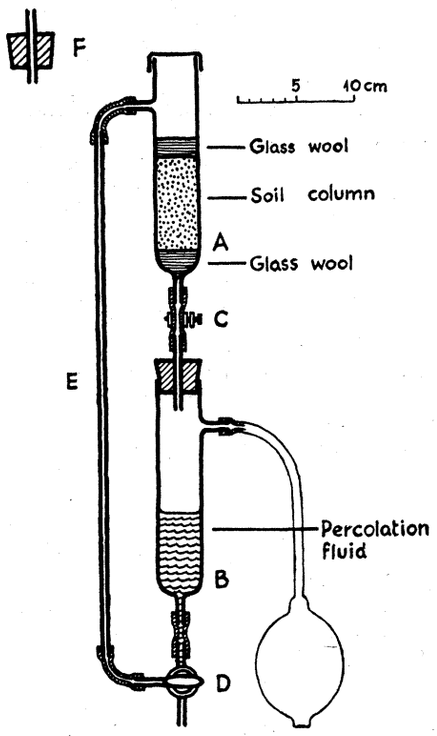

Fig. 1. Soil percolator.

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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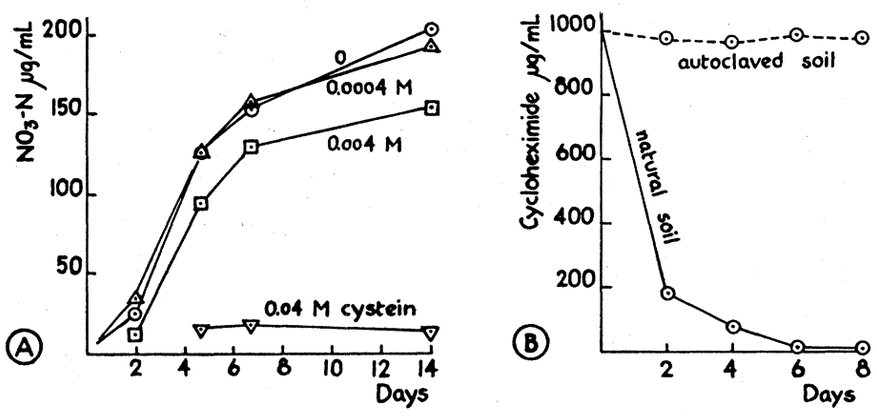

Fig. 2. (*A*) Effect of *l*-cystein on nitrification in a compost soil, *p*H 7.1. The percolation fluids were made up from 0.02*M* ammonium sulfate. (*B*) Decomposition of cycloheximide (actidione) in soil from spruce forest, *p*H 4.2. Prior to the experiment the soil had been percolated with cycloheximide 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

demonstrated experimentally (*3*). Thus, the oxidation of galactose by the soluble fraction of kidney, a tissue known to be low in alcohol dehydrogenase (*8*), is not inhibited by alcohol but is inhibited by the addition of glycolaldehyde or an aldehyde-generating system such as propylene glycol and horse liver alcohol dehydrogenase. Progesterone ($10^{-4}M$) largely overcomes this inhibition, presumably by inhibiting DPNH formation via the aldehyde dehydrogenase reaction (*3*). Menthol ($10^{-3}M$), under comparable conditions, completely overcomes this inhibition of galactose oxidation. Moreover, menthol is oxidized by horse liver alcohol dehydrogenase in the presence of DPN, presumably to menthone. DL-Menthone also markedly inhibits the oxidation of acetaldehyde, glycolaldehyde, glyceraldehyde and propionaldehyde by aldehyde dehydrogenase activity present in the fraction obtained between 40- to 60-percent saturation of rabbit liver and kidney supernatant with ammonium sulfate. The concentration of menthone giving 50-percent inhibition is $6 \times 10^{-3}M$. No inhibition on horse liver alcohol dehydrogenase is exerted by progesterone (*3*), menthol, or menthone.

