establishment of partly aquatic territories; (ii) fights limited to two males; (iii) incessant barking by established territorial males; (iv) the use of physical force for displacement of an opponent; (v) the stereotyped boundary ceremony; (vi) the intrusion of smaller males into the resident male's territory so long as they maintain silence and remain visually inconspicuous; and (vii) interference by a dominant bull in "noisy squabbles" of females and pups.

Thus these experiments demonstrate that during its breeding season barking by a large *Zalophus* male serves as a cue for restricting movement and barking by other smaller males in the vicinity and accessible to attack. Intraspecific status or class recognition was demonstrated by the fact that, among the three more mature sea lions, barking and attack were primarily directed toward animals of most nearly equal size. The cues for identification among individual males are both visual and auditory. Although size is certainly an important cue, males also develop with growth a clearly discernible head crest as well as a lower-pitched bark.

Our experiment and recent field observations (*7*) demonstrate that barking by immature *Zalophus* males (in this study, 3 to 6 years old) is highly significant in the development of social communication patterns leading to adult displays of aggressive and sexual behavior. There is experimental evidence that *Zalophus* can learn to inhibit or emit its underwater click vocalizations in the presence of exteroceptive cues (*8*). That barking may also come under the control of exteroceptive cues, which are social in nature, is suggested by the finding that the sight and sound of a larger animal often suppressed vocalization and movement by the male of the next smaller size, when a larger male was capable of physically attacking and punishing smaller males.

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5. Throughout these experiments we saw *Zalophus* submerge and resurface in the pool while emitting a series of barks. On the basis of previous work, in which we have monitored their underwater sounds, it seems likely that during such vocal episodes *Zalophus* was barking while submerged.
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Selective Advantage of the Sickle-Cell Trait

Wiesenfeld's article "Sickle-cell trait in human biological and cultural evolution" (*1*) showed how a change in cultural practices may have far-reaching effects on the genetic composition of a population. His mathematical analysis, however, contains a common error which causes him to understate the importance of the effect.

In examining the relationship between intensity of endemic malaria (sporozoite rate) and selective advantage of the "sickler" heterozygote, Wiesenfeld derived an equation,

$$w_{12} = 1.075 + 1.289s$$

based on the estimated values for these variables; w_{12} is the selective advantage and s is the sporozoite rate.

If this equation is taken at face value it indicates that the sickle-cell trait confers a substantial selective advantage even when the sporozoite rate is zero. If this were true the trait would be common even in areas or among ethnic groups with no history of malaria. This, of course, is contrary to the known facts. What *is* known is that the gene results in significantly increased mortality among homozygotes and is associated with a number of deleterious conditions in heterozygotes (*2*). The selective advantage is only one of comparison, in that, on the average, persons with the sickle-cell trait are not as badly affected by the malarial parasite as persons lacking the trait are.

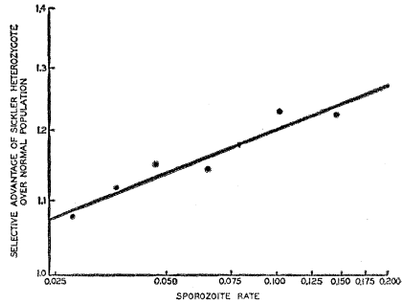

Fig. 1. Relationship between the intensity of endemic malaria (the sporozoite rate) and the selective advantage of the "sickler" heterozygote over the normal population.

The error in analysis which leads to this inconsistency is the assumption that the relationship is linear. Actually, logarithmic scales are almost always more appropriate for describing biological dose-response relationships (*3*). In this case, logarithmic scales not only give a visibly better fit (compare Fig. 1 given here with Fig. 4 of Wiesenfeld's article) but also yield a relationship in better conformity with known facts (*4*):

$$\log_{10} w_{12} = 0.1571 + 0.0774 \log_{10} s$$

Extrapolation of this result indicates that the selective advantage disappears at a sporozoite rate of 0.009; at rates below this the heterozygote is at a relative disadvantage. This is consistent with what is known of this condition.

This result adds weight to Wiesenfeld's argument by showing quantitatively how the rising prevalance of malaria, resulting from changes in agricultural practice, has caused a potentially harmful characteristic to become selectively advantageous. It further indicates that present and future efforts to control the incidence of malaria will have a long-term effect on the gene pool; when the sporozoite rate is reduced below approximately 0.009, the sickling trait will become disadvantageous and should be reduced to very low levels in a relatively few generations (*5*).

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4. Values were read from Fig. 4 of Wiesenfeld's paper. Recalculation from these values gave good agreement with his equation. For the raw data, $(s_y^2 - s_{y.x}^2)/s_y^2 = .79$; that is, the regression "accounts for" approximately 79 percent of the variance. Using the logarithmic

transformation, we obtain $(s_y^2 - s_{y.x}^2)/s_y^2 = .90$, which is a substantial improvement.

5. Modern medical treatment may reduce the severity of illness caused by the malarial parasite, but it may also prevent or reduce the severity of deleterious conditions associated with the sickle-cell trait. Where malaria is prevalent, the advantage would seem to remain with the heterozygote.

