include a section on timed release products, it is recommended that this rotating bottle apparatus be considered for use for *in vitro* control testing of such products, with the understanding that the speed of rotation used on certain products may have to be changed to increase the sensitivity of the test for control purposes; and that the time intervals and the pH and other characteristics of the immersion fluids used, must be given individual consideration so as to be appropriate for each product tested.

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Notes

Effect of Ionized Air on Early Growth of Black Mustard Seedlings

By ROBERTSON PRATT

Mammalian systems, bacteria, and fungi have previously been reported to be affected by ionized air. The present paper records an effect of such air on a spermatophyte, black mustard *Brassica nigra* (L.) Koch. Neither positive nor negative air ions influence germination of the seeds, but both types of ions depress the early growth of roots and shoots of the seedlings.

NUMEROUS EFFECTS of ionized air on biologic systems, including human beings, have been reported for more than 200 years, and attempts to apply some of these effects clinically were made as early as 1754. However, most of the reports must be classed as testimonial or, at best, speculative. Within the past decade possibly more reliable, but nonetheless subjective, clinical appraisals have indicated an ameliorating effect of negative air ions and an aggravating effect of positive ions on sinusitis, rhinitis, asthma, pollenosis, and related conditions. The literature up to 1935 has been reviewed extensively (1) and the more recent literature briefly (2).

Among quantitatively measurable effects of air ions on other biologic systems are reduction in succinoxidase content of the adrenal gland of the intact rat exposed to positively ionized air (3); killing of staphylococci by both positive and negative air ions (4); increase in ciliary movement in mammalian trachea, both *in vivo* and *in vitro*, when exposed to negative air ions and the reverse effect, or even abolition of movement, when exposed to positive ions (5–7); and reduction of germination of spores, growth of mycelium and elaboration of penicillin in cultures of *Penicillium notatum* exposed to an atmosphere enriched with air ions, negative ions having a greater effect than positive ones in reducing production of penicillin but positive ions exerting the greater depressing effect on spore germination and mycelial growth (2). The data below, taken from a larger continuing study, record a quantitatively measurable effect of air ons on early seedling growth of a spermatophyte, black mustard (*Brassica nigra*).

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EXPERIMENTAL

Seeds, surface sterilized by immersion for 20 minutes in a dilute solution of calcium hypochlorite, were rinsed several times in sterile distilled water, then were soaked for 1 hour in same, and finally were placed aseptically on moist sterile filter paper strips about 2 cm. wide adhering to the inner surfaces of the germination chambers, consisting of 600-ml. Pyrex beakers. The strips (placed one in each beaker about 6 cm. from the top) were kept moist by a wick which consisted of a "tail" about 1.5 cm. wide that extended to the bottom of the chamber where it dipped into sterile distilled water. Seeds of black mustard are small enough to adhere easily to moist filter paper, even on a vertical surface. Soaking the seeds in water not only removed traces of hypochlorite, it also improved adhesiveness. Experiments were conducted in diffuse daylight; the temperature ranged from 20 to 23°.

A sterile ion generator head,[1] which has been described elsewhere (2), was supported about 1 cm. below the top of each beaker, and then the beaker was aseptically closed with sterile aluminum foil. The details and theory of the ion generators have been reviewed (8–10). At the rectifier voltage employed (860 v.), the atmosphere of the closed beakers was enriched with about 9.5×10^6 ions of appropriate charge/ml./sec. Radiation controls, similar to those run in earlier experiments on *Penicillium* (2), failed to reveal any evidence of direct radiation effects on the seeds or seedlings.

The data in Table I (averages computed from 5 replicate experiments totaling 500 seeds in each of the four environmental conditions) indicate that

[1] The ion generators were kindly provided by the Wesix Electric Heater Co., San Francisco, Calif.

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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