togenicity is not required by law. It is very difficult to ascertain what chemical additives are present in a garment. Our study demonstrates the absorption of a biologically hazardous chemical from fabric into people. This absorption and the presence of a wide variety of additives in fabrics suggests the need for fabric labeling to identify additives, and toxicological testing before marketing.

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References and Notes

1. A. Blum and B. N. Ames, *Science* **195**, 17 (1977).
2. M. J. Prival, E. C. McCoy, B. Gutter, H. S. Rosenkranz, *ibid.*, p. 76.
3. *National Cancer Institute Carcinogenesis Technical Report Series No. 76* (U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, 1978), Publication No. (NIH) 78-1326.
4. R. K. Haroz, personal communication; B. L. VanDuuren, G. Leowengart, I. Seidman, A. C. Smith, S. Melchionne, *Cancer Res.*, in press.
5. R. E. Osterberg, G. W. Bierbower, R. M. Hehir, *J. Toxicol. Environ. Health* **3**, 979 (1977).
6. R. J. Feldman and H. I. Maibach, *J. Invest. Dermatol.* **54**, 339 (1970); also, see (*11*) for further references and calculations on skin absorption.
7. R. W. Morrow, C. S. Hornberger, A. M. Kligman, H. I. Maibach, *Am. Ind. Hyg. Assoc. J.* **37**, 192 (1976).
8. A. G. Ulsamer, W. K. Porter, R. E. Osterberg, *J. Environ. Pathol. Toxicol.*, in press.
9. F. A. Daniher, Information Council on Fabric Flammability, Tenth Annual Meeting, 10 December 1976.
10. L. E. St. John, M. E. Eldefrawi, D. J. Lisk, *Bull. Environ. Contam. Toxicol.* **15**, 192 (1976).
11. N. K. Hooper and B. N. Ames, in *Regulation of Cancer-Causing Flame-Retardant Chemicals and Governmental Coordination of Testing of Toxic Chemicals*, Serial No. 95-33 (Government Printing Office, Washington, D.C., 1977), pp. 42–45.
12. R. C. Dougherty and K. Piotrowska, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1777 (1976).
13. E. C. Horning, M. G. Horning, D. I. Carroll, I. Dzidic, R. N. Stillwell, *Anal. Chem.* **45**, 936 (1973).
14. I. Dzidic, D. I. Carroll, R. N. Stillwell, E. C. Horning, *ibid.* **47**, 1308 (1975).
15. E. C. Horning, D. I. Carroll, I. Dzidic, S-N. Lin, R. N. Stillwell, J-P. Thenot, *J. Chromatogr.* **142**, 481 (1977).
16. Sleepwear, size 6X, Bates-Jama, flame retardant, 100 percent dacron polyester, was purchased at Capwell's Department Store in November 1976. Urine samples were collected between 2 and 14 December 1976.
17. On the assumption that the child weighed 20 kg and produced 600 ml of urine per day containing 20 ng/ml, then the 2,3-dibromopropanol excreted would be 12 μg/day or 0.6 μg/kg per day.
18. A. G. Ulsamer, W. J. Porter, Jr., R. E. Osterberg, Seventeenth Annual Meeting of the Society of Toxicology, 12 to 16 March 1978, San Francisco, Calif.
19. H. I. Maibach, R. J. Feldman, T. H. Milby, W. F. Serat, *Arch. Environ. Health* **23**, 208 (1971).
20. B. Gutter and H. S. Rosenkranz, *Mutat. Res.* **56**, 89 (1977).
21. R. A. Valencia, Ninth Annual Meeting of the Environmental Mutagen Society, 9 to 13 March 1978, San Francisco, Calif.
22. H. F. Stich, personal communication.
23. M. Furukawa, S. F. Sirianni, J. C. Tan, C. C. Huang, *J. Natl. Cancer Inst.* **60**, 1179 (1978).
24. NIOSH Conference on DBCP, 20 and 21 October 1977, Cincinnati, Ohio; D. Whorton, R. M. Krauss, S. Marshall, T. H. Milby, *Lancet* **1977-II**, 1259 (1977).
25. H. S. Rosenkranz, *Bull. Environ. Contamin. Toxicol.* **15**, 192 (1976).
26. W. A. Olson, R. T. Huberman, E. K. Weisburger, J. M. Word, J. H. Weisburger, *J. Natl. Cancer Inst.* **51**, 1993 (1973).
27. T. R. Torkelson, S. E. Sadah, V. R. Rowe, J. K. Kodama, H. H. Anderson, G. S. Anderson, G. S. Loquvam, C. H. Rine, *Toxicol. Appl. Pharmacol.* **3**, 545 (1961).
28. Fyrol FR2 is the commercial name of tris(1,3-dichloro-2-propyl)phosphate, manufactured by Stauffer Chemical Company. Apex Chemical Company sells this compound under the name of Emulsion 212 for use in children's sleepwear. Fyrol FR2 has been shown to be a mutagen in *Salmonella* [M. D. Gold, A. Blum, B. N. Ames, *Science* **200**, 785 (1978)].
29. We thank Lois Gold, Robert H. Harris, Kim Hooper, Karen Kenyon, and Cedric Shakleton for their considerable help on the project. Supported by DOE contract EY-76-S-03-0034-PA156 to B.N.A.; NIH grants GM-13901 and HL-17269 and grant Q-125 of the Robert A. Welch Foundation to E.C.H.

