a fresh fall of sand or mud; this would increase the probability of the proboscis making a recognizable trace on the sediment. If successive sediment falls thick enough to preserve the feeding trace, the animal might be buried and unable to dig itself out. Some echiuroids do not burrow well in soft, newly deposited sediment; influx of a thick layer could either kill them by suffocation or cause them to leave. In either case, because of their restricted openings the burrows would not be filled in by the new sediment, but instead would collapse. The only remnant in the subsurface would be a thin carbonaceous film, evidence of the mucous lining. Several carbonaceous films are present in the Thorold sections, but there are many possible origins aside from collapsed echiuroid burrows. There are also several linear depressions near the feeding traces that may be the result of collapsing burrows.

Echiuroids are exclusively marine, and are found from the intertidal zone (*4–6*) to more than 10,000 m down in the Philippine Trench (*8*). Most are burrowing filter feeders (*Urechis*) or deposit feeders (*Listriolobus, Echiurus*) with a wide range of body types, while some burrow into shells of other benthic invertebrates. Joysey (*9*) described possible echiuroid borings in a Cretaceous echinoid test. Most burrowing echiuroids (*Listriolobus* is an exception) construct U-shaped tubes of varying depths: about 14 inches (35 cm) for *Urechis* (*10*) and 50 cm for *Echiurus* (*11*).

High population densities of echiuroids are probably characteristic of shelf depths. In the deep sea, the transition from a bathyal to a hadal or ultra-abyssal fauna is characterized by an increase in the number of echiuroid species (*12*).

If these traces have been made by echiuroids, their major paleoecological contribution may lie in the fact that echiuroids are stenohaline (*4*); there are few known occurrences of echiuroids in shallow water subject to periodic feshwater influxes. Boron analyses of shale interbeds in the Thorold have already indicated a normal marine environment (*13*).

I have recently collected traces from the Guelph and Lockport dolomites in the vicinity of Dundas, Ontario, which closely resemble the Thorold traces. It is possible that echiuroids were significant members of the bottom fauna of the shallow Paleozoic seas, and that their presence has not been recognized. Even if feeding traces are absent, the presence of echiuroids could be established by carefully searching for their characteristically sickle-shaped ventral or anal setae.

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References and Notes

1. T. E. Bolton, *Geol. Surv. Can. Mem. 289* (1957).
2. C. J. Sarle, *Proc. Rochester Acad. Sci.* **4**, 203 (1906).
3. A. Seilacher, *Neues Jahrb. Geol. Palaeontol. Abh.* **96**, 421 (1953).
4. T. Gislén, *Lunds Univ. Arsskr. Afd. 2* **36** (No. 10), 1 (1940).
5. W. K. Fisher, *Proc. U.S. Nat. Mus.* **96**, 216 (1946).
6. A. C. Stephen, *Proc. Roy. Phys. Soc. Edinburgh* **22**, 159 (1934).
7. G. E. Farrow, *Symp. Zool. Soc. London No. 28* (1971), p. 455.
8. L. A. Zenkevitch, *Galathea Rep.* **8**, 175 (1966).
9. K. A. Joysey, *Palaeontology* **1**, 397 (1959).
10. G. MacGinitie and N. MacGinitie, *Natural History of Marine Animals* (McGraw-Hill, New York, ed. 2, 1968), p. 184.
11. W. Schäfer, *Ecology and Palaeoecology of Marine Environments* (Oliver & Boyd, Edinburgh, 1972), pp. 308–309.
12. T. Wolff, *Deep-Sea Res.* **17**, 983 (1970).
13. I. P. Martini, thesis, McMaster University (1965).
14. I thank G. V. Middleton and G. E. G. Westerman for bringing the traces to my attention, and A. Bidder, K. Fauchald, R. Fay, R. Given, J. Pilger, R. Woollacott, and R. Zimmer for helpful discussions. K. Fauchald, H. J. Hofmann, G. E. G. Westermann, R. L. Zimmer, and C. Teichert critically reviewed the manuscript. Supported by the Research Board of McMaster University. Contribution No. 8 from the Santa Catalina Marine Biological Laboratory.

