explanation for these results. The inability of bone marrow cells treated with antiserum to Thy 1.2 plus C′ to give rise to TSRC may indicate that at least certain thymic lymphoid cells are not derived from the hematopoietic stem cell. On the other hand, Wu *et al.* (*13*) infused W/W^v mice with irradiated +/+ bone marrow cells and found that a proportion of cells from the bone marrow, spleen, thymus, and lymph nodes of the recipient mice all showed the same radiation-induced chromosomal abnormalities. Based on these observations, they reasoned that hematopoietic colony-forming cells, erythroblasts, granulocytes, thymic cells, and cells of the lymph nodes may all constitute the same clone. Further experiments are necessary to resolve these differences.

Our data also indicate some of the characteristics of the TSRC. These cells are sensitive to treatment with antiserum to Thy 1.2 serum, are present in 10^7 of +/+ bone marrow cells, and may constitute less than 1 percent of the total bone marrow, since it has been reported that the frequency of cells sensitive to Thy 1 in mouse bone marrow is below 1 percent (*14*). Also, there must be a sufficient number of such cells present in 10^7 thymocytes and in 10^7 spleen cells.

One of the critical questions in the present study is the use of antiserum to Thy 1.2 prepared by immunizing AKR mice with CBA/J thymocytes, which also may contain another specificity, namely, antiserum to Ly 3.2 (*15*). The Ly 3.2 antigen is present in C57B1/6 parents and, therefore, this specificity may be responsible for the observed effect. It must be mentioned, however, that both Ly 3 and Thy 1 antigens are carried exclusively by T lymphocyte cells. It is also possible that this serum contains other specificities directed against unknown surface antigens expressed on CBA/J but not on AKR thymocytes.

It has been reported (*14*) that mouse bone marrow does not contain mature T cells; therefore, it seems that the TSRC is possibly a T-lymphoid precursor cell type. Moreover, Harrison and Cherry (*16*) and Harrison and Astle (*17*) recently have found, using a T6 chromosome marker, that the thymus in W/W^v anemic mice injected (60 to 90 days after transplantation) with +/+ bone marrow cells is completely repopulated by cells of the donor type. This suggests that the T-cell precursor of the W/W^v mouse is unable to compete with normal T-cell precursors from the +/+ mouse.

Our evidence on the role of TSRC in the correction of the anemia in W/W^v mice prompted us to examine the hematological data available concerning athymic *nu/nu* mice. Values for Hct and RBC in the *nu/nu* mice were reduced but not statistically different from their +/*nu* littermate controls (*18*). However, there is evidence for a reduced number of nucleated cells in the bone marrow, a reduced number of hematopoietic stem cells in the bone marrow, and a reduced life-sparing capacity of the bone marrow in irradiated recipients (*19*). This, together with numerous reports of the stimulatory effect of thymocytes on erythropoiesis (*6–8*), suggests that collaboration of a hematopoietic stem cell (as determined by the CFU-S assay) with the TSRC may be a normal event, at least in mice, and for the erythropoiesis, not only in the W/W^v mice but also in normal strains.

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10. Since treatment of bone marrow cells with antiserum to Thy 1.2 plus C′ results in less than 1 percent net cytotoxicity, which is too small to be detected by routine methods, the efficiency of our preparation of antiserum to Thy 1.2 and C′ in killing theta-sensitive cells was determined by evaluation of the ability or inability of treated spleen cells to respond to the T-cell specific mitogenic agents, such as phytohemagglutinin and concanavalin A. Since the proportion of erythroid precursors in the mouse bone marrow is about 30 percent (*1*), the observation that antiserum to Thy 1.2 and C′ kills an undetectable (net less than 1 percent) proportion of cells in the bone marrow also suggests that such treatment has no substantial cytotoxic effect on erythroid precursors.
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Neoplastic and Possibly Related Skin Lesions in Neotenic Tiger Salamanders from a Sewage Lagoon

Abstract. *Tiger salamanders (*Ambystoma tigrinum*) inhabiting a sewage sedimentation lagoon become neotenic, and approximately one-third develop neoplastic skin lesions including cancer. Circumstances suggest a chemical etiology for the neoplasms.*

