TABLE I.—PER CENT GERMINATION OF SEEDS AND ROOT AND SHOOT LENGTH OF SEEDLINGS OF BLACK MUSTARD EXPOSED TO IONIZED AIR

Ionization	Per Cent Germination	Average Root Length, mm. 48 hr.	72 hr.	96 hr.	120 hr.	Average Shoot Length, mm. 72 hr.	96 hr.	120 hr.	Root/Shoot Ratio 72 hr.	96 hr.	120 hr.
None (no unit)	89.7	9.4	27.1	63.0	93.0	6.0	16.9	19.7	6.5	6.0	5.1
None (unit not connected to power)	89.2	9.8	28.0	63.2	92.3	5.5	17.6	20.1	6.9	6.2	5.4
Negative	90.1	6.6	22.3	46.1	67.7	2.9	12.5	16.5	12.3	7.2	4.1
Positive	89.6	6.1	22.4	45.8	65.2	3.0	12.2	16.4	12.0	7.3	4.4

germination of black mustard seeds was not affected by an atmosphere enriched with ionized air (at the level employed), but that subsequent early growth of roots and shoots of the seedlings was depressed. The root/shoot ratios (last 3 columns of Table I) were obtained by dividing the total length of roots by the total length of shoots. The high values at 72 hours for plants exposed to ionized air (first of the 3 columns) reflect the delay in emergence of shoots in these seedlings. But, during the next 2 days, the increase in shoot length with respect to increase in total root length was greater for seedlings exposed to air ions than for control seedlings. Thus, the early differences in root/shoot ratio were eliminated, and there is indication of a reversal in the trend by the fifth day. For technical reasons, it was not practical to continue measurements beyond the fifth day.

DISCUSSION

Positive and negative air ions exerted qualitatively and quantitatively similar effects on seedlings of black mustard. This finding is similar to results obtained with staphylococci (4) but contrary to observations on succinoxidase content of rat adrenal gland (3), on ciliary movement in mammalian trachea (5–7), and on spore germination, mycelial growth, and penicillin production in cultures of *Penicillium* (2), in all of which significant differences were noted between the effects of negatively and positively charged air ions.

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Effect of Small Variations in Heat Stimulus Temperature on the Tail Flick Response of Rats in Analgesimetry

By J. JUSZKIEWICZ-DONSBACH and GERHARD LEVY

The heat-induced tail flick reaction time of rats is affected by small changes in temperature of the heat stimulus. A crossover experiment, using temperatures of 48 and 50°, respectively, showed significant differences in reaction times in a group of 31 rats. The results demonstrate the need for accurate temperature control of heat sources.

VARIOUS METHODS employing thermal stimuli are used widely in some phases of the evaluation of analgesics (1). Such techniques include the use of electric hot plates, hot wires, and metal tubing heated by circulating hot water. In our hands, the determination of the reaction time after immersion of the rat's tail in a beaker of hot water maintained at constant temperature proved to be the most reproducible method.

In many instances, the thermal sources used in the methods cited above maintain a given temperature within a range of ±1 or ±0.5°. During preliminary experiments we found that such relatively small fluctuations in temperature of the heat stimulus appear to have a significant effect on the reaction time of the animals. In order to investigate this factor in greater detail, the heat-induced reaction time of a number of rats was determined at 48 and at 50°. Both temperatures were maintained within ±0.05°. It was also of interest to determine the effect of repeated stimuli at 15-minute intervals on reaction time as well as the likelihood of "conditioning" due to regular application of the heat stimulus over a period of several weeks.

EXPERIMENTAL

Female Wistar rats weighing 50–90 Gm., starved for about 12 hours, were placed in individual plexiglas holders (Fisher Scientific Co., item 1-280) for 30 to 60 minutes. After an animal had calmed

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down, one-third of the length of its tail was immersed in a beaker of water maintained at either 48 or 50 ± 0.05°. The beaker was an especially constructed, double-wall container of 400-ml. capacity, which permitted the circulation of water at constant temperature with a circulatory pump connected to a thermostatically controlled water bath. The water in the beaker was agitated with a magnetic stirrer to reduce any temperature gradient. The magnetic stirrer was stopped immediately prior to and during the time an animal's tail was immersed.

The animals reacted to the thermal stimulus with a typical contraction of their tails into circles. The time of this reaction was measured with a stop watch which was turned away from the operator to reduce a possible subjective influence upon him. Reaction times were determined at 15-minute intervals for a total of three determinations. The same individual carried out all measurements in order to assure proper interpretation of the end point. From 4 to 8 animals were used at a time. Half of these received a 50° heat stimulus; the others were subjected to a heat stimulus of 48°. Two days later the conditions were reversed, so that the response of each animal could be measured at both temperatures.

A number of animals were subjected to the described thermal stimulation twice a week for three weeks in order to determine a possible conditioning of their response.

RESULTS AND DISCUSSION

The first-week data obtained from several groups, totaling 31 animals, are shown in Table I. A significant difference ($P < 0.01$) in response to the 48° as compared to the 50° heat stimulus was observed. The reaction times during the third week of animals subjected to heat stimulation twice weekly for three weeks are listed in Table II. No significant differences between the first week and the third week reaction times of these animals at a given temperature were found, but the results obtained during the third week again show the effect of a 2° temperature difference on reaction time. It appears that the animals are not "conditioned" by repeated stimulation over a period of three weeks. This is in agreement with the findings of D'Amour and Smith (2).

TABLE I —TAIL FLICK REACTION TIME OF RATS AT 48 AND 50°

Time of Stimulus, min.	Reaction Time, sec.[a] 48°	50°
0	4.8(1.9)[b]	2.9(0.9)
15	5.4(1.5)	3.4(1.2)
30	5.4(1.7)	3.5(1.5)
Mean	5.2(1.7)	3.3(1.3)

[a] Average of 31 animals. [b] Standard deviation in parentheses.

TABLE II —TAIL FLICK REACTION TIME OF RATS AT 48 AND 50° AFTER REPEATED EXPOSURE TO STIMULUS FOR THREE WEEKS

Time of Stimulus, min.	Reaction Time, sec.[a] 48°	50°
0	5.6(1.5)[b]	3.3(0.7)
15	5.4(1.9)	3.5(1.0)
30	5.5(1.3)	3.9(1.1)
Mean	5.5(1.6)	3.6(1.0)

[a] Average of 21 animals. [b] Standard deviation in parentheses.

The animals used in this investigation neither were especially selected for their normal response, nor were trained in any special manner. According to some investigators (3–5), preselection and training of the experimental animals yields more consistent data, but even without these precautions it was possible in this study to demonstrate the effect of small temperature differences on reaction time. The results show that the temperature of the heat source used for thermal stimulation must be closely controlled if the sensitivity and reproducibility of thermal analgesimetric methods is to be assured.

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ERRATUM

In the paper titled "Toxic Saponin from *Elvira biflora*" (1), qualitative test result (*f*), page 780, column 2, should read (*f*) the same water solution boiled with cholesterol did not hemolyze blood cells of the guinea pig in phosphate buffer.

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