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Genetic Abnormality of the Visual Pathways in a "White" Tiger

Abstract. *"White" tigers show an inherited reduction of pigment, produced by an autosomal recessive gene. The brain of one of these tigers shows an abnormality of the visual pathways similar to abnormalities that are associated with albinism in many other mammals. There is a close relationship between the reduced pigment formation, the pathway abnormality, and strabismus.*

From time to time, tigers with a reduced amount of pigmentation are seen in the wild. In 1951 one of these "white" tigers was captured in India, and from it a line of white tigers was bred by the Maharaja of Rewa (*1–3*). In many respects the gene controlling coat color in these tigers resembles *chinchilla*, an allele of the *albino* series (*4*). The white tigers have the gray-brown stripes characteristic of a normal tiger, but in place of the normal yellow, they have an off-white stripe. The white tends to be darkened by low environmental temperatures (*3*). The tigers have reduced pigment in the iris, which looks blue, and the gene controlling these features appears to be an autosomal recessive (*2*).

One of these tigers was brought to the Smithsonian Institution's National Zoological Park, Washington, D.C., and several tigers have been bred from her. One of these, Rewati (see cover), is currently there, and we become interested in the white tigers when it was pointed out to us that Rewati has a strabismus (*3, 5*). Siamese cats, which are homozygous for an allele of the *albino* series (*4*), also commonly have a strabismus, and we have shown that this abnormality is related to an abnormality of the central visual pathways (*6, 7*). In Siamese cats, some of the nerve fibers that come from the temporal part of the retina go to the opposite side of the brain, instead of staying on their own side as is normal. We have found this abnormality in all Siamese cats, even those that are not obviously cross-eyed. It is probable that the size of the abnormality is related to the strabismus (*7*).

The abnormality is not confined to Siamese cats. Among carnivores, similar abnormalities have been found in albino ferrets (*8*) and mink (*9*), and albinos of every other mammalian species that has been studied also show the abnormality (*9, 10*). In addition, some mink that have a wild-type gene at the *albino* locus, but lack pigment due to the action of other genes, also have the abnormal pathway (*11*).

In carnivores, which have a clearly laminated lateral geniculate nucleus, the geniculate layers that normally receive their input from the ipsilateral eye are broken up in the abnormal individuals. These layers, instead of forming a continuous cell mass as is normal, form distinct islands, and the islands that receive an abnormal contralateral input tend to fuse with adjacent layers that normally receive a contralateral input. Thus, it is possible to look at the laminar