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Phagocytosis in the Retinal Pigment Epithelium of the RCS Rat

Abstract. *The retinal pigment epithelium of RCS rats, previously thought not to phagocytize photoreceptor outer segments, exhibited a peak of phagocytosis in vivo when animals were kept under conditions of cyclic lighting (12 hours of darkness and 12 hours of light). This peak occurred at 1 hour after the onset of light, with maximum and minimum levels of phagocytosis averaging about 5 percent of that found in the pigment epithelium of Osborn-Mendel rats used as a control. Eyecups that were obtained from Osborn-Mendel rats and maintained for up to 3 hours in organ culture demonstrated levels of phagocytosis that were sevenfold greater than those of unincubated controls. Likewise, a tenfold increase occurred in incubated as opposed to unincubated RCS eyes, raising the possibility that phagocytosis could be experimentally stimulated in vivo.*

Although the retinal pigment epithelium of the RCS rat has been thought not to phagocytize the debris shed from rod outer segments (*1*), we now present evidence that such phagocytosis occurs, but at a greatly diminished rate relative to that of the normal rat. Furthermore, the apparent rate of phagocytosis in RCS pigment epithelium varies with a diurnal rhythm similar to that of unaffected animals (*2*), and the rate can be increased by maintaining the eyecup in organ culture.

Table 1. Number of phagosomes in pigment epithelium of OM and RCS rats. Each value represents the mean ± standard error of the mean of phagosomes per 180-μm field from six sections per eye for two eyes. In all experiments the lights were on between 0700 and 1900. The RCS peak at 0800 is significantly ($P < .005$, by one-way analysis of variance) different from the other levels.

Time of enucleation	Large phagosomes per 180 μm	
	OM	RCS
0600	4.9 ± 0.36	0.54 ± 0.03
0630		0.23 ± 0.03
0700	4.4 ± 1.7	0.49 ± 0.14*
0730	9.3 ± 1.4	0.65 ± 0.29*
0800	29.0 ± 5.2	1.58 ± 0.23*
0830	29.4 ± 1.8	0.90 ± 0.20*
0900		0.40 ± 0.12*
0930	12.0 ± 8.0	0.52 ± 0.16*
1100	6.3 ± 0.7	0.44 ± 0.15

*Average of two separate experiments (four eyes).