23 January 1968

Hexter states that there is an error in my analysis of the relationship between the intensity of malaria and the selective advantage of the "sickler" heterozygote, because extrapolation of the equation to low levels of malarial parasitism reveals that the "sicklers" still have a selective advantage. I appreciate Hexter's effort and interest; however, no such extrapolation was made in my article. Hexter argues from the assumption that Eq. 1 in my article may be taken at its face value; however, this assumption is not valid. I stated (*1*) that the relationship approximated by the equation was valid (i) only in east and west subsaharan Africa; (ii) only for Negro agricultural communities of many centuries' duration; and (iii) only for hyperendemic falciparum malaria. Any extrapolation of the relationship to evaluate the response of the sickle-cell trait at very low levels of malarial parasitism is unrealistic because low levels of malarial parasitism are commonly associated with vivax malaria, with different species of mosquitoes, and with different environments. Also, mild malarial parasitism is characteristically epidemic, seasonal, and therefore associated with sporozoite rates much higher than those of stable, endemic malaria (*2*). In areas where levels of parasitism are low, the deleterious factors of the sickle-cell trait play a relatively greater role in morbidity and mortality.

Within these limits of the relationship, Hexter's logarithmic transformation is a better approximation of the data than the straight-line equation. However, the differences are small.

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7 March 1968

Unmineralized Fossil Bacteria: A Retraction

I have described (*1*) unmineralized fossil bacteria from two sources, widely separated in time and space. The first occurrence was in present-day calcite crystals that grew, in part at least, in the black mud at the bottom of Green Lake, near Fayetteville, New York. The second was in a black, lacustrine limestone stratum from the Lower Cretaceous Newark Canyon Formation from an area south and east of Eureka, Nevada. When two friends to whom I had sent parts of my sample of the Newark Canyon limestone failed to find the coccoid bacteria, I reexamined the sample and made the embarrassing discovery that the minute spheres were fluorite artifacts produced during the preparation of the material for microscopic examination.

The organic matter in the black limestone of the Newark Canyon Formation had been isolated by dissolving the rock in dilute HCl. A small portion of this finely divided organic matter was wholly destroyed when treated with warm 30 percent hydrogen peroxide, which demonstrated that all the organic matter was unmineralized. Because the finely divided organic matter resisted dispersion, I supposed that aggregates of the particles may have been held together with small amounts of silica. They were therefore treated with HF and, to eliminate any remaining free acid, the treated material was taken to dryness on a steam bath. When taken up with distilled water, the organic particles dispersed satisfactorily in a sonicator and were then centrifuged to remove the coarser particles. Fractions of the very fine dark particles remaining in suspension were removed with a pipette to microscope slides; the sample was dried on the slide and mounted in glycerin. These fractions consisted largely of the minute spheres shown in Fig. 1 of my earlier report (*1*). These spheres I mistook for coccoid bacteria. Instead they were fluorite, which formed because I had failed to wash all the calcium out of the organic matter before treating it with HF. To complete my confusion, the fluorite spheres had occluded enough organic matter to color them dark brown. I was as completely taken in as Don Quixote when he blamed "The Enchanter" for changing "that giant into a windmill at the last moment" (*2*).

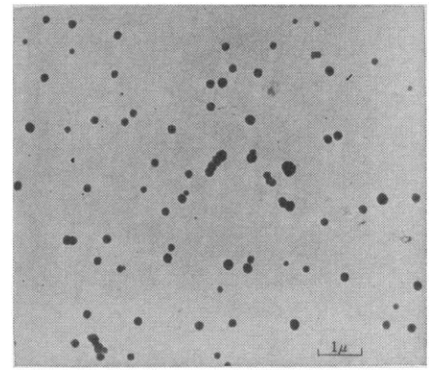

Fig. 1. Electron micrograph (shadowed with uranium) of fluorite spheres precipitated from dilute $Ca(NO_3)_2$ solution with HF in the presence of "humic" organic matter. The spheres, which have a median diameter of 0.2 μ, have a tendency to clump and to arrange themselves into short chains that stimulate certain bacteria. [E. J. Dwornik, U.S. Geological Survey]

It is easy to duplicate the minute fluorite spheres (Fig. 1) by adding HF to a dilute $Ca(NO_3)_2$ solution, provided that organic matter is present. Without organic matter, one gets only minute, ill-formed crystals of fluorite. I was not successful, however, in reproducing the dark-brown color of the fluorite despite the addition of various deeply colored "humic" leachates to the calcium nitrate solution.

Having been shaken by this experience, perhaps I should question also my interpretation of the dark-brown, nearly black, spherical particles that I found in the calcite crystals from the bottom mud of Green Lake, New York. Certainly they are not fluorite spheres. On the other hand, exhaustive efforts by Vallentyne and Brunskill (*3*) to establish viability of these particles failed. Could it be that the particles showed no spark of life because they were not whole microorganisms before being trapped in the calcite crystals?

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27 February 1968