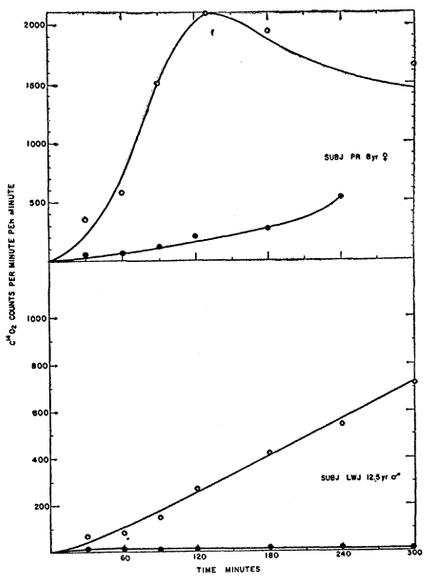

Fig. 1. Excretion of $C^{14}O_2$ by two prepubertal galactosemic subjects after intravenous administration of galactose-1-C^{14}. Subject P.R. was an 8-year-old female; L.W.J. was a 12.5-year-old male. Each child received 2 μc of galactose-1-C^{14} (4.70 μc/mg) just prior to the collection of expired carbon dioxide. Menthol (13 mg/kg), as a 25-percent solution in peanut oil, was given orally in three equal doses at 12-hour intervals during the 36 hours preceding administration of labeled galactose. The technique for collection and assay of $C^{14}O_2$ has been described (*4*). Solid circles, excretion of $C^{14}O_2$ prior to administration of menthol; open circles, excretion after administration of menthol.

Evidence of a more direct nature relating the rate of galactose oxidation to the rate of the UDPGal-4-epimerase reaction as controlled by the DPNH level has been presented elsewhere (*2*). Thus, the oxidation of UDPGal-1-C^{14} by the soluble fraction of rabbit liver was accelerated by progesterone when propylene glycol, a good substrate for alcohol dehydrogenase, was present. However, under identical conditions, the rate of oxidation of UDPG-1-C^{14} was not affected by progesterone. Presumably the hormone inhibited the oxidation of lactic aldehyde which was generated from propylene glycol (*9*). Menthol ($8.7 \times 10^{-5}M$), under comparable conditions, also stimulated the oxidation of UDPGal-1-C^{14} (by 65 percent) as compared to a propylene glycol control but had no effect on the metabolism of UDPG-1-C^{14}.

The in vitro effects described above can be summarized as follows. Galactose oxidation is inhibited in tissue preparations which are metabolizing certain aldehydic substrates. Progesterone and menthone inhibit liver and kidney aldehyde dehydrogenase activity, thereby decreasing the amount of DPNH generated by this reaction. The rate of galactose metabolism reflects this phenomenon—that is, a decrease in DPNH level permits the UDPGal-4-epimerase reaction to proceed at a rate comparable to that which obtains in the absence of aldehydic substrates.

Since menthol seemed to simulate some progesterone effects in vitro, it was of interest to determine whether the similarity extended to the galactosemic state. It can be seen from Fig. 1 that this is indeed the case. The calculations for cumulative excretions indicated that subject P. R. converted 2 percent of the administered galactose-1-C^{14} to $C^{14}O_2$ in 6 hours in the control experiment but was able to metabolize 17 percent to $C^{14}O_2$ during a comparable period after treatment with menthol. Subject L. W. J. produced essentially no $C^{14}O_2$ during the control study, but about 6 percent of the injected galactose-1-C^{14} was oxidized to $C^{14}O_2$ after menthol administration. It is of interest to note that the same subject oxidized 7 percent of the labeled sugar after progesterone administration (*4*). Normal adult subjects in similar studies oxidized 35 percent of the galactose-1-C^{14} in the control experiment but showed no increased capacity to metabolize galactose after ingestion of menthol.

The metabolic block in congenital galactosemia is at the level of P-Gal transuridylase (*10*), the enzyme which catalyzes the formation of UDP-Gal from galactose-1-phosphate. The in vitro effect of progesterone and menthol is at an enzymatic locus one step beyond the transferase level. It is not yet known whether or not the mechanism of the in vivo effect is the same as that which has been shown to operate in vitro. Further clinical trials will be necessary to determine whether these agents have any therapeutic value in congenital galactosemia.

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Lethal Effect of Visible Light on Cavernicolous Ostracods

Abstract. Light intensities of the order of 1/20th that of normal sunlight are sufficient to kill ostracods from two different caves. Loss of physiological protection against light has probably occurred through the same mechanism which has resulted in the much better known loss of morphological characters in cave animals.

Loss of characters such as eyes and integumentary pigment is common in animals which have become adapted, in the evolutionary sense, to the lightless environment of caves (*1–3*). Cave animals also frequently lose physiological characters such as the ability to withstand temperature fluctuations characteristic of surface waters (*3*). In addition, they generally become more tolerant to conditions of semistarvation and may become more euryphagous. Some animals are also reported, by Absolon (*4*), to lose physiological characteristics which enable them to survive irradiation by visible light. Absolon states that a few minutes of sunlight is lethal to some cave-dwelling mites and springtails. His report, however, does not discriminate between the effects of light and of light-induced heat.

Ostracods (*Candona* sp., a new species described by Charles D. Wise) have been collected in Valdina Farm