Phagocytosis is an integral part of the process of photoreceptor renewal, in which new discs are added to the base of the rod outer segment and packets of old discs are shed at the apex (*3*). The shed discs are phagocytized by the retinal pigment epithelium and, if the animals are maintained under cyclic lighting (12 hours of light and 12 hours of darkness), the bulk of this shedding occurs in a peak within 2 hours of the beginning of the light phase of the cycle (*2*, *4*). If the process of phagocytosis is defective, an imbalance can result between synthesis and degradation of outer segment membranes, leading to an accumulation of outer segment debris and eventual photoreceptor death. Such a defect occurs in the RCS rat (*1*), in which a genetic abnormality leads to the accumulation of rod outer segment debris from postnatal day 12 through 3 months of age, when few photoreceptor cells survive. No evidence has been reported of phagocytosis in the pigment epithelium of eyes of intact, affected animals (*1*), although the pigment epithelium of such animals can phagocytize carbon particles and, in rare cases, exogenous outer segment fragments which have been introduced subretinally (*5*). Since it has been demonstrated that the genetic lesion is in the pigment epithelium (*6*), cultures of RCS pigment epithelium were studied and

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found to exhibit normal phagocytosis of polystyrene spheres and infrequent phagocytosis of outer segment fragments (*7*).

To determine whether phagocytosis occurs in the pigment epithelium of the RCS rat under in vivo conditions, we tested whether a peak occurs in the normal rat retina soon after the onset of light and at various times in the light cycle. Having observed elevated levels of phagocytosis in the pigment epithelium of normal rats when the eyecups were maintained in vitro for 1 to 3 hours, we used organ culture to evaluate shedding and phagocytosis in eyecups from RCS rats.

All animals in this study were supplied by the NIH Small Animal Facility, were 3 weeks of age, and were raised from birth under conditions in which the lights were on between 0700 and 1900 hours. Littermates from either the dystrophic, pink-eyed, tan-hooded (PETH) strain of RCS rats or the normal Osborn-Mendel (OM) strain of albino rats were used in each experiment. Eyes were enucleated and processed for electron microscopy directly or maintained in culture (*8*) before being processed (*9*). Phagosomes in the pigment epithelium were counted by light microscopy (*10*).

Phagocytosis in the pigment epithelium of OM rats in vivo shows a sixfold increase in phagosomes at 0830 or 1½ hours after the onset of light (Table 1). Phagosome counts were slightly lower than those reported by LaVail (*2*) because of our more stringent scoring criteria and the use of 0.5-μm sections instead of the 1.5-μm sections of LaVail. Table 1 also shows that a peak of phagocytosis occurs in the RCS at 0800, 1 hour after the onset of light, at a level three times above baseline. This difference is statistically significant ($P < .01$ by t-test for two means; $P < .005$ by one-way analysis of variance). Phagosomes in the RCS pigment epithelium (Fig. 1a) strongly resemble those of the OM pigment epithelium (Fig. 1b). The RCS phagosomes can be recognized in various stages of ingestion (Fig. 2a) by electron microscopy. Phagosomes demonstrating a typical lamellar pattern (Fig. 2b) could be recognized which were totally surrounded by cytoplasm and embedded 1 μm or deeper into the cell. Residual bodies displaying compressed lamellar features (Fig. 2c) were visible at all times, but were most numerous in tissue taken later in the cycle.

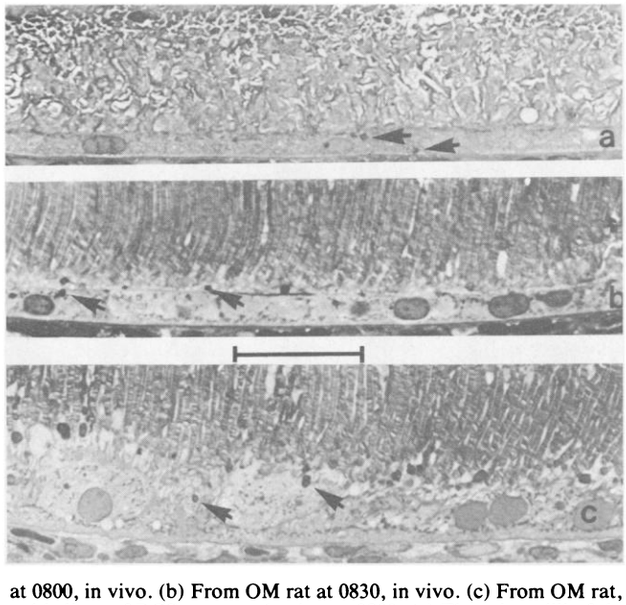
Table 2. Phagosomes in pigment epithelium from OM and RCS rats. The eyecups were either intact (0 hours of incubation) or incubated in vitro. Counting and lighting conditions are as in Table 1.

