placed about 18 in. directly above the animal's abdomen and remained in that position throughout the entire experiment. The heat lamp alone in this position never elicited a significant sweating response.

The pharmacological substances to be tested were dissolved in water at various concentrations. They were then applied (0.1–0.2 ml.) directly on the foot pads of the anesthetized (sodium phenobarbital, 125 mg./Kg. i.p.) cat by use of an applicator stick. The material was then allowed to dry before the paw was placed into the glass chamber. Another paw, enclosed in a separate chamber, served as a

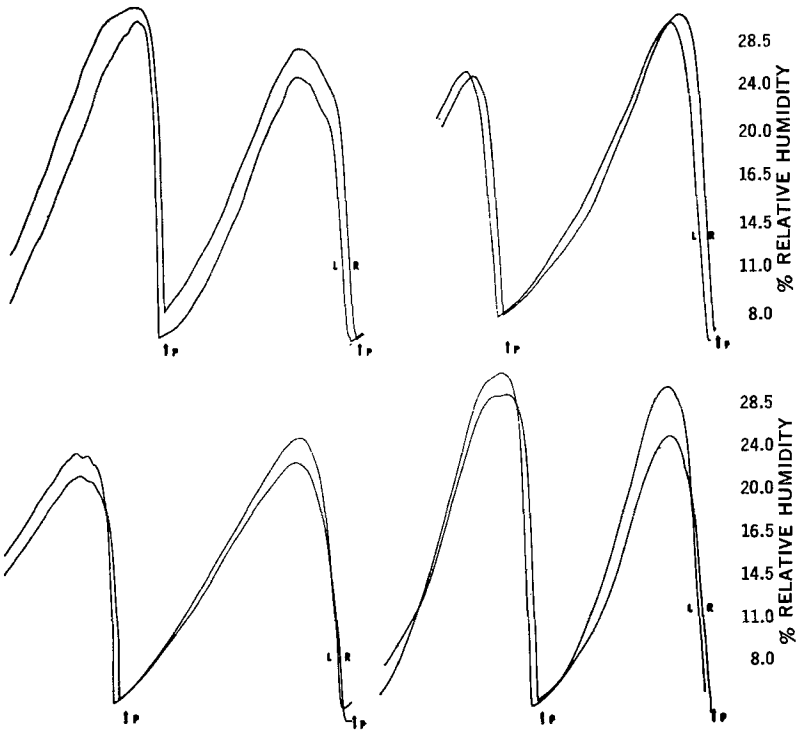


*Fig. 4—Control sweating responses to the intravenous administration of pilocarpine (0.1 mg./Kg.) in four cats. The graphs read from right to left. The arrows indicate the time of pilocarpine injections. The time interval between the two injections was approximately 2 hr. Key: top right, cat No. 192-66; top left, cat No. 162-66; bottom right, cat No. 233-66; bottom left, cat No. 198-66.*

control. Sweating was induced by the administration of pilocarpine hydrochloride (0.1 mg./Kg.) *via* a jugular vein previously cannulated with P.E. 50 tubing.

The duration of action produced by various substances can also be determined using this method simply by increasing the length of time between application and the injections of pilocarpine.

## RESULTS

Figure 4 shows the sweating responses obtained in four cats following the intravenous administration of pilocarpine. In control experiments, the difference in sweating response between the two paws never varied more than 4 to 5%. This small difference made it possible to determine the effect of various substances, when applied topically, on the sweating response. The response to pilocarpine in the majority of tests has been consistent and reproducible over a period of about 5 to 6 hr. Whenever the time has been extended to 8 and 10 hr., the pilocarpine response may be diminished. This diminution in the response may be noted even though the body fluid lost through sweating and salivation is replaced during the entire experiment by the intravenous infusion of normal saline.

To illustrate that the system is capable of detecting differences in sweating rates, the effects of a commercially available aluminum salt preparation,[1] applied as previously described, are shown in Fig. 5. The aluminum salt preparation was applied 3–5 hr. prior to the injections of pilocarpine. It can be seen that the maximum relative humidity provided by the control paw was between 30 and 36%, whereas the treated paw showed values of between 12 and 15% following two injections of pilocarpine. Upon further extending the time between application and the injection of pilocarpine, the effect was greatly diminished or could no longer be demonstrated.

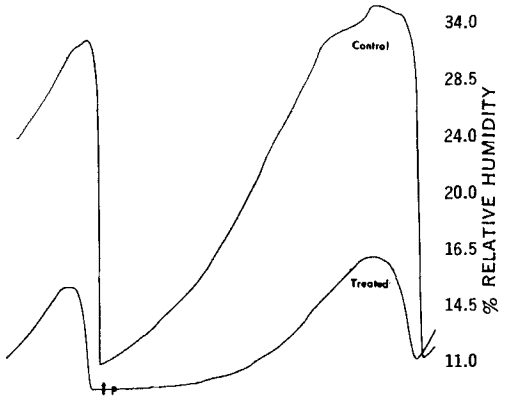


*Fig. 5—The sweating response obtained after the local application to one foot pad of a commercial aluminum salt preparation 3–5 hr. prior to pilocarpine. The graph reads from right to left. The arrows indicate the time of the pilocarpine injections (0.1 mg./Kg. i.v.). Time interval between pilocarpine injections was approximately 2.5 hr. (cat No. 250-66.)*

## DISCUSSION

There have been few methods reported in the literature suitable for the rapid evaluation of pharmacological substances on sweating in laboratory animals. The adapted method of resistance hygrometry reported in this study makes it practical to investigate a relatively large number of such substances in anesthetized cats. The same general method has also been used in this laboratory to study the sweating response in dogs. The duration of the antihidrotic effects of a substance may be easily determined simply by varying the time between application and the injections of pilocarpine.

The choice of the anesthetic agent used is important in sweating rate studies involving cats. The longer-acting barbiturates are the most desirable since additional drug is usually avoided during the experiment. In most experiments involving the use of supplemental doses of a shorter-acting agent, there was an initial increase in the sweating response. The reason for the increased sweating rate following barbiturate administration is not clearly understood.

The sweating rate measuring system described is readily adaptable for the measurement of sweating produced under a variety of environmental conditions. Finger tip sweating in man has also been determined by this method.

[1] Marketed as Ban by Bristol-Myers Co., New York, N. Y.

## REFERENCES

(1) Kuno, Y., "The Physiology of Human Respiration," Churchill, London, England, 1934.
(2) Adams, T., Funkhouser, G. E., and Kendall, W. W., *J. Appl. Physiol.*, **18**, 1291(1963).
(3) Nakayama, T., and Takagi, K., *Japan. J. Physiol.*, **9**, 359(1959).
(4) Bullard, R. W. J., *J. Appl. Physiol.*, **17**, 735(1962).
(5) Lawton, R. W., Prouty, L. R., and Hardy, J. D., *Rev. Sci. Instr.*, **25**, 370(1954).