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.

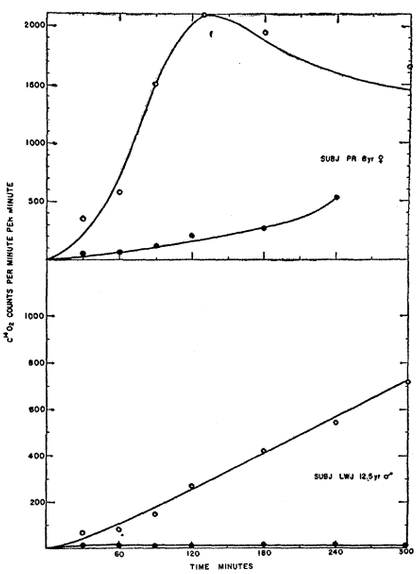

Fig. 1. Excretion of $C^{14}O_2$ by two prepubertal galactosemic subjects after intravenous administration of galactose-1-C^{14}. Subject P.R. was an 8-year-old female; L.W.J. was a 12.5-year-old male. Each child received 2 μc of galactose-1-C^{14} (4.70 μc/mg) just prior to the collection of expired 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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29 April 1960

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

Cave (16 mi north of D'Hanis, Medina County, Tex.) and in Cave X (about 12 mi south of Austin, Travis County, Tex.) and have been successfully cultured at 24°C ± 2° in constant darkness. They were fed dried yeast and small pieces of lettuce and celery. These animals grow and reproduce vigorously under these conditions. Young ostracods from a Valdina Farm culture were randomly divided among four bottles. Two of these bottles, containing a total of 89 animals, were placed about 2 ft from a north-facing window, in an opaque black box; the other two, containing a total of 101 animals, were placed next to the box, where they received the light coming through the window. Within 9 days all the animals which had been in the light were dead, while only 2 of the 89 controls had died.

Apparatus was then constructed so that animals could be exposed to known and constant light conditions. This apparatus consisted of cardboard boxes, with black interiors, which were 12 by 8 by 102 cm and which had a 7.6 cm square opening in one end. These boxes were so arranged that their open ends directly faced a 100-watt light bulb which was immersed in a beaker through which water constantly flowed. The light was thus filtered by 3 cm of water which removed almost all of the infrared (*5*) before it reached the animals.

Seven animals were placed in each of 13 containers with optically flat sides; these containers were oriented within the boxes so that the flat sides were perpendicular to the light. Animals from Valdina Farm Cave were put in some of the containers and animals from Cave X were put in the remainder. Placement of each animal among containers for animals from a given cave was randomly determined except for the last few from each cave. The containers were placed at various distances from the light so as to produce the exposure intensities indicated in Fig. 1. These intensities were considerably less than the intensity of direct sunlight (about 10,000 ft-ca). The control animals, cultured in continuous darkness, were kept in a completely closed box similar in size, shape, and orientation to the boxes described above. The temperature in the boxes that were open at one end would have been about 2°C higher at the end near the light than at the far end if the far end of the box had not been heated slightly. This was accomplished with appropriately adjusted gooseneck lamps.

The results, summarized in Fig. 1, show that a light intensity of approximately 1/20th that of direct sunlight is lethal to these cave-dwelling ostracods. They also show that an intensity of 10 ft-ca does not kill these animals. Furthermore, in one container, in which light intensity was 10 ft-ca, at least four young were produced after 60 days of exposure and three more were produced about 13 days later.

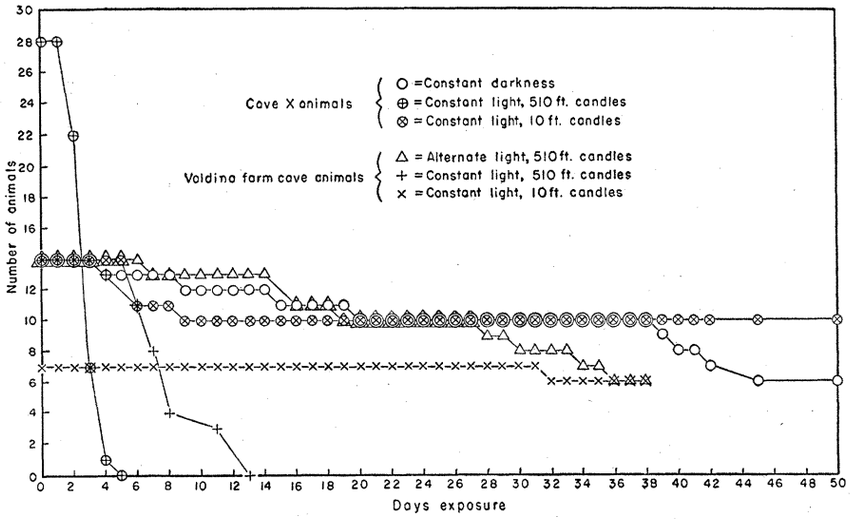

Fig. 1 Number of animals alive as a function of duration of exposure to experimental conditions.

Sensitivity to light was greater in small animals than in larger ones, and the order of death of animals within a given container was correlated with the order of size of the animals. This differential sensitivity may be responsible for most of the difference in the exposure required to kill animals from the two caves, since the average size of the Cave X animals was 0.28 mm and that of the Valdina Farm Cave animals was 0.44 mm.

One light and one dark period per day were used during the first 5 days of the alternating light and dark phase of the experiment. After this, the alternating periods were very close to 24 hours each. During the first days the dark periods were considerably longer than the light periods, and it was not until the 31st day of the experiment that the animals exposed to alternating light had accumulated a number of light-exposure hours (312) equal to the number of hours of exposure that resulted in the death of all animals exposed to constant light. At this time, 8 of the original 14 animals exposed to alternating light were still alive. There are three plausible explanations (which are not mutually exclusive) for their survival. (i) At least partial recovery from light-induced damage occurs during the dark periods. (ii) At least partial adaptation to light occurs, and with alternate dark periods there is more time for this adaptation to proceed after it is initiated. (iii) The extension of exposure over a longer period permits the animals to become older and larger, with increased resistance to the effects of light.

Sensitivity to light as is shown by the ostracods, and which I have found also, though in lesser degree, in cave-dwelling copepods (*Paracyclops fimbriatus*), is not universal in cavernicolous animals (*1, 6*).

The loss in cave animals of physiological characteristics that are adaptive to the epigeal environment is probably brought about by the same mechanism that is responsible for the much better known loss of morphological characters in these animals. The mechanism is evidently one in which mutations which are destructive of the developmental sequences leading to pigment formation, eye formation, physiological protection from light, and so on, accumulate and persist in cave populations, primarily because there is no natural selection against animals carrying them (*7*).

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7. This work was supported in part by the Research Institute of the University of Texas.

28 March 1960