Time of enucleation	Hours of incubation	Large phagosomes per 180 μm
	OM strain	
0600	0	14 ± 1.5
0600	3	57 ± 2
0700	3	55 ± 13
0800	3	55 ± 0.5
0800	0	29 ± 5
1100	0	6 ± 0.7
1100	1	23 ± 2
1100	2	42 ± 7
1100	3	43 ± 3
	RCS strain	
1100	0	0.4 ± 0.2
1100	3	4.6 ± 0.7

Shedding and phagocytosis of ROS fragments appear to be stimulated when OM eyecups are maintained in organ culture. For example, intact eyes showed the expected increase in the number of phagosomes between 0600 and 0800 (Table 2), but eyecups isolated at these times and incubated for 3 hours all had identical numbers of phagosomes at a level almost double that of the control eyes at 0800. In addition, incubation of the eyecups isolated at 1100, when the phagosome number in intact eyes had dropped to preshedding levels, produced an elevated number of phagosomes, but only about two-thirds of that seen earlier in the day. The number of phagosomes under these conditions showed a distinct elevation by 1 hour and reached a maximum by 2 hours of incubation. When the incubation was performed with RCS eyecups, an increase of phagocytosis of about tenfold was observed (Table 2), showing that phagocytosis can be enhanced in the RCS pigment epithelium.

The mechanisms of the enhancement of phagocytosis in vitro are not clear. The increase in phagocytosis may reflect changes due to the separation of the eye from the hormonal system of the body, or may merely indicate the presence of trauma to the eyecup.

It has been reported that the OM rat undergoes a spontaneous retinal degeneration (*11*), with an onset at between 7 and 10 months of age. Since the animals in this study were only 3 weeks of age,



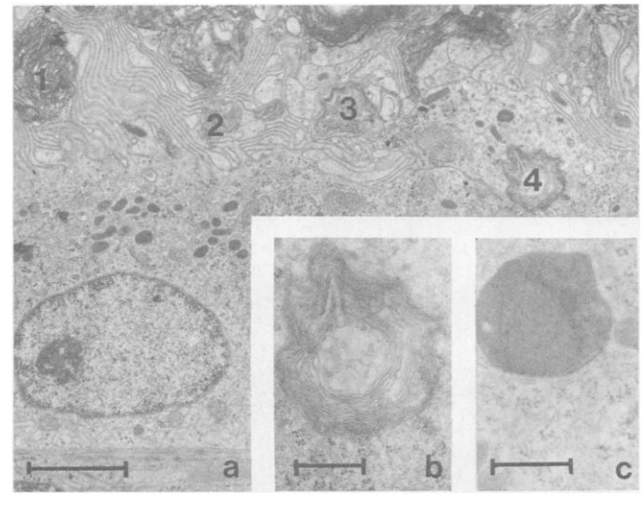


Fig. 1 (left). Phagosomes (arrows) in pigment epithelium of eyes enucleated at various times and either fixed immediately or maintained in vitro. The bar represents 25 μm in all micrographs. (a) From RCS rat, at 0800, in vivo. (b) From OM rat at 0830, in vivo. (c) From OM rat, enucleated at 0700, maintained in vitro for hours. Fig. 2 (right). Pigment epithelium of RCS rat, enucleation at 0800. (a) The microvilli of the pigment epithelium enclose packets (1 to 3) of lamellar debris. One phagosome (4) has been totally engulfed by the cell. The bar represents 2 μm. (b) Phagosome at a higher magnification. (c) Residual body showing compressed lamellar features. The bars in (b) and (c) represent 0.5 μm.

this degeneration was not a factor in our experiments. No evidence of such damage was observed in the eyes we studied, and the patterns of shedding and phagocytosis obtained with the OM rats were similar to those obtained by LaVail (*2*) in Fisher rats.

