cur a day later. Even so, it is curious that these differences in adjacent areas of a colony exist for a period despite considerable cytoplasmic streaming throughout the *Neurospora* colony.

The total pyridine nucleotide content of the two areas was approximately the same (3.9 ± 0.2 μmole/g), but the composition of these two areas differed. The ratio of NAD plus NADH to NADP plus NADPH changed from approximately 3 : 1 in the interband area to approximately 5 : 1 in the band area. More important, the ratio of oxidized to reduced nucleotides differed from one area to another. In the interband area, the ratio of NAD to NADH was approximately 4 : 3; whereas in the band area it was approximately 9 : 3. On the other hand, the ratio of NADP to NADPH was approximately 2 : 3 in the interband region and approximately 1 : 3 in the band region. It is not clear why the two redox couples changed in opposite directions (the NAD ratio toward the oxidized, and the NADP ratio toward the reduced). In contrast, previous studies indicated that neither of these ratios changed in mutants with an altered glucose-6-phosphate dehydrogenase or in strains starved of or supplemented with nicotinic acid, a precursor of the pyridine nucleotides (*11*). The redox changes reported here differ from those in yeast cells in that the latter had a periodicity in minutes and only involved approximately 10 percent of the total pyridine nucleotide content (*12*).

Although the 30 to 50 percent change in content of some of the pyridine nucleotides may seem too small to be of general significance, even smaller changes in pyridine nucleotide levels can lead to a dramatic and gross change in the growth rate and morphology of a *Neurospora* colony (*9*). It may be hard to detect these spatial differences in organisms with a different life-style. In *Neurospora*, the spatial localization of the band areas from the interband areas permits one to physically separate and assay these two areas. If the entire colony rather than the separate areas were assayed at 53 or 77 hours, one might expect an NADH value intermediate between the values for the two areas. Furthermore, the NADH level in the total colony might change only slightly as the proportion of bands to interbands changed; that is, as the colony expanded. Therefore, measurements of total NADH content as a function of time might not indicate changes as large as those observed here. It would be interesting to test these predictions, as well as to see if there are any finer spatial differences or gradients in pyridine nucleotide composition within a given band or interband area.

The finding of these spatial differences raises the questions of whether these changes (i) are caused by the rhythmic conidiation; (ii) trigger the conidiation process; or (iii) are just correlated with this process. None of these three questions can be definitively answered now. In reference to the first question, the enzyme NAD glycohydrolase, which is found in large quantities in conidia and is associated with the conidiation process (*13*), does not appear to play a significant role in causing these spatial differences. This conclusion is based on the observation that the NAD level is higher in the conidiating areas than in the interband region (Table 1). To determine if other factors, triggered by the conidiation process, bring about the changes in the pyridine nucleotide ratios will require other experimental approaches. The opposite approach—to determine whether there are spatial differences in a growing colony of the wild-type strain with no obvious pattern of conidiation—might also be fruitful.

Regardless of whether the pyridine nucleotide levels are responding to the conidiation rhythm or to some other metabolic oscillation, one can speculate about the types of metabolic changes produced by the changed pyridine nucleotide ratios. In the band region as compared to the interband region, one might expect an increase in the rate of many NAD-requiring reactions, since there would be more substrate (NAD) and less product (NADH). Reactions of enzymes such as NAD-dependent isocitritic dehydrogenase might then produce more CO_2 under these conditions, provided their substrates were not limiting. Another possible effect of the changed ratios would be on the configuration of an enzyme, since pyridine nucleotides can affect the aggregation state of certain enzymes (*14*). Many other possible effects could be listed, such as the role of pyridine nucleotides as allosteric feedback effectors (*15*), but further speculation is probably unwarranted, since it is uncertain whether the changes in the pyridine nucleotide levels or ratios have any of these effects in vivo.

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Leukemia Virus Activation during Homograft Rejection

Abstract. *Activation of murine leukemia viruses, as detected by the mixed culture cytopathogenicity (XC) assay, followed the transplantation of A/J skin onto immunosuppressed BALB/c mice. Virus was found in most of the mice receiving both skin grafts and antilymphocyte serum, but not in animals receiving either the serum alone, skin graft alone, or no treatment.*

Among patients receiving organ transplants, there is an increased incidence of malignant tumors arising de novo months to years after transplantation (*1*). The most common tumors in this population are malignant lymphomas, particularly reticulum cell sarcomas, estimated to occur approximately 4000 times in excess of what would be expected in the general population (*1, 2*). This extraordinary incidence of reticulum cell sarcomas is thought by some investigators to be a result of therapeutic immunosuppression, reducing immune surveillance of malignant cell mutants that are normal-

