it would never be shocked. After the daily feeding, 1 hour of avoidance training was alternated with 1 hour of rest for a total of 20 hours (10 hours of avoidance training plus 10 hours of rest). An uninterrupted 3-hour rest period followed the last avoidance session of the day. A red, flashing light in front of the animal was on during avoidance sessions and off during rest sessions. The same daily sequence of water, alcohol, and alcohol-water conditions was followed as during the preavoidance phase.

In the final, "postavoidance," phase, which lasted 56 days, the avoidance schedule was no longer in effect and the red, flashing light was never turned on.

Each animal's alcohol and water intake was recorded daily. Table 1 shows the mean alcohol and water intake, in milliliters per 23 hours, during (i) the last 3 preavoidance weeks; (ii) the last 3 avoidance weeks; (iii) the first 3 postavoidance weeks; and (iv) the last 3 postavoidance weeks. Transitions from one intake level to the next were gradual.

When alcohol solution alone was available (except for crackers and water during the feeding period), both animals drank considerably more alcohol per day when they had to press the lever to avoid shocks than during the preavoidance phase. Their alcohol intake remained at a high level for the first 3 postavoidance weeks. By the beginning of the last 3 postavoidance weeks monkey No. 2 had returned to its preavoidance level of alcohol consumption, whereas monkey No. 1 did not return completely to its initial level.

Two factors argue against the possibility that the elevation in alcohol intake during the avoidance phase reflected an increased caloric demand caused by the large amount of work performed by the animals to avoid shocks: (i) alcohol intake remained high during the first 3 postavoidance weeks, even though the monkeys rarely pressed the lever; and (ii) the amount of solid food eaten by the animals each day did not change during the avoidance phase.

When water was the only fluid available to the monkeys, monkey No. 2 did not change its water intake in any consistent fashion throughout the experiment. During the avoidance phase monkey No. 1 showed a surprising drop in water intake, which persisted through the postavoidance phase. Neither animal changed its water intake during the 1-hour feeding periods. Since water consumption either remained the same or decreased during the avoidance phase, the increase in alcohol consumption does not reflect a general elevation in fluid intake by the monkeys.

Even when both fluids were available to the monkeys, their alcohol consumption increased during the avoidance phase. It remained high throughout the first 3 postavoidance weeks but returned to approximately the preavoidance base line by the beginning of the final 3 postavoidance weeks. During the avoidance phase, animal No. 1 again drank less water than before, and it continued to do so thereafter. However, animal No. 2 showed no consistent changes in water intake.

Drinkometer records showed striking differences between the animals' preavoidance- and avoidance-phase drinking patterns. On an alcohol-only or an alcohol-and-water regimen the monkeys, before avoidance conditioning, drank alcohol at a fairly uniform rate throughout the day, but during the avoidance phase and the first 3 postavoidance weeks, they drank the major portion of their daily alcohol within the first 2 or 3 hours. Also, on an alcohol-and-water regimen the animals invariably drank 20 to 30 milliliters of water before taking any alcohol during the preavoidance phase, but during the avoidance phase they consumed large amounts of alcohol before drinking any water. Paralleling their return to the base-line levels, the subjects also returned to their preavoidance-phase drinking patterns by the beginning of the last 3 postavoidance weeks.

The appearance of the animals on both the alcohol-only and the alcohol-and-water regimens during the avoidance phase indicates that they became intoxicated within the first few hours after feeding. They seemed heavy-lidded and lethargic, failed to display the aggressive responses typical of rhesus monkeys, and were easily petted and handled. They were quite normal throughout the preavoidance period and during the avoidance phase of the water-only regimen. The monkeys displayed no appreciable changes in their rate or pattern of lever pressing on days when alcohol was available, although they did receive slightly fewer shocks on days when they had only water.

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3 March 1960

An All-round Soil Percolator

Abstract. A description is given of a soil percolator which has been used both for instructional purposes and for microbiological research and has been found accurate and easy to operate. It could be used for aerobic and anaerobic experiments with a soil under water-saturated conditions.