An estimated 28,000 (*1*) neotenic tiger salamanders, *Ambystoma tigrinum*, are the only vertebrates inhabiting a small isolated lagoon which is heavily polluted with secondary treated domestic sewage. On an annual basis 30 to 50 percent of this population have had skin lesions, 84 percent of which are neoplasms. The neoplasms are of epidermal, fibrocytic, and melanocytic origin; while most are well differentiated and superficial, some are poorly differentiated and invasive. In contrast, tiger salamanders from uncontaminated lagoons in the same general vicinity metamorphose normally (*1*), and no neoplasms were discovered among 12,600 larvae sampled from 16 proximal nonsewage lagoons over the past 6 years.

The contaminated lagoon, a natural playa centrally located in a grass field on Reese Air Force Base, Hurlwood, Lubbock County, Texas, covers 13 ha at an average depth of 2 m. It was chosen in 1970 for an in-depth ecological study because of its many desirable features: its size, depth, and the density of the salamander population facilitated seining; its

location on a military base gave good long-term stability and protection from the public; and the failure of many animals to metamorphose (*2*) provided a unique opportunity to study the cause of neoteny. During the course of routine sampling in 1970, one larva out of the 2430 sampled was discovered to have a conspicuous fibroma about 30 mm in diameter on the dorsal surface of the tail. Despite a detailed external examination no growths were discovered on any of the other larvae. In 1971, the frequency of obvious external neoplastic and nonneoplastic abnormalities rose sharply to 397 (25 percent) of 1588 larvae examined. Based on five samples in excess of 1000 larvae each (*3*), taken between early 1972 and early 1974, the frequency of external abnormalities held constant. However, following a drought in 1974 which reduced the total water volume an estimated 40 percent below normal, the frequency of abnormalities climbed to 42 percent of 3120 larvae examined in late 1974 and to 53 percent of the larvae examined in March 1975.

Nonneoplastic lesions were ascites, serous cysts, and epidermal inclusion cysts. Ascites was the only condition found in larvae from nonsewage lagoons as well as the sewage lagoon, and the frequency from all localities was identical at 0.2 percent. Ultimately this condition is lethal, for the visceral cavity becomes so distended with fluid that the individual starves. Serous cysts, probably originating from lymphatic vessels, were the most bizarre condition but occurred at a low frequency of less than 0.8 percent. All but one occurred dorsally on the head. They were uni- or bilateral and sometimes became so enormously large (Fig. 1) that feeding behavior was impaired. Epidermal inclusion cysts, which ranged up to 5 mm in diameter, were frequently multiple and sometimes occurred in combination with any of the other skin lesions. Their prevalence was < 1 to 12 percent.

Neoplastic lesions arose from skin epithelium, dermal fibroblasts, or dermal melanocytes. Those arising from epidermis had shaggy surface features and occasionally reached several centimeters in diameter. The smaller, apparently earlier lesions showed prickle-cell hyperplasia (acanthosis), while larger, apparently more advanced lesions consisted of papillary growths with pegs of tumor cells extending deeply within the papillary layer (stratum spongiosum) of the dermis. In contrast to the normal skin, mitoses were much more frequent, goblet cells were absent, basement membrane appeared thinner, and subepidermal mucus glands were small or absent. Normally, the subepidermal mucus glands are composed of cells with small densely chromatic nuclei and exceptionally voluminous cytoplasm packed with eosinophilic, mucinous, intracytoplasmic granules. These glands project from the epidermis into the spongy layer of the dermis and apparently discharge through a fine canal to the surface. Proliferating tumor cells appeared to follow these canals and to replace the subepidermal mucus glands. Invasion of the underlying layer (stratum compactum) of the dermis was not observed. More advanced epidermal growths of this type were interpreted as epidermal papillomas.

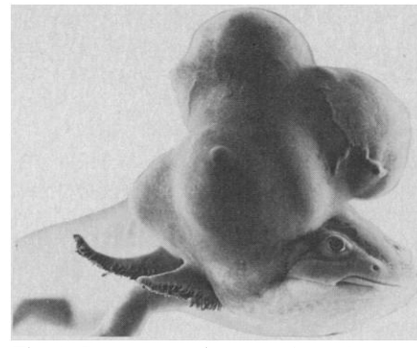

Fig. 1. Serous cyst of a neotenic *Ambystoma tigrinum* collected from a sewage lagoon at Reese Air Force Base.