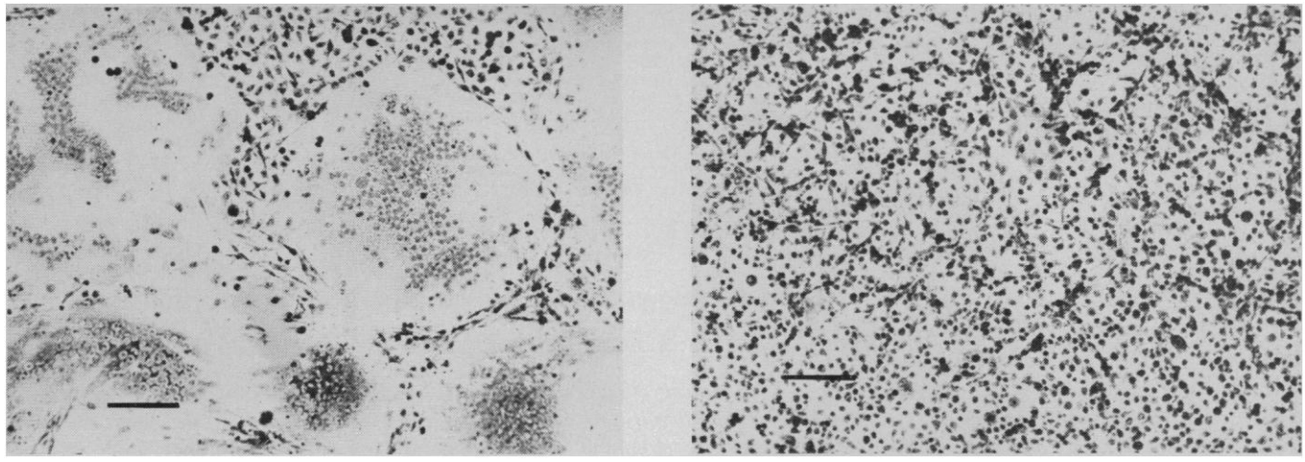

Fig. 1. (Left) Positive mixed culture cytopathogenicity (XC) assay on spleen cell preparation from BALB/c mouse treated with ALS and carrying an A/J skin graft. Multiple giant cells with 50 to 200 nuclei per cell are shown. The scale represents 100 μm. (Right) Control XC assay on spleen cell preparation from normal BALB/c mouse. The scale represents 100 μm.

ly detected and destroyed (*1*). Others have proposed that this mechanism alone is inadequate to explain the skewed distribution of tumor types and the apparently increased incidence of tumors in transplant recipients when compared with other immunosuppressed populations (*2, 3*). It has recently been shown that, in experimental animals, chronic immunological stimulation by foreign histocompatibility antigens can activate latent or repressed leukemia virus genetic material, with subsequent production of infectious oncornaviruses and development of reticulum cell sarcomas (*4*). Thus, transplant recipients are faced with two major assaults on their immunological responsiveness, chronic rejection reactions against histocompatibility antigens and therapeutic immunosuppression, both of which are capable of facilitating viral oncogenesis.

Our experiments were designed to approach the question of which of these two mechanisms, if either, is important in leukemia virus activation and oncogenesis in murine transplant recipients. BALB/c and A/J mice, 6 to 8 weeks of age, were obtained from Jackson Laboratories, Bar Harbor, Maine. The BALB/c mice were divided into four groups. One group was left untreated. A second group was grafted with A/J skin according to the technique of Billingham and Medawar (*5*). Plaster casts were removed on day 6 after the operation and the skin grafts were scored daily by visual and tactile inspection until destruction was complete. A third group received antilymphocyte serum (ALS) alone: 0.25 ml twice weekly intraperitoneally for 2 weeks, and then 0.1 ml twice weekly for 2 weeks. The ALS was prepared in New Zealand rabbits (*6*), and its immunosuppressive activity was tested by its ability to prolong first-set C3H/HeJ skin grafts on A/He mice (*6*); the ALS was also found to be free of neutralizing antibody against AKR leukemia virus using the mixed culture cytopathogenicity (XC) assay of Klement *et al.* (*7*). A fourth experimental group received both an A/J skin graft and twice-weekly ALS as described above, beginning 1 day before grafting.

Four weeks after skin grafts were applied, all animals were killed. Spleens were aseptically removed, individually weighed, and single cell spleen suspensions were prepared. These suspensions were assayed for murine leukemia viruses by the XC assay (*4*); controls in this assay were uninfected NIH Swiss mouse embryo cultures overlain with XC cells. A given sample was considered positive for leukemia virus if the number of multinucleated giant cells per dish was 15 times the mean of the number of multinucleated cells in the control untreated dishes. As an additional control, homogenized skin preparations from A/J mice were also tested for leukemia virus by the same assay.

Leukemia viruses were detectable in the spleens of seven of nine BALB/c mice that maintained their A/J skin grafts for 1 month; all of these animals received ALS during this entire period. Leukemia viruses were also found in the spleens of four of ten mice of this group who had lost their grafts sometime between 12 and 30 days after transplantation despite continued ALS treatment. One of ten mice receiving grafted skin, but no ALS, had detectable splenic leukemia virus; in this unimmunosuppressed group the mean survival time of the grafts was 10 days. None of 20 mice in the control group or in the group receiving only ALS had detectable leukemia viruses by the XC criteria described above. No virus was detected in A/J skin (four mice were tested).

Actual virus titers in the spleens of grafted animals were not determined. However, in virus-positive XC cultures,

Table 1. Incidence of leukemia virus activation after grafting of A/J skin onto BALB/c mice with or without immunosuppression. Spleen weights are mean ± standard error.

