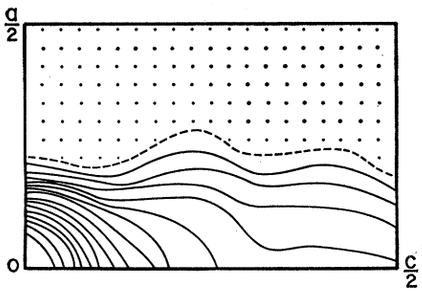

Fig. 4. Patterson section along the zero level of the *b*-axis. In addition to the maximum at the origin there are two other bulges in the function.

ented roughly parallel to the *c*-axis. These calculations thus reinforce the interpretation of the Patterson calculation which indicates that the molecule lies parallel to the *c*-axis.

A three-dimensional Patterson calculation is an intermediate stage in the crystallographic analysis of tRNA. At 12-Å resolution it is clear that we are unable to proceed very far in interpreting the detailed folding of the molecule. However, with a favorable unit cell, the Patterson function at this resolution is able to depict some of the gross features of the molecule and indicates the manner in which they are packed together in the unit cell. Analysis of the 12-Å data has lead us to the conclusion that the molecule has an elongated form and is dimerized in an antiparallel fashion with partial overlapping along the length of the molecule. The length of the dimer is about 109 Å. An individual molecule has a length of 80 ± 10 Å and a width near 25 Å in one direction and 35 Å in another direction. The molecule is thus not rounded in cross section but has irregular features, the detailed nature of which will have to await the result of a higher resolution x-ray diffraction analysis.

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Mouse Leukemia Virus Activation by Chemical Carcinogens

Abstract. *The induction of lymphomas in C57BL mice by methylcholanthrene, urethan, or diethylnitrosamine was accompanied by the development of murine leukemia viral antigen in most of the lymphoid tumors. The cell-free transmission of lymphomas induced by methylcholanthrene and the development of antibody to murine leukemia virus prior to the detection of overt lymphoma in these mice suggest that unmasking of a latent leukemia virus is an indigenous actuating cause of the lymphomas.*

Many chemical carcinogens have been studied in mice, but the mechanism of neoplastic transformation by these chemicals remains unclear. Intermediary events preceding transformation seem likely because of the multiplicity of cellular and humoral effects of chemical carcinogens in vivo. Several investigators who recovered leukemogenic agents from tumors induced by chemicals, including methylcholanthrene (*1*), urethan (*2*), and 7,12-dimethylbenz(*a*)anthracene (*3*), have suggested that activation of a latent oncogenic virus may play a role in chemical carcinogenesis. These studies were based on the demonstration of leukemogenic activity of very limited numbers of cell-free preparations, and thus the frequency and significance of finding leukemogenic virus in chemically induced lymphomas is subject to question.

The activation of a latent leukemogenic virus and induction of lymphoma in C57BL mice by radiation is well documented. Leukemogenic activity of cell-free filtrates from lymphomas induced by radiation in C57BL mice has been shown (*4*) and confirmed (*5*). The presence of a latent leukemia virus in the C57BL strain, which has a relatively low incidence of spontaneous leukemia, provides a model system for the study of chemically induced lymphomas.

During several long-term experiments on virus-chemical cocarcinogenesis with C57BL mice, we found that murine leukemia virus (MuLV) complement-fixing antigen (*6*) was present in a significant proportion of chemically induced lymphomas. This report concerns the relation between the development of lymphoid tumors and the induction of MuLV antigen in the lymphomatous tissues of chemically treated mice.

Test groups of 60 to 75 newborn C57BL/6 mice (Cumberland View Farms, Clinton, Tennessee) were housed in disposable plastic cages with filter-top lids and fed a standard mouse diet, with the addition of apples. Crystalline 3-methylcholanthrene (*7*) was dissolved in trioctanoin vehicle at concentrations of 10 and 100 μg per 0.05 ml. One group of mice less than 24 hours old received 10 μg of methylcholanthrene intraperitoneally, and another group received 100 μg. In other experiments urethan (*7*) was dissolved in Hanks' basal salt solution. One group was given 5 mg of urethan subcutaneously when the animals were 7 and 9 days old. Another group was given 0.5 mg of urethan per gram of body weight weekly for 10 weeks beginning 1 week after birth. Diethylnitrosamine (*7*) was diluted in Hanks' basal salt solution at concentrations of 1, 4, 16, and 64 μg per 0.05 ml. One group of 1-day-old mice was inoculated intraperitoneally with each of the four concentrations of diethylnitrosamine. Control groups included 1-day-old mice inoculated intraperitoneally with 0.05 ml trioctanoin, mice inoculated subcutaneously with 0.05 ml of Hanks' basal salt solution on days 7 and 9, and mice inoculated subcutaneously with 0.05 ml of Hanks' basal salt solution on day 1. All mice were weaned and separated by sex at 3 weeks of age; they were palpated twice weekly for detection of tumors or enlarged lymph nodes. Lymphomatous mice were killed, and clarified 10 percent extracts of various tissues were screened at a 1 : 2 dilution for the presence of complement-fixing antigen against rat antiserum group reac-

Table 1. Summary of incidences of lymphoma and complement-fixing MuLV antigen in chemically treated and control C57BL/6 mice. The incidence of MuLV antigen is expressed as a ratio of the number of 10 percent tissue extracts containing MuLV antigen to the number of extracts tested in that group. The lymphomas tested for antigen were harvested 5 to 18 months after chemical treatment; normal spleen and thymus (S-T) tissues were pooled from considerably older mice because these extracts were prepared from the survivors in each test group at the termination of the experiments (15 months for the MC groups and 20 months for the DEN groups). N.T., Not tested; MC, methylcholanthrene; HBSS, Hanks' basal salt solution; DEN, diethylnitrosamine.