Neoplasms arising from the dermal fibrous tissue appeared grossly as smooth, gray, often multilobular protuberances. Microscopically they consisted of randomly oriented bundles of fibrocytes. The overlying epidermis was seldom affected. When confined to the dermis, these lesions were interpreted as polypoid dermal fibromas and polypoid dermal myxofibromas, depending on their cellular density. Some fibrous neoplasms breached the basement membrane, and large spindle-shaped cells with pleomorphic, but elongate, basophilic nuclei infiltrated, enveloped, and destroyed the subcutaneous skeletal muscle. These lesions were interpreted as fibrosarcomas.

Neoplasms arising from dermal melanocytes ranged grossly from small, unraised, darkly pigmented spots in the skin to black dome-shaped growths up to 5 cm in diameter. Microscopically, the less extensive lesions consisted of accumulations of elongated branching melanocytes within the spongy outer layer of the dermis and were interpreted as intradermal melanocytomas. Larger lesions extended laterally just under the epidermis and appeared successively to penetrate the compact layer of the dermis, the subcutaneous tissue including the muscle fascia, and even in some cases the skeletal muscle. Examples were seen where neoplastic melanocytes penetrated all the way from one side of the tail to the other. Although metastases were not observed, these advanced lesions were interpreted as invasive melanomas.

Several factors require consideration in relation to the etiology of the lesions observed. The sewage lagoon receives all the domestic sewage from the air force base, but no other direct sources of pollution are known. It is not geographically situated to receive runoff from agricultural lands, and runway wash is supposedly discharged into another lagoon, where salamanders are not found. Prior to this study, foreign material was dumped into the lagoon on four occasions: (i) in 1963 a large pile of asphalt was dumped in, but was removed in 1968 except for numerous fragments still strewn along the bottom; (ii) in 1959 an attempt was made to destroy the salamanders with toxaphene, which can no longer be analytically detected; (iii) on several occasions runway foam was dumped around the lagoon, but, being proteinaceous, it was presumably rapidly degraded; and (iv) diesel oil mosquitocide was occasionally used up to about 1972.

Initial chemical analyses of water, sediment, and tissues were negative for 14 volatile nitrosamines. However, analyses of sediments from the lagoon for polycyclic aromatic hydrocarbons (*4*) revealed 300 parts of perylene (a component of jet fuel) per million and a trace of benzpyrene. Perylene alone is not known to be carcinogenic but, in combination with other polycyclic aromatic hydrocarbons, has been shown to cause low tumorigenic activity in mice (*5*) and rats (*6*). In light of this large concentration its tumorigenic potential needs to be retested with salamanders in an aqueous environment.

Two possibilities could account for the presence of perylene in the lagoon: a small, apparently natural drainage ditch suggests that (i) wash from the runways may have reached the lagoon in the past; and (ii) jet exhausts may contaminate the lagoon. A runway that is heavily used for training flights is located only approximately 100 m away on the windward side. Exhaust odors have been frequently experienced in the vicinity of the lagoon.

Failure of metamorphosis is also an obvious factor in tumorigenesis, since it allows the 14 to 17 months exposure time needed for larval skin to develop neoplasms. Rain-dependent nonsewage la-

goons are intermittent, and selection favors salamanders with the ability to metamorphose early. The larvae inhabiting these natural lagoons characteristically metamorphose at a small size around 6 months of age (*7*). The sewage lagoon, in contrast, has a constant inflow of sewage water, and although the water level fluctuates somewhat with seasonal rainfall, the need for rapid metamorphosis to survive has been eliminated (*7*). Thus the fact that no tumors were found on the thousands of larvae examined from nonsewage lagoons has little value, since the two types of populations are not readily comparable. For the same reasons, it has been impossible to compare animals transferred from one environment to another. When transfers are made from the sewage lagoon to an unpolluted environment, individuals metamorphose in 6 to 9 months and papillomas regress.

The possibility of either an exogenous or endogenous tumor virus being activated is being studied by electron microscopy. So far, several epidermal papillomas have been examined but no virus has been discovered.