Group	Positive/ total	Percent positive	Spleen (mg)
Normal BALB/c controls	0/10	0	108 ± 3
ALS alone*	0/10	0	132 ± 4†
Graft alone	1/10	10	112 ± 2
Graft + ALS*			
Graft maintained	7/9†	78	191 ± 26†
Graft lost	4/10‡	40	197 ± 14†
Total	11/19†	58	194 ± 8†

* The ALS was administered as described in text intraperitoneally twice a week. † $P < .01$ compared with normal BALB/c controls. ‡ $P < .05$ compared with normal BALB/c controls.

the fused cells were not only numerous, but extremely large and bizarre, with nuclei ranging from 40 to 200 per cell (Fig. 1). Since the size and number of multinucleated cells in such cultures are dependent on the amount of virus in the culture (*8*), it can be concluded that significant amounts of leukemia virus were activated in these mice. Spleen weights were also larger in the group of mice receiving both grafts and ALS than in any other group ($P < .01$, *t*-test), although there was no clear correlation in individual animals of this group between spleen weight and recoverable virus.

These results are the first to show activation of leukemia viruses after a homograft transplantation. They also demonstrate that activation of leukemia viruses by immunologic reactions to histocompatibility antigens is not confined to long-term graft-versus-host disease (*4*), but applies to host-versus-graft reactions as well. The observation that both immunosuppression and rejection reactions appeared necessary for maximum virus activation suggests that these two processes may complement each other in the mouse transplant recipient. It would appear that virus becomes activated by the immune reaction and once activated becomes amplified by the recipient's inability to immunologically eliminate it. Whether this sequence will subsequently lead to an increased incidence of malignant lymphomas in these mouse transplant recipients remains to be determined. It is also intriguing to speculate that a similar series of events may account for the frequent appearance of various viral infections and malignant lymphomas in human transplant patients undergoing therapeutic immunosuppression (*1*, *9*).

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Arrested Development in Human Hookworm Infections: An Adaptation to a Seasonally Unfavorable External Environment

Abstract. *Contrary to general belief, larvae of* Ancylostoma duodenale *do not always develop directly to adulthood upon invasion of man. In West Bengal, India, arrested development appears to be a seasonal phenomenon which results in (i) reduction of egg output wasted in seeding an inhospitable environment and (ii) a marked increase in eggs entering the environment just before the monsoon begins.*

In man, arrested development of helminths is generally believed to occur among abnormal parasites, which, depending on the species, may survive for extended periods of time. This phenomenon is recognized clinically as larva migrans. As regards hookworms specifically, the larvae of *Ancylostoma braziliense*, a parasite of dogs and cats, wander without maturation in the skin of man, and recognition of their survival and movement is possible since they leave visible, raised, erythematous tracks. Some larvae become temporarily inactive and drug resistant, and Stone and Willis (*1*) suggest that these enter a dormant state, during which their metabolic rate is decreased. They speculate that man's own species of hookworms may become dormant, but that if this happens, it would be difficult to demonstrate, as visible signs (tracks) do not occur. If such dormancy occurs in normal hosts, the epidemiology of human ancylostomiasis is much more complicated than presently believed.

That the parasitic larvae of one of the anthropophilic species of hookworms, *Ancylostoma duodenale*, have the capacity to become latent and subsequently resume development in man is suggested by our recent investigations of ancylostomiasis in West Bengal, India. Larvae acquired during the rainy season of one year appear to remain dormant until just before the monsoon of the following year, when they resume development and mature.

A seasonal investigation of the variation in egg counts in Indian villagers was conducted in Hooghly District, West Bengal, in 1969–1970 (*2*). The monsoon began in June, but the fecal egg counts had already shown a sharp, statistically significant ($P < .005$) increase during the round of stool examination begun in late April and completed in early June (Fig. 1A); in fact, the most marked increase occurred at this time. Were this rise attributable to a proximal increase in exposure, then, considering the 6- to 8-week period required for maturation within the host, the density of infective larvae on the soil surface should have increased sharply in March and April. A parallel investigation of larval abundance and distribution (*3*), based on Beaver's gauze-pad technique (*4*), recovered *no* larvae in March, although larvae were recovered by comparably intensive sampling during the rainy season (Fig. 1B). Meteorological data (Fig. 1C) support Chandler's opinion (*5*) that the premonsoon showers do not provide enough moisture to sustain infective larvae in sufficient abundance to cause a marked increase in the rate of acquisition. The showers of March fell on parched soils which had been losing water since November; furthermore, in March the mean maximum air temperature attained 34°C and the mean weekly daytime temperature at the soil surface ranged from 30° to 39°C. The impact of 30.5 mm of precipitation was inconsequential, and the failure to recover larvae at this time is explicable.

The inference that the premonsoon increase in worm burden reflects transmission which occurred in the previous year is supported by seasonal trends in the rate at which children converted from negative to positive for hookworms (*6*). Some conversions occurred throughout the year (Fig. 2), but a statistically significant increase in the