Albino-beige mice were produced in order to combine accumulations of ingested membranes were not observed in the pigment epithelium of albino-beige mice formed giant granules. Additional, incompletely processed membranes accumulated as the mice aged or were exposed to 150 foot-candle light for longer periods. Such membranes needed to control phagocytosis experimentally are more reproducible in albino than in pigmented animals, an attempt was made to produce mice which were albino yet had the inheritable lysosomal disorder which has been well characterized in beige mice and Chediak-Higashi patients. The development of a new animal model—the albino-beige mouse—should allow more definitive studies on the effects of light on the phagocytosis and processing of rod outer segment disks by the retinal pigment epithelium.

**Materials and methods.** All mice used were homozygous nonagouti (a/a) and were congenic descendants of albino (c<sup>32</sup>/c<sup>32</sup> +/+ ) and/or beige ( +/+ bg<sup>d</sup>/bg<sup>d</sup>) mice of the C57BL/6J strain obtained from Jackson Laboratory, Bar Harbor, Maine. They were raised from birth on standard laboratory diet and unlimited water under 12 hr cyclic illumination of less than 4 foot-candles at temperatures of 21° to 27° C. The genetic crosses are described in the next section (Results).

Experimental animals were exposed for the times indicated to 150 foot-candles of white light supplied by three 12-inch Circline fluorescent bulbs (cool white) that were dimmed by a voltage control and shielded by a light diffuser around a 9-inch wire cage. Fans maintained the cage temperatures at 25° to 27° C. Control mice were exposed to 4 foot-candles at the same temperatures.

The mice were sacrificed between 8:00 and 10:00 A.M. by intraperitoneal injections of sodium pentobarbital (0.2 ml). Retinas were fixed and incubated histochemically as described previously, using only regions within 1.0 mm of the optic disc.

**Results**

Origin of the albino-beige mouse. Albino-beige mice were produced by a dihybrid cross from congenic parental strains. First, C57BL/6J-c<sup>32</sup>/c<sup>32</sup> -/+ and C57BL/6J-+/+bg<sup>d</sup>/bg<sup>d</sup> were crossed. The F<sub>1</sub> generation, C57BL/6J-c<sup>32</sup>/ + bg<sup>d</sup>/+, was black, since normal dominant genes were present at the albino (c<sup>e</sup>) and beige (bg<sup>d</sup>) loci.

The gene for pigment formation was contributed by the beige mouse, which can synthesize melanin normally but is grayish rather than black because the pigment is packaged in larger than normal granules.

The gene for standard pigment granule size was contributed by the albino mouse, which is unable to synthesize pigment but has normal-size premelanosome granules.


Albino-beige mice were produced in order to combine two experimentally useful characteristics, albinism and lysosomal dysfunction, in the same animal. The retinal pigment epithelium of albino-beige mice formed giant intracellular granules. Exposure of albino-beige mice to white light of 150 foot-candles for 3 to 10 hr induced marked phagocytosis of rod outer segment fragments by the retinal pigment epithelium, resulting in intracellular accumulations of undigested disk membranes within the giant granules. Additional, incompletely processed membranes accumulated as the mice aged or were exposed to 150 foot-candle light for longer periods. Such accumulations of ingested membranes were not observed in the pigment epithelium of exposed or aging albino mice heterozygous for the beige gene. Because of its altered processing of ingested outer segment membranes, this new albino mouse should be useful for studying the possible roles of the retinal pigment epithelium in the maintenance of photoreceptor cells and in their recovery from light damage and other insults.

Beige mice exhibit anomalous lysosomes and delayed breakdown of phagocytized materials, thus providing an opportunity for studying a retinal pigment epithelium which may show altered processing of ingested rod outer segment membranes. Since the light exposures of the pigment epithelium needed to control phagocytosis experimentally are more reproducible in albino than in pigmented animals, an attempt was made to produce mice which were albino yet had the inheritable lysosomal disorder which has been well characterized in beige mice and Chediak-Higashi patients. The development of a new animal model—the albino-beige mouse—should allow more definitive studies on the effects of light on the phagocytosis and processing of rod outer segment disks by the retinal pigment epithelium.