For a diversity of studies within the field of soil microbiology the percolation technique has proved useful. In principle the technique consists in letting a solution of known composition filter through a soil column held in a tube of glass or other material and, by analysis of the percolated solution, describing the biological or nonbiological transformation it has undergone when in contact with the soil. Lees (*1*) introduced an automatic soil percolator, which was later modified (*2*), and Audus (*3*) described an apparatus which could be used for the measurement of soil-produced CO_2. More recently, Greenwood and Lees (*4*) obtained good results with a rocking respirometer, based on the percolation principle, which makes possible the measurement of both gas-exchange and reaction products from a soil sample. Theories on the percolation technique have been discussed by Lees (*2*).

Although the percolation technique might be a valuable tool in soil microbiology, percolation studies have not become very popular. This may be due mainly to the fact that percolation apparatus are not available commercially, and that the construction of one of the percolators described in the literature seems somewhat complicated.

A rather simple and inexpensive soil percolator which has proved useful and adequately accurate for nitrification and decomposition experiments is described below. The apparatus has also been used in laboratory exercises in microbiology at the University of Gothenburg for some years and has been found convenient and instructive as a means of demonstrating the microbiological processes in soil.

A mounted percolator is shown in Fig. 1. It consists of two identical round-bottomed Pyrex glass tubes *A* and *B* with a side outlet near the open end and a bottom outlet. Tube *B* is closed with a bored rubber stopper and connected with *A* by a glass tube and rubber tubing. The passage through this connection is controlled by a screw clip *C*. The second connection between *A* and *B* is through the three-way stopcock *D* and a long capillary glass tube *E* (bore 0.75 to 1.0 mm), all parts being assembled with not-too-heavy vacuum rubber tubings. Air pressure or suction is applied through the side outlet in *B*. The dimensions of the ap-

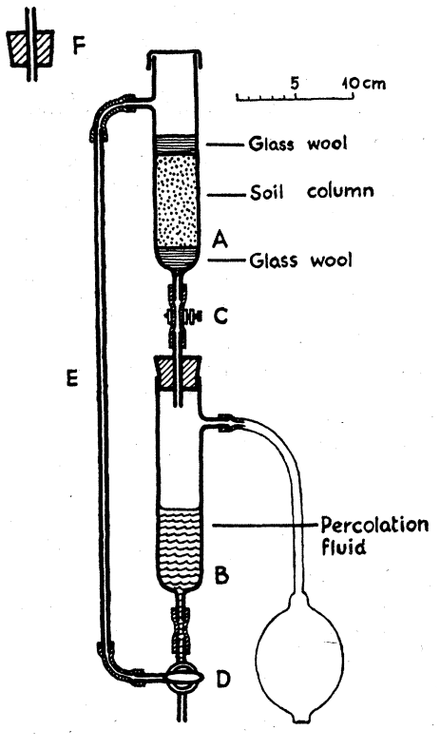

Fig. 1. Soil percolator.

paratus can be chosen to fit any purpose. However, the size of the standard percolator (Fig. 1) has proved practical for most purposes.

The soil collected for investigation should be dried in air, sieved, and mixed well before use. Fresh and untreated soil is not sufficiently homogeneous to allow several percolators to be used simultaneously, since the physical conditions of the soil vary too much from percolator to percolator. Also, with untreated soil the water flow through the columns is rendered difficult because of the packing of small particles at the bottom. The most convenient particle size has been found to be 1 to 2 mm. The amount of soil used in one percolator varies with the properties of the soil. With "garden soil," 50 gm is practical for the standard percolator, but with sandy soils, more, and with soils rich in humus, less than this quantity should be used. The quantity of solution varies with the amount of soil and the water-holding capacity of the soil used.

The soil is placed in tube *A* between two glass wool plugs and allowed to settle; settling is hastened by gently tapping the glass wall of the tube with the finger. In order to exclude light from the soil the tube should be partially covered with a piece of aluminum foil or heavy white drawing paper.

