

in alcohol on dilution with broth gave slight turbidity. However, concentrations below 100 mcg./ml. were all clear solutions. The tubes were then inoculated with 0.05 ml. of the suspension of *S. aureus* in broth and incubated at 37°. These were then examined after 24 hours for visible growth.

EXPERIMENTAL

The seeds of the drug were coarsely powdered and subjected to successive extractions with solvents in a Soxhlet apparatus. The per cent residues were: petroleum ether, 8.6; chloroform, 5.2; and alcohol, 2.8.

The petroleum ether extract inhibited the growth of *S. aureus* in the concentration of 10 mcg./ml. Since the chloroform and alcoholic extracts did not show antistaphylococcal activity, these extracts were not taken up for further study.

Isolation of Antistaphylococcal Principle.—Ten grams of petroleum ether extract was chromatographed over 100 Gm. of alumina (Brockmann grade I) using, successively, petroleum ether, benzene, ether, alcohol, and their appropriate mixtures (Table I) as elution solvents. All the solvents were previously dried and purified. Ninety 30-ml. fractions were collected. The solvents were removed *in vacuo*, and the antistaphylococcal activity of the various fractions recorded (Table I). After the elution, the chromatographic column showed the presence of six different bands, which were extruded separately and refluxed with methyl alcohol on a water bath. The residues were tested and did not show antistaphylococcal activity.

The eluates consisting of petroleum ether-benzene mixture (1:1) were pooled and designated as fraction A. After refrigerating for a few days, it deposited needle-shaped crystals which were washed with cold petroleum ether to remove the adhering liquid. A white crystalline compound was obtained which on repeated crystallization from hot dilute alcohol gave a pure compound. It was identified as psoralen and did not show activity

against *S. aureus*. The mother liquor was further concentrated, and 1 Gm. of this liquid was again passed through a column containing 5 Gm. of alumina. The column was then eluted with petroleum ether-benzene mixture (1:1). The solvent was removed *in vacuo*, and the residual liquid was distilled under reduced pressure; the fraction B, b.p. 155°/0.3 mm., was collected. It was of a pale, viscous oily nature and inhibited the growth of *S. aureus* in the concentration of 0.5 mcg./ml.

Anal.—Calcd. for $C_{12}H_{16}O$. Found: C, 82.22; H, 8.85, mol. wt. (Rast) 186.

The benzene eluates were also mixed together and did not deposit crystals when cooled. The liquid was distilled under reduced pressure and the fraction, b.p. 155°/0.3 mm., was obtained. The compound on analysis was identical to B ($C_{12}H_{16}O$) and had equal inhibition against *S. aureus*.

The activity of compound B was also tested against *S. aureus* resistant to penicillin, streptomycin, chloramphenicol, and tetracycline, in which it was equally effective.

A survey of the literature has revealed that compound B ($C_{12}H_{16}O$) has not been reported previously in *P. corylifolia*. The preliminary characterization tests have revealed the aldehyde nature of this compound; therefore, the name psoralaldehyde is proposed for it. Detailed work about its characterization and constitution will be communicated later.

REFERENCES

- (1) Chopra, R. N., et al., "Chopra's Indigenous Drugs of India," 2nd ed., U. N. Dhur and Sons, Calcutta, India, 1958, p. 391.
- (2) Kirtikar, K. R., and Basu, B. D., "Indian Medicinal Plants," Vol. 1, 2nd ed., Lalit Mohan Basu, Allahabad, India, 1933, p. 718.
- (3) East, J., *J. Endocrinol.*, **12**, 261(1955).
- (4) Jois, S. H., and Manjunath, B. L., *Ber.*, **69B**, 964(1936).
- (5) Seshadri, T. R., and Venkattarao, C., *Proc. Ind. Acad. Sci.*, **5A**, 351(1937).
- (6) Chakravarti, K. K., Bose, A. K., and Siddiqui, S., *J. Sci. Ind. Res. India*, **7B**, 24(1948).
- (7) Gupta, K. C., et al., *Bull. Reg. Res. Lab.*, **1**, 59(1962).
- (8) Jois, S. H., Manjunath, B. L., and Venkattarao, C., *J. Ind. Chem. Soc.*, **10**, 41(1933).

Semiautomatic System for Timing Rotarod Performance

By NATHAN WATZMAN, HERBERT BARRY, III, JOSEPH P. BUCKLEY, and WILLIAM J. KINNARD, JR.

An electronic circuit automatically stops the timer when the animal falls to a platform beneath the rod. This system improves the accuracy and convenience of recording rotarod performance time. The investigator may test several animals simultaneously with only periodic observation.

THE ROTAROD is a device which has been used extensively for the evaluation of drug effects on the central nervous system. Rats and mice perform a task similar to log-rolling; neurological deficit induced by a drug is indicated by the inability

of an animal to remain on the rod for a specified period of time (1). Various types of central nervous system depressants (2, 3) and stimulants (4) may be evaluated and compared by the increase or decrease in performance times they produce.

The rod is generally divided into compartments by thin circular disks, larger in diameter than the roller, so that several animals can be tested simultaneously. The experimenter starts a timer as soon as he places each animal on the rod and stops the timer as soon as he sees the animal fall to the platform beneath.

In situations where as many as six animals are being tested at one time, it is necessary for the experimenter to give his undivided attention. Under these circumstances, it is quite possible that, if more than one animal falls in quick succession, the experimenter might not be able to observe each fall

Received April 6, 1964, from the Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pa.