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**Results**

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The gene for pigment formation was contributed by the beige mouse, which can synthesize melanin normally but is grayish rather than black because the pigment is packaged in larger than normal granules. The gene for standard pigment granule size was contributed by the albino mouse, which is unable to synthesize pigment but has normal-size premelanosome granules.
Fig. 1. A, Retinal pigment epithelium of a 45-day-old albino mouse (C57BL/6J-c2J/c2J/+ +) showing normal-sized pigment granules (P) with limited pigment deposition, in the apical region near the rod outer segment tips (ROS). (x18,900.) B, Retinal pigment epithelium of a 45-day-old albino-beige mouse (C57BL/6J-c2J/c2J bgJ/bgJ) showing a giant pigment granule (P), which is near the cell base and has limited pigment deposition, an irregular shape, and a heterogeneous composition. (x18,900.)

loci. Also, the F1 generation had lysosomes of normal size and would not be expected to show delayed breakdown of phagocytized materials. Next, C57BL/6J-c2J+/bgJ+/ were intercrossed, producing mice that were black, beige, or albino depending on gene segregation.

As would be predicted by Mendelian genetics, approximately one fourth of the F2 mice were albinos, and one fourth of these albinos were homozygous for the recessive beige allele. These albino-beige mice (C57BL/6J-c2J/c2J bgJ/bgJ) could be distinguished from the other F2 albinos by examination of blood smears. Their leukocytes exhibited the giant lysosomes which are typical of the beige mouse and now are present in albinos which are homozygous for the beige allele.

The albino-beige mice developed in this manner should have most of their genes in common with the original parents and with their litter mates. Nevertheless, they were inbred by brother-sister matings for two generations. Next they were crossed with albinos of the F2 generation that were heterozygous for the beige gene (C57BL/6J-c2J/c2J bgJ/+) so that both heterozygous and homozygous littermates would be produced for the experiments. Presently we have stocks of albino-beige mice that have been inbred for nine generations. They are healthy and have good fertility.

**Unusual granules with pigment.** Although the C57BL/6J-c2J/c2J +/+ mice used for this study appeared to be typical albinos, partially developed pigment granules were found in their retinal pigment epithelium. Several granules found in the apical region of essentially every cell were ovoid to needle-shaped, averaging 0.5 by 1.5 μm. Usually the granules contained filaments which were

*These were albinos which had both black and beige offspring upon crossing with a beige mouse.*
thickened and electron opaque like newly formed melanosomes in black mice (Fig. 1, A). However, these filaments did not continue to thicken nor become compacted to form homogeneously dense granules as did those found in black mice.

The albino-beige mice that were developed in this study also had significant numbers of pigment granules in their retinal pigment epithelium. These granules, like those in c2J/c2J +/+ mice, appeared to have limited deposition of melanin. However, they were much larger (3 by 5 μm), fewer in number, more centrally located, and were very irregular in shape (Fig. 1, B). Like the giant pigment granules of beige mice these enlarged granules contained distinct groups of melanosomes in different stages of pigment deposition, suggesting that they originated by multiple fusions of normal-sized pigment granules. Unlike pigment granules of beige mice they never became completely opaque, always showing a fibrilar or granular matrix typical of developing pigment granules.

The giant granules were larger in mice over 100 days of age than in younger mice, and the membranous and lamellar material as well as pigment. The proportion of the granules composed of densely packed pigment was much less in albino-beige (Fig. 2, A) than in beige mice (Fig. 2, B).

Often, tightly packed groups of membranes, presumably deriving from phagocytized outer segment disks, were included in the giant, partially pigmented granules of albino-beige mice but were not associated with pigment granules in albino or black mice. As albino-beige mice aged, the giant granules of their retinal pigment epithelium exhibited more heterogeneity and a higher proportion of membrane remnants in their contents (compare Figs. 1, B, and 2, A).

Alterations of retina by light. Exposure for 3 to 10 hr to 150 foot-candles of homogeneous illumination appeared to accelerate phagocytosis, in that more phagosomes were found in the retinal pigment epithelium. These phagosomes were larger than normal, especially in albino-beige mice where they averaged 3.5 μm in diameter, were more heterogeneous in content, and appeared to arise by fusions with other phagosomes. Under this condition of induced phagocytosis the giant pigment granules were generally larger and more heterogeneous, being composed of regions having the structure of phagosomes, the structure of pigment, and the structure of lysosomes (Fig. 3).