Regardless of the factor or factors ultimately determined to be responsible for the etiology, tiger salamanders appear to be sensitive indicators for at least certain types of environmental carcinogens. This suggests they have value for tropical screening of potential carcinogens. Since they can live in sewage, by virtue of having both lungs and gills, perhaps sewage ponds could be stocked as a monitor for carcinogens. Finally, it also suggests that other larval forms of vertebrate and invertebrate animals could be developed for screening carcinogens by hormonally prolonging the time spent in the immature stages.

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Niemann-Pick Disease Experimental Model: Sphingomyelinase Reduction Induced by AY-9944

Abstract. *Organs of rats treated with the drug AY-9944 for 5 days showed a significant reduction in sphingomyelinase activity. Evidence is presented which suggests that the reduction is due to impaired enzyme synthesis.*

The heritable metabolic disorder known as Niemann-Pick disease is characterized by a deficiency of sphingomyelinase activity resulting in the accumulation of sphingomyelin in organs and tissues of afflicted individuals (*1*). Studies with AY-9944 (*trans*-1,4-bis[2-chlorobenzylaminomethyl]cyclohexane dihydrochloride), a compound that causes the accumulation of 7-dehydrocholesterol through its inhibitory effect on 7-dehydrocholesterol reductase (*2*), have revealed lamellated cytoplasmic inclusions and pathological degeneration in oligodendroglial cells (*3*). It recently has been found that cytoplasmic inclusion bodies appear in the retina and optic nerves of rats treated with AY-9944 and that they are similar to those in patients with Niemann-Pick disease (*4*). We now report a significant reduction in sphingomyelinase activity in various organs of rats that received AY-9944 over relatively short periods of time. This reduction in enzymatic activity was accompanied by an accumulation of sphingomyelin in the livers of the treated rats. We present evidence here that suggests that the reduction of sphingomyelinase is due to impaired synthesis of the enzyme. Animals treated with AY-9944 may be useful as experimental models of Niemann-Pick disease.

Albino rats of the Sprague-Dawley strain received daily intraperitoneal injections of 50 mg of AY-9944 per kilogram of body weight, beginning on day 2 after birth. Individual animals were killed from 3 to 25 days after the first injection. Chemical and enzymatic analyses were carried out on liver, kidney, spleen, brain, retina, and lens obtained from injected rats and age-matched controls.

After the administration of AY-9944, lamellar inclusion bodies appeared in the retina, lens, and various ocular cells. Prolonged administration of the compound caused degeneration of the retina and cataractous changes in the lens. Similar lamellar inclusion bodies were found in both glial and neuronal cells in the brain, in reticuloendothelial cells in the spleen, and in the Kupffer cells in the liver. The inclusion bodies consisted of concentrically arranged membranes measuring 75 Å. Many appeared as conglomerate large masses. The inclusion bodies are similar to those that occur in sphingolipidoses such as Niemann-Pick disease, GM_1 and GM_2 gangliosidosis, and metachromatic leukodystrophy.

There was a 30 percent reduction in

Table 1. Sphingomyelin in the livers of rats treated with AY-9944 from day 2 after birth. Phospholipids were extracted and analyzed according to Marinetti (*10*). Sphingomyelin was quantified as described (*11*) after separation into the faster (SP-L) and slower (SP-C) migrating molecular species. The values represent the mean and standard deviation of four determinations.

Item (wet weight)	Age of rats					
	7 days		12 days		19 days	
	Control	Treated	Control	Treated	Control	Treated
Total phospholipids (mg/g)	20.6 ± 1.0	23.9 ± 0.88	23.8 ± 0.6	22. ± 0.5	28.7 ± 1.1	43.9 ± 2.3
Total sphingomyelin (mg/g)	1.5 ± 0.1	2.0 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	1.6 ± 0.2	3.0 ± 0.3
SP-L (mg/g)	1.14 ± 0.14	1.4 ± 0.09	1.22 ± 0.08	1.35 ± 0.05	1.3 ± 0.18	2.04 ± 0.13
SP-C (μg/g)	402. ± 7	568. ± 60	380. ± 62	669. ± 83	346. ± 71	1011 ± 147