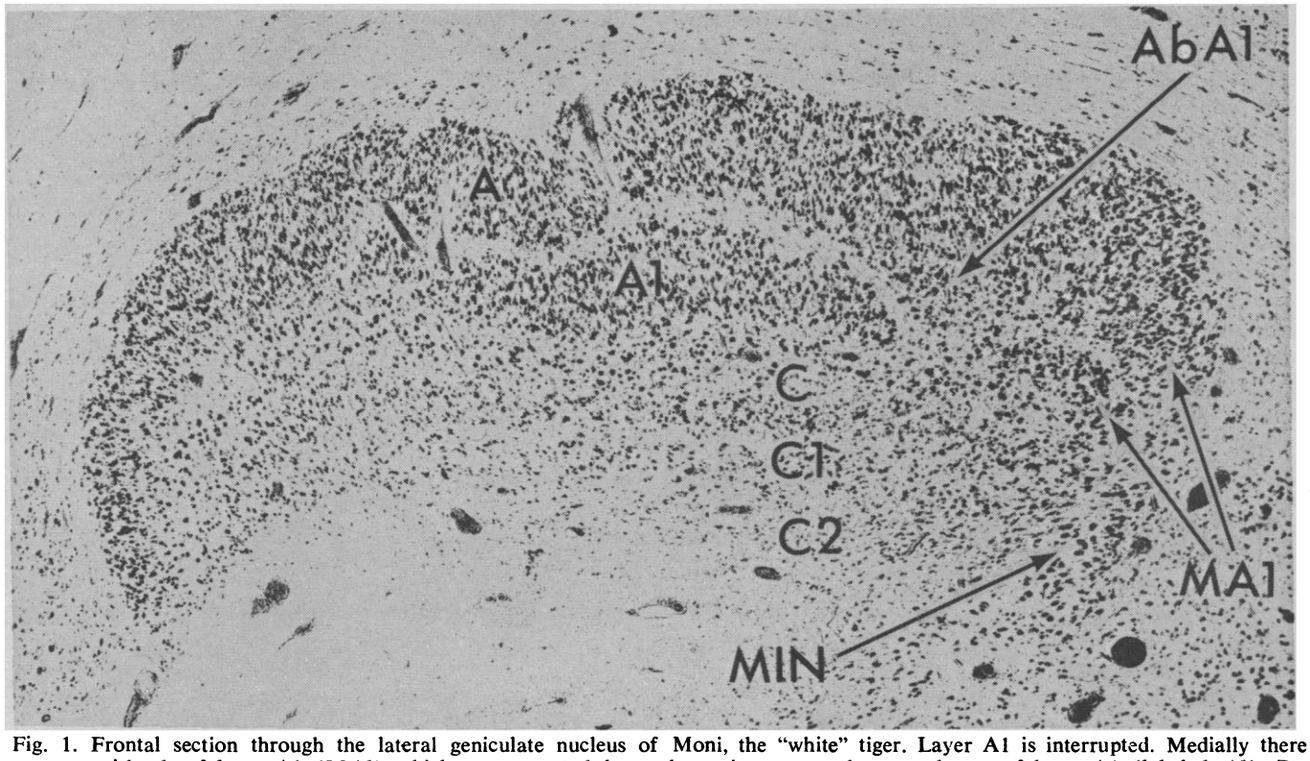

Fig. 1. Frontal section through the lateral geniculate nucleus of Moni, the "white" tiger. Layer A1 is interrupted. Medially there are some islands of layer A1 (*MA1*) which are separated from the main, apparently normal part of layer A1 (labeled *A1*). Between these two there is a region where layer A1 appears to be fused with layer A (*AbA1*). The C layers are labeled in the ventral parts of the nucleus; *MIN*, medial interlaminar nucleus.

structure in a carnivore brain and determine whether it shows the "albino" abnormality.

We have been able to obtain the brain of one of the white tigers, Moni, a younger brother of Rewati. His eyes were normally aligned, and he died at the National Zoological Park when he was 16 months old. The brain was embedded in celloidin, and frontal sections were stained with thionin. The overall laminar pattern (Fig. 1) is very much like that of other felines (*12*). The layers that characterize the feline brain—A, A1, C, C1, and C2 (*13*)—stand out particularly well. However, layer A1 is clearly disrupted, as in a Siamese cat. There are medial islands (*MA1* in Fig. 1) and a large lateral island (*A1*) of layer A1, and in between there is an unusually broad piece of layer A (*AbA1*), which probably represents the region where the abnormal part of layer A1 fuses with layer A. The disruption of layer A1 is less extensive than that generally seen in a Siamese cat, an observation suggesting that the abnormal patch of the retina is smaller. However, there can be little doubt that layer A1 is abnormal. Although we have not been able to compare this brain with another brain from a normally pigmented adult tiger, we have looked at an infant tiger brain, the brains of several adult lions, and a variety of other normal carnivores, and have never seen an interrupted layer A1.

This tiger's brain is of some interest because it demonstrates the "albino" abnormality in yet another species. Beyond this, however, the tiger is the most highly pigmented individual that we have found with an abnormal visual pathway. Burmese cats and silver Persians are thought to be homozygous for the *chinchilla* gene (*4*), and in these we have as yet found no clear evidence for any abnormality of the visual pathways. Chinchilla rabbits are normal as well (*14*), but chinchilla mice remain to be studied.