Exposure for 5 or 6 days to continuous or cyclic (12 hr + 12 hr) light of 150 foot-candles left the retinas of both albino and albino-beige mice with greatly shortened or absent rod outer segments (Fig. 4, A). In albino-beige mice, the giant granules of the pigment epithelium were larger and more numerous in exposed retinas. Moreover, these granules had higher proportions of membranous material (Fig. 4, A and B).

Lipid droplets were more abundant along the basal and lateral cell boundaries in exposed mice than in unexposed mice and in albino-beige mice than in albino mice.
Fig. 3. Retinal pigment epithelium of a 45-day-old albino-beige mouse (C57BL/6J-c2J/csu bg Jlb^) which was exposed for 10 hr to white light of 150 foot-candles. Note the anomalous granule of heterogeneous content apparently composed of distinct regions with the structure of phagosomes (ph); the structure of lysosomes (Ly); and the structure of pigment (P). Uningested rod outer segments (ROS) showed some disk disorganization. (×25,200.)

Discussion. A strain of albino mice with giant intracellular granules was produced by crossing albino mice with beige mice of the same inbred strain. This new animal provides another model for the Chediak-Higashi syndrome and may prove useful experimentally in studies involving the effects of light on the retina, especially in regard to interrelationships between epithelial and photoreceptor cells.

Albino-beige mice formed giant granules in their retinal pigment epithelium, which contained stacks of membranes as well as pigment and which probably arose from multiple fusions as did the previously described giant granules in beige mice. The formation of melanosomes in the standard-sized pigment granules of albino mice as well as in the giant granules of albino-beige mice indicates that incomplete granules of albino-beige mice must be a characteristic of the c2l mutation, although this has not been reported to our knowledge. Why the albino-beige mice reported by Hearing et al. did not form any giant granules in the retinal pigment epithelium is not known, but apparently almost no melanin deposition occurred in the c2l mutant mice they used.

The giant granules in the retinal pigment epithelium of albino-beige mice derived from the c2l mutant probably represent anomalous lysosomes, suggesting a lysosomal dysfunction like that reported in the renal cells of beige mice and in the leukocytes of patients with the Chediak-Higashi syndrome. In the c2l/c2l bg J/bgJ albino-beige mice, material having the structure of partially degraded membranes, perhaps representing remnants of ingested rod outer segment disks accumulated in giant granules. Normally, membranes ingested by the pigment epithelium disappear within a few hours.

How the phagocytosis of different amounts of membrane would influence its processing by the retinal pigment epithelium of normal and mutant mice was investigated. Phagocytosis by the pigment epithelium had occurred longer in older mice and presumably had occurred more rapidly in mice exposed to 150 foot-candle light. In normal albino mice this additional load of membranes ingested by the pigment epithelium disappeared, leaving only traces. However, in albino-beige mice aging or exposing to light increased the numbers and sizes of giant granules which contained membranous material. Impaired degradation would explain the additional accumulation of what appeared to be membrane remnants associated with the longer times or the accelerated rate of phagocytic activity. Such accumulations of unprocessed debris in the retinal pigment epithelium of albino-beige mice may provide a means for estimating the amount of phagocytosis which has occurred in mice of different ages or following different experimental treatments.

Photoreceptor cells undergo a regular turnover of their light-absorbing membranes. Disk addition at the proximal ends of rod outer segments and disk shedding at the distal ends are influenced by light, temperature, and daily rhythms. But how photoreceptor membrane turnover might be influenced by a defective pigment epithelium remains largely unknown. Albino-beige mice should be useful for investigating this and other questions related to photoreceptor maintenance by the retinal pigment epithelium.
Fig. 4. A, Retina and choroid of a 45-day-old albino-beige mouse \(\text{C57BL/6J-c}^2\text{J/c}^2\text{J bg}^1\text{J}^1\text{bg}^1\text{J})\) which was exposed for 6 days to continuous white light of 150 foot-candles. Essentially all of the outer segments and most of the inner segments \((\ast)\) are gone, and many of the photoreceptor nuclei which remain are pyknotic (asterisks). Note the giant granules (arrows) in the pigment epithelium. \((\times 1,250).\) B, Retinal pigment epithelium of a 45-day-old albino-beige mouse \(\text{C57BL/6J-c}^2\text{J/c}^2\text{J bg}^1\text{J}^1\text{bg}^1\text{J})\) which was exposed to cyclic white light of 150 foot-candles for 12 hr a day for 5 days. No outer segments remained in the adjacent neural retina. The giant granules were composed mainly of putative membrane remnants \((R),\) probably originating from phagocytized outer segment membranes. Note the microperoxisomes (arrows) and lipid droplets (asterisks). This and all other tissue of this report was incubated for the peroxidatic activity of catalase \(^5\) in order to demonstrate microperoxisomes. \((\times 13,000).\)