Accepted for publication April 24, 1964.

This investigation was supported by Grant MH-06540 from the National Institute of Mental Health, U. S. Public Health Service, Bethesda, Md.

The authors gratefully acknowledge the technical assistance of Mr. Robert Butchko.

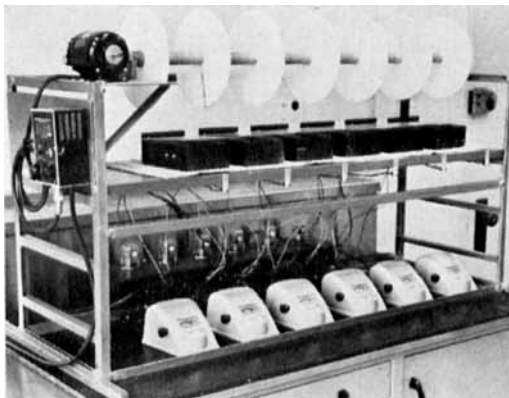


Fig. 1.—Rotarod.

and stop the timers at the exact moment. Since performance time data, under such a system, become dependent on the alertness and reflex abilities of the experimenter, the authors have devised a semiautomatic system for stopping the timers.

APPARATUS

The rotarod is shown in Fig. 1. The supporting structure is composed of 0.75-in. angle iron fastened to a wooden base $24 \times 48 \times 0.5$ in. The wooden catch platform is $43.25 \times 11.5 \times 0.75$ in. and can be adjusted at several heights. A 115-v. d.-c. motor (Bodine Electric Co., NSH-12R; 1/50 H.P.) with speed control (Minarik Electric Co., model No. 5H-14) drives the rod at any selected speed, ranging from 4.1 to 83 r.p.m.

The circuitry for the semiautomatic recording of performance times is shown schematically in Fig. 2. S-1 is a pushbutton switch (S.P.S.T.-N.O. Grayhill 30-1). S-2 is a microswitch (S.P.S.T.-N.C.; Unimax 2 HBW-1) which is mounted on a $6.5 \times 4 \times 2$ in. wooden frame as shown in Fig. 3. The 5×7 in. lid, made of Bakelite or plastic, is hinged to the frame and rests lightly on the arm of the microswitch. K-1 represents a 115-v. a.-c. relay (S.P.S.T.-N.O.; Potter and Brumfield KRP5A) which is set in an octal socket housed on a frame ($20 \times 5 \times 3.5$ in.) located at the base of the rotarod. The timer is a

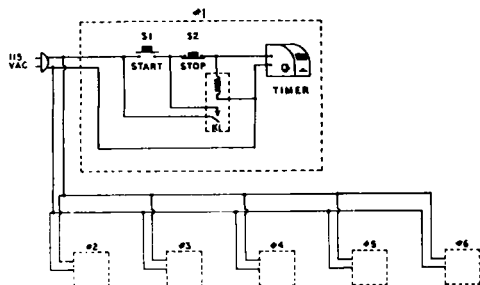


Fig. 2.—Rotarod timing circuit.

Phipps and Bird precision Time-It electrical stop-clock (78-760) with a manual reset. Each animal compartment has a separate pushbutton switch (S-1), microswitch (S-2), relay coil (K-1), and timer.

OPERATIONAL PROCEDURE

As soon as the experimenter places the animal on the rod, he presses the pushbutton start-switch (S-1) which activates the relay (K-1) and the timer through S-2 (normally closed). The relay contacts, being closed, keep the relay activated and the timer on. When an animal falls from the rod, its weight forces the Bakelite platform downward on the microswitch (S-2), opening it. This de-energizes the relay and stops the timer.

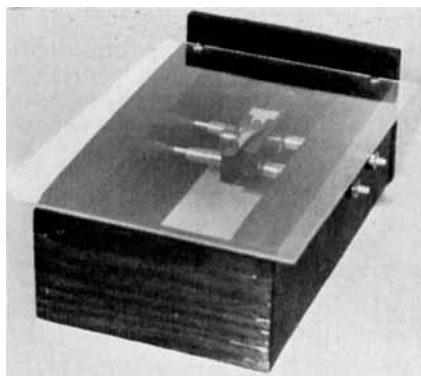


Fig. 3.—Microswitch platform.

CONCLUSIONS

A semiautomatic system for recording rotarod performance times, described in this paper, measures the success or failure of the animal to stay on the rod, independent of the alertness and reflexes of the experimenter. Therefore, the data more accurately reflect the forced coordinated motor ability of the animal. Furthermore, the method does not demand rigid undivided attention on the part of the experimenter; and, indeed, the animals need be observed only periodically. The system is electronically simple and inexpensive to build and repair. It may be expected to function for long periods of time without needing repairs, because the number of operations (on-off conditions) on a typical test day is small compared with the number for which the components are constructed.

We have used this system in our laboratory for several months with completely satisfactory results.

REFERENCES

- (1) Dunham, N. W., and Miya, T. S., *THIS JOURNAL*, 46, 208(1957).
- (2) Kinnard, W. J., and Carr, C. J., *J. Pharmacol. Exptl. Therap.*, 121, 354(1957).
- (3) Weaver, J. E., and Miya, T. S., *THIS JOURNAL*, 50, 910(1961).
- (4) Plotnikoff, N., Reinke, D., and Fitzloff, J., *ibid.*, 51, 1007(1962).