Initially, the percolation solution is poured in at the top of the soil column, and the flow rate is adjusted with clip *C*. Subsequent percolations are carried out by closing *C* and turning stopcock *D* so that the fluid will pass through tube *E* back to *A* when air pressure is applied. The soil is effectively aerated if *C* is again opened and air is blown through. Vigorous aeration is to be avoided, since this might lift the soil column. If aeration by pressure is made difficult because of packing of the soil, suction should be used. Surplus free water around the soil particles can also be removed by suction or, eventually, by applying pressure from above the soil column through a bored rubber stopper, *F*. If the same percolation fluid is to be used throughout an experiment of long duration, and especially if the fluid contains added organic matter, it should be removed after each percolation and refrigerated.

The percolator can be used for several types of investigation, including adsorption experiments, biological transformation of organic and inorganic compounds, enrichment of desired physiological groups of microorganisms followed by isolation in pure culture, and so on. Results of two representative percolation experiments are presented in Fig. 2. The percolator was developed for studies of the soil in a water-saturated state under aerobic conditions, but air could be replaced by any other gas in a closed system for anaerobic experiments. Further, the whole percolator could be autoclaved for experiments with sterile soil. For such use, cotton plugs should be supplied for all free outlets.

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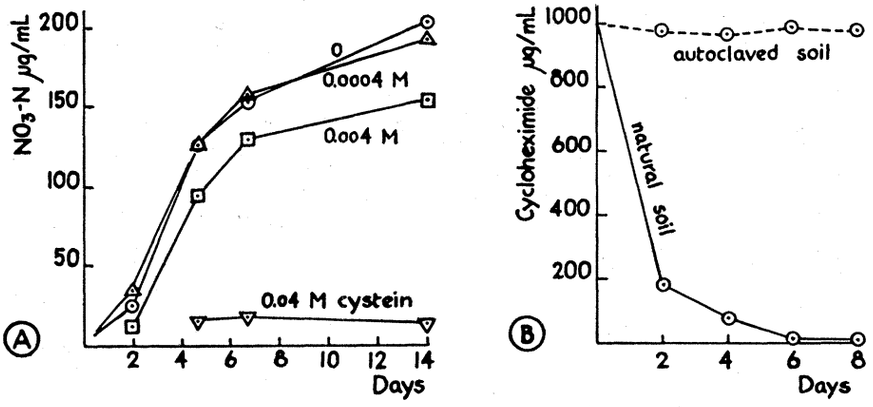

Fig. 2. (*A*) Effect of *l*-cystein on nitrification in a compost soil, *p*H 7.1. The percolation fluids were made up from 0.02*M* ammonium sulfate. (*B*) Decomposition of cycloheximide (actidione) in soil from spruce forest, *p*H 4.2. Prior to the experiment the soil had been percolated with cycloheximide solutions (100 µg/ml) during 8 weeks. Finally, two cycloheximide-decomposing bacteria and one fungus were isolated from the percolator soil.

Some Steroid Hormone-like Effects of Menthol

Abstract. Menthol or menthone, or both, like progesterone, have been shown to have the following biological activities: (i) an inhibitory action on liver and kidney aldehyde dehydrogenase activity which, under certain circumstances, is reflected in an increased rate of oxidation of D-galactose, and (ii) a stimulatory effect on the oxidation of D-galactose by two prepubertal congenitally galactosemic subjects.

We have reported on some biochemical effects produced by progesterone and androgens, both in vitro and in vivo (*1–4*). It has been possible to demonstrate that a different type of molecule—namely, DL-menthol or DL-menthone, or both—can simulate these effects of the steroids (*5*). This report presents a comparison of a number of parameters of progesterone and menthol activity.

It has been shown that the rate-determining reaction in the conversion of galactose-1-C^{14} to $C^{14}O_2$ by the soluble fraction of rabbit liver homogenate is the epimerization of uridine diphosphogalactose (UDPGal) to uridine diphosphoglucose (UDPG) (*2*). This reaction requires a catalytic amount of diphosphopyridine nucleotide (DPN) and is strongly inhibited by reduced diphosphopyridine nucleotide (DPNH) (*6*). Therefore, any agent which increases the level of DPNH would be expected to decrease the rate of galactose oxidation, whereas a decrease in the level of DPNH might be expected to accelerate galactose oxidation (*7*). This has been