tivity showed no expansion of interlayers during the entire treatment. Similarly, no interlayer expansion was detected when biotite particles less than 50 μ in diameter were kept in 0.1N or 0.01N oxalic acid solutions that were changed frequently. The apparent initial stage of acid alteration was removal of iron, resulting in a light-colored weathering edge that gradually advanced inward. The bleached fragile matrix disintegrated with shaking and dissolved in the acid, thus reducing the size of the particle (Fig. 1, B, C, and D). Chemical analyses of the acid extracts at varying periods of the reaction showed that the constituents released were in the same proportion as in the original biotite, except that slightly larger amounts of iron and aluminum were extracted. This is attributed to the chelating abilities of the acids we used. Indeed, the greater the chelating ability of the acid, the more iron and aluminum it removed. Thus, acid alteration of biotite involves breakdown of the edges and not expansion of interlayers, as is attained by alteration with salts. This conclusion is supported by recent work of Marshall and McDowell (*5*) and Sawhney and Frink (*6*), which suggests that edges of lattice minerals break down in acid solutions.

The rate at which alteration proceeded was primarily a function of the concentration of acid or salt at a given temperature. In some instances treatments were carried out at elevated temperatures, and substantial rate increases in alteration for a given concentration of salt or acid solution were noted. Cultures inoculated with soil suspensions were much slower in producing weathering edges than those inoculated with *A. niger*, indicating differences among different groups of soil organisms. Chromatographic analysis indicated that *A. niger* produced both oxalic and citric acids from the glucose medium. Alteration of biotite by this organism, however, was more closely related to changes caused by oxalic than by citric acid. Similar alterations were observed on biotite particles in close contact with tree roots, both in soil and in inoculated quartz sand cultures.

The contribution of the observed alteration of biotite particles to pedogenesis or to the maintenance of ecosystem nutrient status cannot be assessed at present. However, acid weathering occurs in varying degrees wherever plant roots and their associated microorganisms permeate the substratum and thus constitutes an important mechanism for mineral weathering.

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Detergents in Membrane Filters

Abstract. *Membrane filters from several manufacturers contain detergents. Cells cultured in media filtered through washed filters had higher plating efficiencies and a higher percentage of differentiation than cells cultured in media filtered through unwashed filters.*

Membrane filters from several manufacturers (Millipore, Gelman, and Schleicher and Schuell) are widely used in many areas of biology, biochemistry, and pharmacology. These filters contain 2 to 3 percent of their dry weight as detergent, a fact not contained in the descriptive brochures of any of the manufacturers. Millipore filters contain Triton X-100 or a similar detergent. The type of detergent used in the other filters has not been determined. The manufacturers state that the detergent is added to promote efficiency of filtration and to allow filters to be sterilized by autoclaving. Without the detergent, the filters are unwettable, and excessive pressures are needed to effect filtration. I wish to point out the presence of detergent in the filters and to demonstrate at least one toxic effect of eluates from unwashed filters containing detergent.

The fact that the filters contain a detergent is not immediately obvious when media containing serum or solutions containing proteins are filtered, but it becomes immediately recognizable when distilled water or a saline solution is filtered. The resulting filtrates develop a persistent foam not obtained with the solutions before filtration. The concentration of detergent in eluates of the filters is high enough to cause damage to cells cultured in filtered media and probably has as yet unrecognized effects on other biological systems.

Millipore filters (Millipore Filter Corp., Bedford, Mass.) were used in pressure or vacuum filtration apparatuses as described by the manufacturer. The filters were assembled and autoclaved before use. They were then extracted with 200 ml of glass-distilled water (90° to 100°C) and rinsed with 100 ml of ice-cold saline before use. These eluates were discarded. The hot water effectively removes most of the detergent in the filter. Saline was used simply to cool the apparatus. This treatment does not remove all the detergent from these filters, but if filtration is carried out rapidly after the extraction a negligible amount of detergent is included in subsequent filtrates. It is important that the actual filtration be carried out cold to minimize extraction of detergent.

Although the manufacturers would not disclose the process of incorporation of the detergent into the filters, it is likely that the filters of some manufacturers contain detergent within the substance of the filter and not just on its surface. This may account for the continued, slow leaching of detergent into later filtrates. Filters free of detergent may be obtained from some manufacturers (Millipore, Gelman) upon special request. These filters are not wettable with physiological solutions and must be wet with 50 percent ethanol before use. The use of these filters may eliminate the necessity for the procedure of extraction described above. However, filters made without detergent are difficult to sterilize. They buckle and are considerably weakened after being autoclaved. Furthermore, ethanol is quite toxic to most cultured cells.