The results obtained from mink show that the *albino* locus is not the only one that can produce the abnormality (*11*). However, the abnormality has so far always been found in association with a reduced amount of retinal pigment. It is of interest to determine how much retinal pigment the tigers have and to find out more precisely how pigment formation can be related to the abnormality. It is possible that the abnormality is related to pigment formation in some simple and quite general manner, and that in man, some forms of strabismus may be produced by a similar deviation of retinogeniculate fibers associated with a reduced amount of pigment formation, either in the body in general, but more probably in the eye itself.

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References and Notes

1. E. P. Gee, *Animals* **3** (No. 11), 282 (1964).
2. I. W. B. Thornton, K. K. Yeung, K. S. Sankhala, *J. Zool.* **152**, 127 (1967).
3. P. Leyhausen and T. H. Reed, *Smithsonian* **2**, 24 (April 1971).
4. A. G. Searle, *Comparative Genetics of Coat Colour in Mammals* (Logos, London, 1968).
5. R. W. Doty first drew our attention to this.
6. R. W. Guillery, *Brain Res.* **14**, 739 (1969); R. Kalil, S. Jhaveri, W. R. Richards, *Science* **174**, 302 (1971)
7. R. W. Guillery and J. H. Kaas, *J. Comp. Neurol.* **143**, 71 (1971).
8. R. W. Guillery, *Brain Res.* **33**, 482 (1971).
9. K. J. Sanderson, *Anat. Rec.* **172**, 398 (1972).
10. D. J. Creel and R. A. Giolli, *Exp. Neurol.* **30**, 411 (1972); R. A. Giolli and M. D. Guthrie, *J. Comp. Neurol.* **136**, 99 (1969); R. W. Guillery, C. Sitthi-Amorn, B. B. Eighmy, *Science* **174**, 831 (1971); R. W. Guillery and M. W. Orsini, unpublished observations on normal and albino hamsters; R. D. Lund, *Science* **149**, 1506 (1965).
11. K. J. Sanderson and R. W. Guillery, in preparation.
12. J. H. Kaas, R. W. Guillery, J. M. Allman,

Brain Behav. Evol. **6**, 253 (1972); K. J. Sanderson, in preparation.
13. R. W. Guillery, *J. Comp. Neurol.* **138**, 339 (1970).
14. K. J. Sanderson, unpublished observations.
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Rejection of Tumor Cells in vitro

Abstract. *The emergence of lymphoblast-like cells, capable of rapidly destroying tumor cells, was observed in primary cultures of an antigenic sarcoma transplantable in strain 13 guinea pigs. It is likely that these cytotoxic cells represent the progeny of lymphocytes sensitive to tumor antigens that had infiltrated the tumor tissue.*

Although the weight of experimental evidence indicates that most tumors are antigenic and elicit an immune response in the host, in only a few cases is the tumor rejected after the onset of tumor growth, and most tumors progress until the host's death (*1*). A number of workers have shown that some tumors are infiltrated with lymphocytes and other types of leukocytes and have ascribed to these cells a role in the host's resistance to the tumor (*2*). We report here that tumor-reactive lymphocytes infiltrating a solid tumor could outgrow the tumor cells in primary cultures through a cytotoxic reaction against the tumor cells.

The tumor used in these studies was a strongly antigenic methylcholanthrene-induced sarcoma D (MC-D), transplantable in inbred Sewall Wright strain 13 guinea pigs (*3*). For the preparation of cell suspensions, nonnecrotic pieces of MC-D were treated with 0.25 percent trypsin (Difco) in spinner-modified Eagle's medium (Difco) at room temperature for 30 minutes. The released cells were collected, sedimented (400*g*, 4°C, 10 minutes), and resuspended at 10^5 to 10^6 cells per milliliter in the complete medium described below. The cells were then cultured in Eagle's minimal essential medium that had been supplemented with nonessential amino acids (Difco) and 200 ml of heat-inactivated fetal calf serum (Gibco) per liter of medium. Penicillin and streptomycin (Difco) were added to final concentrations of 100 unit/ml and 100 μg/ml, respectively; the final medium is referred to as the complete medium (CMEM). All cultures were incubated at 37°C in a water-saturated atmosphere containing 5 percent CO_2. Cell lines of MC-D cultured for 40 days were able to form tumors on reinjection into syngeneic guinea pigs, resulting in death of all animals within 50 days.