We have demonstrated that not only is the RCS pigment epithelium capable of phagocytosis, but that it normally phagocytizes small amounts of endogenous outer segment debris in a daily burst similar to that of the normal rat. The levels in vivo average about 5 percent of that of OM rats under comparable conditions. Furthermore, the phagocytosis can be increased in vitro to a level comparable to that in normal intact rat eyes before the burst of shedding (that is, about 5 phagosomes per 180 μm). The observations of residual bodies in the dystrophic pigment epithelium strongly suggest that not only does phagocytosis take place, but that the digestion of these phagosomes also occurs. Thus it is clear that the pigment epithelium of the RCS rat is impaired but not lacking in its ability to phagocytize the debris from the shed rod outer segments. This raises the possibility that the phagocytic rate could be manipulated (in vivo) to achieve normal levels and, thus, retard or prevent the progress of the retinal degeneration.

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References and Notes

1. W. L. Herron, Jr., B. W. Riegel, O. E. Myers, M. L. Rubin, *Invest. Ophthalmol.* **8**, 595 (1969); D. Bok and M. O. Hall, *J. Cell Biol.* **49**, 664 (19➞ M. M. LaVail, R. L. Sidman, D. O'Neil, *ibid.* **53**, 185 (1972); M. M. LaVail and B. A. Battelle, *Exp. Eye Res.* **21**, 167 (1975).
2. M. M. LaVail, *Exp. Eye Res.* **23**, 277 (19➞ *Science* **194**, 107 (1976).
➞ R. W. Young, *J. Cell Biol.* **33**. 61 (1967); _____ ➞ D. Bok, *ibid.* **42**, 392 (19 ➞ M. O. Hall, D. Bok, A. D. E. Bacharach, *J. Mol. Biol.* **45**, 397 (1969); R. W. Young, *J. Cell Biol.* **49**, 303 (1971).
4. S. Basinger, R. Hoffman, M. Mathes, *Science* **194**, 1074 (1976).
5. N. V. Custer and D. Bok, *Exp. Eye Res.* **21**, 153 (1975).
6. R. J. Mullen and M. M. LaVail, *Science* **192**, 799 (1976).
➞ R. B. Edwards and R. B. Szamier, *ibid.* **197**, 1001 (1977).
8. Animals were anesthetized with sodium pentobarbital, the eyes were enucleated, and the anterior segments were removed in the dark for the dark-adapted animals and in the light otherwise. The eyecups were placed gently in Krebs-Ringer bicarbonate buffer with glycerol as the carbon source and incubated in the dark at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2. After incubation for 1 hour the eyecups were transferred to RPMI-1640 medium (Gibco) to maintain the structural integrity of the retinas. The initial use of the Krebs buffer was dictated by an additional set of experiments on the same tissue, but not reported here.
9. Tissue was fixed either in 3 percent glutaraldehyde and 0.5 percent formaldehyde for 2 hours at room temperature or 1 percent glutaraldehyde and 1 percent formaldehyde overnight at 4°C. With both fixatives we used 85 m*M* phosphate buffer, *p*H 7.2. There was no change in phagosome counts that could be attributed to the effect of the fixative. Tissue was postfixed in 1 percent osmium tetroxide, dehydrated in a series of ethanol concentrations, and finally embedded in Araldite 502 resin. Thin (0.5 μm) sections were collected for light microscopy and sections of a silver interference color were collected for electron microscopy.
10. Phagosomes were counted across 290-μm lengths of pigment epithelium, the field of view of the 63X Zeiss Planapochromat oil immersion objective used for the counting. Phagosomes in OM rats were defined as densely staining bodies measuring at least one outer segment diameter in the smallest dimension and located either in the pigment epithelium somas or microvilli. Phagosomes in the RCS rats were defined as being completely contained within the pigment epithelium and having a minimum diameter of at least 1 μm. Although the diameter of an outer segment was found to be 1.5 μm, these bodies are inserted into the pigment epithelium at an angle, so that the projection of an average outer segment onto the pigment epithelium was 1.8 ± 0.3 μm (based on measurements of 37 eyes). Counts are reported as phagosomes per 180 μm, which approximates phagosomes per 100 outer segments.
11. L. von Sallman and P. Grimes, *Arch. Ophthalmol.* **88**, 404 (1972); *Invest. Ophthalmol.* **13**, 1010 (1974).