Table 1. Effects of eluates from Millipore filters on clonal platings of chicken cartilage cells in vitro. Cells were clonally cultured and assayed for differentiation as previously described (*2*), and the filters were washed as described in the text.

Filters	Plating efficiency (%)	Cartilage-making colonies (%)
Washed	33.2	65
Unwashed	18.4	45.6

Chick embryo cartilage and pigmented retina cells were cultured by previously described methods (*1*, *2*) under conditions that allowed high plating efficiency (25 to 50 percent) and a high percentage of differentiation of the cells (20 to 70 percent). The cells were grown either in medium filtered through washed Millipore filters or in medium filtered through untreated Millipore filters (Table 1).

Tissue culture medium filtered through unwashed membrane filters considerably lowers plating efficiency and differentiation in clonal cultures of various kinds of cells (Table 1). It is not known how the inhibitory eluate acts, but it is reasonable to suppose that the detergent may be acting on the plasma membrane or other membranes of the cell. It seems advisable in light of these results that membrane filters from all manufacturers be washed prior to use in filtering medium for cell cloning and other sensitive studies of cell cultures. Washed filters or filters made without detergent may have to be used in studies in which the adsorptive properties of membrane filters are used, such as those of complementary binding of RNA and DNA (*3*).

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Radiant Solar Energy and the Function of Black Homeotherm Pigmentation: An Hypothesis

Abstract. *White zebra finches exposed to artificial sunlight used an average of 22.9 percent less energy after they were dyed black. The hypothesis that black homeotherm coloration functions primarily to maximize absorption of radiant solar energy is suggested. This hypothesis may explain the dark skin pigmentation of certain human populations.*

The black coloration of birds has been a puzzle (*1*) because dark colors absorb more solar radiation than pale colors and should contribute to the heat stress of black birds exposed to solar radiation. Since homeotherms, including birds, maintain a nearly constant body temperature, there might seem to be little advantage in absorbing additional heat from the environment. Under certain conditions, however, it is possible that the "cost" of energy to maintain body temperature might be reduced by obtaining environmental heat from sunlight. Black coloration may, on the average, reduce the metabolic costs of staying warm and be of positive advantage.

To test this hypothesis, we measured the energy expenditure of domesticated zebra finches, *Poephila castanotis*. Energy expenditure was measured with and without artificial sunlight. Then they were colored with a black dye and the same comparison was made. Dyeing was done with W. J. Stange Co. 92 percent Black Shade food dye. The birds were recolored weekly with a solution of 1 g dye, 0.2 ml wetting agent, and 40 ml water. At least 2 days were allowed after dyeing before a metabolism measurement was made, and no bird was tested more than twice a week.

Experiments were performed with the artificial sun on and off. Each experimental bird was tested under four conditions: (i) color unmodified (white), "sun" on; (ii) white, "sun" off; (iii) dyed black, "sun" on; and (iv) black, "sun" off. This sequence permitted comparisons of the performance of each individual under all conditions.

Oxygen consumption is summarized in Tables 1 and 2. Under artificial sunlight, the birds dyed black used significantly less oxygen than they had required when they were white and exposed to the artificial sun, or when they were black or white and lacked artificial sunlight.

There were no significant differences between dyed and undyed birds with the light source off, but there was a marked difference with the source on.

This result suggests that any slightly greater emissivity related to dark coloration was much less significant to the metabolic economy at the stated temperature than the potential gain from light energy. Experiments with white mice dyed black, and monitored at low temperatures apparently in the absence of light, revealed an increased energy expenditure by the black mice (*2*). The same experiment with rats showed no difference (*3*), but the lighting conditions were not defined and the possibility of two counterbalancing factors, emissivity and absorptance differences, cannot be ruled out.

The radiant energy emitted by an animal falls within the infrared (including the far infrared) portion of the spectrum. A difference in absorption characteristics with respect to the visible part of the spectrum does not establish that there is an emissivity difference in the infrared. Infrared radiometric photographic techniques applied to human subjects show that if there is a difference in infrared emissivity accompanying differences in visible human skin coloration, it is very slight (see *4*).

Oxygen consumption for our active birds under all conditions except black with the "sun" on was higher than Cade, Tobin, and Gold (*5*) determined for resting, naturally colored zebra finches at 10°C. Our active black birds with the "sun" on used less oxygen than those authors reported for their resting birds at the same temperature.

There were no significant differences between birds under the same conditions, with the exception of bird No. 1, which had a significantly higher oxygen consumption while white with the "sun" off than other white birds. There were no significant differences in activity or weight loss between any of the birds, or between any of the conditions.

The experimental arrangement is shown in Fig. 1. Our artificial "sun"