During attempts to derive new tissue culture lines of MC-D, foci of rapidly proliferating lymphoblast-like cells (LBC) appeared in some cultures between days 10 and 20; these cells had the capacity to kill all spindle-shaped tumor cells within the ensuing 2 days (Fig. 1). This phenomenon was observed in 8 of 17 primary MC-D cultures, whereas similar changes were absent in 13 primary testis and 8 primary kidney cultures, although some of these normal cell cultures were prepared from tissues of tumor-bearing animals. Tumor cell suspensions prepared by trypsin treatment of solid tumors and stained with Giemsa contained 1.5 to 2 percent small round cells with lymphoid morphology. When LBC did not appear in the primary MC-D cultures within the first 20 days, new tumor cell lines were successfully derived.

Table 1. Cytotoxic effect of lymphoblast-like cells (LBC) on various target cells (TC). The time between culture initiation and addition of LBC was 24 hours in experiments 1 and 2 and 48 hours in the others. Experiments 1 and 5 were performed in triplicate and the others were done in duplicate; MC-D, methylcholanthrene-induced sarcoma D; GP13, strain 13 guinea pig; GPH, Heston strain of outbred guinea pig; B6, C57BL/6J mice.

Origin of TC	LBC per milliliter ($\times 10^{-5}$)	Incubation of LBC with TC (hours)	TC survival (%)
	Experiment 1		
MC-D	1	72	1.6*
GP13 testis	1	72	10.5
GP13 kidney	1	72	26.3
	Experiment 2		
MC-D	1	72	2.9
GP13 testis	1	72	15.5
GP13 kidney	1	72	11.6
B6 testis	1	72	35.3
	Experiment 3		
MC-D	4.4	24	13.8
B6 melanoma	4.4	24	79.6
B6 polyoma	4.4	24	74.7
	Experiment 4		
MC-D	4.4	72	0.0
B6 melanoma	4.4	72	21.0
B6 polyoma	4.4	72	56.2
	Experiment 5		
MC-D	1	48	11.0
GPH testis	1	48	25.9
GPH kidney	1	48	31.7

* Combined statistical analysis of experiments 1 and 2: survival of MC-D compared to testis, $t = 3.5$ ($P = .010$); survival of MC-D compared to kidney, $t = 3.1$ ($P = .014$).

It was repeatedly observed that LBC did not proliferate in the absence of MC-D cells even when fresh medium was supplied, and gradually lost their viability as judged by dye exclusion (0.2 percent trypan blue). However, their proliferative and cytotoxic potentials were rapidly reestablished on addition of MC-D tumor cells to the culture. This finding is considered to indicate that tumor cells, the probable carriers of the antigenic stimulus, were essential for maintaining the activities of LBC. In fact, LBC could be kept in a state of continuous proliferation and cytotoxicity by a steady supply of MC-D cells for more than 6 months.

The cytotoxic effect of these tumor-derived LBC was investigated on various cell lines established from strain 13 and from outbred Heston guinea pigs or C57BL/6J mice. Cultures of target cells were started with 10^5 cells per milliliter on cover slips in Leighton tubes of 1-ml capacity, and LBC (from an actively destroyed primary tumor culture) were added 24 to 48 hours later. The cover slips (10 by 35 mm) were removed at various times of incubation and stained (Wright stain), and the survival rate of target cells was established by counting the cells in ten microscopic fields (longitudinally 3 mm apart) (Table 1). Although produced only in the presence of MC-D cells, LBC were cytotoxic for syngeneic, allogeneic, or xenogeneic target cells. However, the destruction of MC-D cells was more effective and faster than that of other cell types; normal guinea pig cells were also destroyed completely in 3 to 5 days, but mouse cells were never killed out completely from the culture. In general, target cell destruction could be accelerated by increasing the number of LBC in the system. This cytotoxic effect was found to depend upon the number of viable LBC added to the system; supernatants of LBC