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Virulent *Naegleria fowleri* in an Indoor Swimming Pool

Abstract. *A reservoir of pathogeniw* Naegleria fowleri *has been located in the cracked wall of a swimming pool where repeated outbreaks of primary amoebic meningoencephalitis were observed between 1962 and 1965.*

An indoor swimming pool in northern Bohemia proved to be the source of the infectious agent in four repeated outbreaks of primary amoebic meningoencephalitis (PAME) between 1962 and 1965 (*1*). In epidemiologic investigations the actual location where pathogenic *Naegleria* survived and multiplied to critical concentrations was not traced. The system of water recirculation and technology was improved, and neither *Naegleria fowleri* nor further cases of PAME occurred in the following years (*2*).

After a 12-year interval, in the winter of 1977 to 1978, several strains of *N. fowleri* highly virulent to laboratory mice were isolated from samples of water from this swimming pool. For new examinations the method by Červa (*3*) as modified by Griffin (*4*) was used (*5*). During 3 months 23 strains of *N. fowleri* were found. The first five strains were isolated from the water near the steps, the bottom of the swimming pool, and the surface of the filter in the recirculation system.

Renewed investigations revealed that the inside front wall in the deep part of the pool was cracked in several places. It was found that this wall had been built many years ago to adjust the length of the swimming pool to exactly 25 m. The isolating layer between the main concrete wall of the swimming pool and the inside tiled surface (Moniér's wall) cracked and a layer of free air approximately 10 cm thick resulted. Later the cracks in the facing enabled water to flow between these walls. The temperature in this space was between 27° and 30°C and the water there was relatively isolated from the influence of any disinfectants. Rich populations of pathogenic *Naegleria* developed on the inner walls of the confined area.

For the purpose of competitive swimming, at weekly or monthly intervals, the water level was raised to about 40 cm above the usual level maintained for recreational swimming. We assume that this periodic fluctuation of the water level flushed some of the pathogenic amoebas from the space between the two walls and that these amoebas then infected the swimmers.

Thick layers of organic material containing *N. fowleri* were found on the walls of the confined space. Eighteen strains of *N. fowleri* pathogenic for mice were isolated from samples of this material.

In our opinion this source of the infectious agent survived from the time of the first epidemic occurrence of PAME in 1962. This space served as a reservoir for the *Naegleria* until it was found years later when the cracks in the wall surface became more obvious. Reconstruction of the wall should remove the risk of occurrence of PAME among swimmers in this pool in the future.

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References and Notes

1. L. Červa and K. Novák, *Science* **160**, 92 (1968). See also the review by E. Willaert [*Ann. Soc. Belge Med. Trop.* **54**, 229 (1974)].
2. The swimming pool was examined for *Naegleria* before 1970.
3. L. Červa, *Hydrobiologia* **38**, 141 (1971).
4. J. L. Griffin, *Science* **178**, 869 (1972).
5. This work will be described in more detail in V. Kadlec, L. Červa, J. Škvárová, *Folia Parasitol. (Prague)*, in press.

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