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*).

ROBERT D. CAHN
Department of Zoology,
University of Washington, Seattle

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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"

Table 1. Average oxygen consumption of white zebra finches and the same white zebra finches dyed black. Units are milliliters of oxygen per gram of body weight per minute. The number of trials is in parentheses.

Bird No.	White				Black			
	Sun on		Sun off		Sun on		Sun off	
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
1	10.33 (6)	7.87	13.47 (6)	14.97	6.62 (6)	0.53	9.45 (6)	1.24
2	9.63 (9)	3.37	9.43 (9)	2.04	7.45 (9)	2.54	9.87 (9)	2.06
3	8.14 (6)	1.37	8.55 (6)	2.52	7.25 (6)	3.28	10.29 (6)	5.22
4	8.55 (8)	0.58	8.93 (6)	3.47	7.53 (1)		9.95 (1)	
5	8.76 (2)		10.73 (7)	4.09				
6	10.12 (7)	1.94						
Totals*	9.30 (38)	3.04	9.90 (34)	5.19	7.17 (22)	2.01	9.87 (22)	2.68

* Pooled individual runs.

Table 2. Probability of significance of differences in oxygen consumption. Abbreviations: WS, white bird with "sun" on; WD, white bird with "sun" off; BS, black-dyed bird with "sun" on; BD, black-dyed bird with "sun" off; s, significant at the probability listed; ns, not significant at the .05 level. Two-tailed *t*-tests for differences between two means were used in all cases.

Condition	Bird No.			
	1	2	3	Total*
WS/WD	ns	ns	ns	ns
WS/BD	s, $p < .02$	s, $p < .02$	ns	s, $p < .001$
WS/BD	ns	ns	ns	ns
BS/BD	s, $p < .001$	s, $p < .01$	s, $p < .04$	s, $p < .001$
BS/WD	s, $p < .01$	s, $p < .01$	ns	s, $p < .005$
WD/BD	s, $p < .05$	ns	ns	ns

*Includes data for birds Nos. 4, 5, and 6, which were not tested sufficiently under each condition to be treated separately.

was a 650-watt, 3400°K Sylvania DWY Sun-Gun lamp bulb centered 35 cm from two perches in a metabolism chamber. When the "sun" was off, faint illumination from the fluorescent room lights reached the birds. The 32.5- by 22.5- by 22.5-cm Plexiglas chamber was placed in a 150-gal (about 570-liter) circulating water bath and

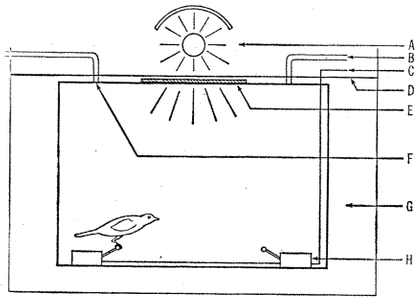

Fig. 1. Experimental arrangement for measuring the effect of light on the metabolism of birds. The components are: *A*, artificial sun; *B*, air intake; *C*, wire lead to operations recorder; *D*, water level; *E*, filter; *F*, air outlet, to oxygen analyzer; *G*, water bath; and *H*, activity monitoring switch and perch.

was maintained within ±0.5° of 10°C during all experiments. Temperature probes at the level of the bird verified these temperatures. The chamber was covered by 1 cm of water. Light reaching the perches also passed through a 12.5- by 12.5-cm window made of optically ground Corning CS9-54 utraviolet-transmitting filter glass 4.0 mm thick. Both perches were mounted on microswitches and perch changes by these birds were monitored by an Esterline Angus operations recorder. Oxygen consumption was monitored with a Beckman paramagnetic oxygen analyzer in an open-circuit system; Depocas and Hart's (*6*) equation was used for calculation of oxygen consumption. Flow rate through the system was 200 ml of oxygen per minute (standard temperature and pressure).

Birds were maintained on an 11-hour light, 13-hour dark photoperiod during the experimental period, January to June 1966. Only one bird was tested each day. Three hours before the experiment, at 0815 hours, the bird to be tested was placed in a small cage with water but no food. Then the bird was transferred to the metabolism chamber, which was immersed in the water bath. A 50-junction Eppley pyroheliometer placed in the cage at the level of a perching finch recorded 1.23 cal of absorbed radiant energy per square centimeter per minute. A measurement of solar radiation of 1.31 cal/cm^2 per minute was obtained at 1400 hours on 12 August 1966 with the same instrument. Average sea-level solar radiation values of 1.07 cal/cm^2 per minute have been obtained by Gates (*7*). Oxygen consumption was recorded when the analyzer indicated a change of no more than 0.04 percent during a 15-minute period. This equilibrium was reached in about 2.5 hours.

We are aware of no other experiments which have attempted to assess the role of solar radiation and surface color in homeotherm energy expenditure. Our experiments establish an average metabolic economy of 22.9 percent for blackened white birds under artificial sunlight. These results indicate that homeothermic animals can absorb and utilize radiant solar energy and that dark pigmentation facilitates this process. Results of the experiments reported here suggest that it is possible that the color of a homeothermic animal is of considerable importance in its energy budget in nature, reducing the metabolic cost of maintaining a constant body temperature. The same evidence is applicable to the coloration of man. Dark human skin coloration may maximize the absorption of solar radiation in situations where energy must be expended to maintain body temperature, as at dawn and dusk in otherwise hot climates.

WILLIAM J. HAMILTON III
FRANK HEPPNER
Department of Zoology,
University of California, Davis

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