The lipid droplets which accumulated in the retinal pigment epithelium of albino-beige and albino mice during light exposure need to be studied for possible relationships with the vitamin A storage droplets common in this tissue. \(^{13}\) It is noteworthy that the lipid droplets, unlike the pigment granules, were not larger in albino-beige mice than in albino mice.

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Key words: retinal pigment epithelium, albino-beige mouse, giant lysosomes, phagocytosis, giant melanosomes, retinal damage by light

REFERENCES


Effects of ascorbate and ATP upon amino acid transport in the toad’s cornea.

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We have examined the effects of ascorbate upon amino acid uptake by the in vitro toad cornea. Physiologic levels of ascorbate increase the uptake of leucine by approximately 35% but have no effect upon the uptake of alanine. Uncouplers of oxidative phosphorylation do not inhibit the stimulation by ascorbic acid of leucine accumulation, indicating the increased synthesis of ATP is not the mechanism; exogenous ATP, unlike ascorbate, stimulates the uptake of both alanine and leucine. Carbon monoxide blocks the effects of ascorbate, whereas 2-heptyl-4-hydroxyquinoline-N-oxide (HOQNO), which inhibits “reverse” electron transfer, enhances the accumulation of leucine. The evidence suggests that ascorbate serves as an energy source for the uptake of leucine.

The mechanisms by which the cornea accumulates amino acids have been examined by several investigators. It has been shown that the rabbit cornea accumulates α-aminoisobutyric acid (AIB) against a concentration gradient. The toad cornea transports amino acids by three separate transport mechanisms. In our studies of the toad cornea we have found that lactic acid causes a large increase in the accumulation of leucine, while inhibiting the uptake of alanine, and that the uptake occurs through the endothelial, but not the epithelial, surface. Because ascorbic acid, as well as lactic acid, serves as an energy source for the electron-transport–linked uptake of organic solutes by some membrane preparations, we have examined the effects of ascorbic acid upon amino acid uptake in the toad cornea. Since we found an ascorbate-induced increase in the uptake of leucine, we also examined the effects of some inhibitors of electron transport upon the process. Our data suggest that ascorbate stimulates leucine uptake by means of an extramitochondrial electron transport system.

Materials and methods. The entire cornea with a ring of sclera was excised from the globe of Bufo marinus of Colombian origin (Tarpon Zoo, Tarpon Springs, Fla.) and incubated at room temperature (about 25° C) as described previously. The incubation medium contained 1.0 µCi of the requisite amino acid ([14C]-labeled AIB, leucine, or alanine), 5.0 µCi of [3H]-inulin (4.21 Ci/gm) or [3H]-mannitol (2.65 Ci/mol), and unlabeled amino acids in concentrations ranging from 10 µM to 10 mM. After a period of incubation, the tissue was removed and prepared for liquid scintillation counting. In each sample, quenching of [3H] and [14C] was corrected by means of the channels-ratio method. In every case, one cornea from each toad was treated as an experimental tissue, and the contralateral cornea of the same animal was used as its control. The extracellular space was calculated for each tissue by means of [3H]-inulin or [3H]-mannitol content, and this value was used to correct for extracellular [14C]-labeled amino acid in each tissue. Antimycin A and 2-heptyl-4-hydroxyquinoline-N-oxide (HOQNO) were obtained from commercial sources. Dehydroascorbic acid was freshly prepared for each experiment by a modification of the method of Frey et al. For the measurement of the levels of ascorbic acid in the aqueous humor, samples were obtained from freshly pithed toads, and ascorbic acid was measured by the diazo coupling method of Smith.

Results. The accumulation of amino acids was measured in paired corneas, with one tissue serving as a control. As indicated in Table I, addition of ascorbic acid to the bathing medium induced a small but significant increase in the amount of AIB accumulated (Table I). Previous studies in this laboratory have shown that AIB is transported by at least two separate systems in the toad cornea.