Inoculum	Lymphoma incidence	MuLV antigen incidence* in:	
		10% Extract of lymphoma	10% Extract of normal spleen-thymus
MC 10 μg	8/55 (15%)†	5/6	6/47
MC 100 μg	20/67 (30%)†	6/13	4/32
Trioctanoin	0/50†		1/40
Urethan 5 mg × 2	8/49 (16%)†	2/2	N.T.
Urethan 0.5 mg/g body wt. × 10	16/62 (26%)†	3/5	N.T.
HBSS 0.05 ml, days 7 and 9	0/55†		N.T.
DEN 1 μg	6/54 (11%)‡	2/4	0/2
DEN 4 μg	4/63 (6%)‡	1/2	0/7
DEN 16 μg	5/62 (8%)‡	2/2	0/8
DEN 64 μg	4/33 (12%)‡	2/3	1/7
HBSS 0.05 ml, day 1	4/62 (6%)‡	1/4	1/15

* The antigen-positive lymphoma extracts from the MC, urethan, and DEN groups had titers of 1:2 to 1:16, with most specimens in the 1:4 to 1:8 range. Titers of antigen-positive nonlymphomatous spleen-thymus extracts from the carcinogen-treated mice were generally in the 1:2 to 1:4 range. The rare occurrence of MuLV antigen in extracts from the control groups was demonstrable only at a 1:2 dilution. † At 15 months. ‡ At 20 months.

tive to mouse leukemia virus. The preparation of this broadly reactive antiserum to MuLV has been described (*6*); it reacts specifically with the soluble group-specific antigen common to all known mouse leukemia viruses, including the radiation-induced leukemia virus from C57BL mice (*8*).

The mice that received 10 and 100 μg of methylcholanthrene and the group treated with trioctanoin alone were observed for 15 months. During this period the only tumors which became apparent clinically were lymphoid neoplasms. However, a few animals in the group treated with 100 μg of methylcholanthrene were cannibalized or died of unknown causes and were not included in the statistical evaluation. The lymphomas most frequently involved the thymus, the spleen, and the cervical and mesenteric lymph nodes. The normal architecture of these organs was obliterated by a diffuse infiltration of relatively mature lymphocytes. Surviving animals in the three groups were killed after 15 months. The only neoplasms, other than lymphomas, noted at this time were a few hepatomas and small pulmonary adenomas in the animals treated with methylcholanthrene. A clarified, 10 percent extract of the pooled spleen and thymus from each nonlymphomatous mouse was prepared for MuLV antigen assay.

Methylcholanthrene at both dosages was leukemogenic (Table 1). The mice inoculated with trioctanoin developed no lymphomas during the test period. Most of the lymphomas induced by methylcholanthrene occurred after latent periods of 5 to 12 months. Eleven of 19 (58 percent) of the extracts of lymphomas induced by methylcholanthrene had MuLV complement-fixing antigen, with titers ranging from 1 : 2 to 1 : 16. Of the 79 spleen-thymus extracts obtained from nonlymphomatous mice that had been treated with methylcholanthrene, 13 percent had MuLV antigen, with titers ranging from 1 : 2 to 1 : 8.

The two groups of mice inoculated with urethan developed 16 and 26 percent incidences of lymphoid neoplasms, respectively, during the first 15 months of observation (Table 1). These animals were held for a longer period, but 15 months was chosen for this report, since almost all lymphomas occurred prior to this time. Five of seven extracts of lymphomas from animals treated with urethan contained MuLV antigen. Mice inoculated with Hanks' basal salt solution on days 7 and 9 did not develop any lymphomas during the 15 months of observation.

The mice inoculated with different dosages of diethylnitrosamine developed high incidences of hepatomas and pulmonary adenomas during the first year (*9*). Lymphoid neoplasms in these groups generally occurred between the 12th and 20th months, and the lymphoma incidences were only slightly greater than those in mice inoculated with Hanks' basal salt solution on day 1 (Table 1). This may be explained at least in part by the fact that many of the mice treated with diethylnitrosamine died of hepatic and pulmonary tumors prior to the development of lymphomas. Seven of 12 extracts prepared from lymphomatous tissues from animals treated with diethylnitrosamine demonstrated MuLV antigen, but only one of 24 extracts of spleen-thymus pools prepared from normal mice after 20 months contained the antigen. Several extracts of pulmonary adenomas and hepatomas from the mice treated with diethylnitrosamine were negative when tested against MuLV antiserum.

Several extracts of lymphomas induced by methylcholanthrene were passed through 0.8-μm Millipore filters which were impervious to a 24-hour culture of *Serratia marcescens*. Newborn C57BL mice were inoculated subcutaneously with 0.05 ml of the cell-free filtrates. All extracts that had an antigen titer of 1 : 8 or greater induced leukemia (mean latency, 7 months). One of four cell-free extracts that had titers of complement-fixing antigen of 1 : 2 to 1 : 4 was leukemogenic. Three extracts from the spleens of normal mice failed to induce leukemia. It appears that the presence of complement-fixing antigen is a more sensitive indicator of activation of leukemia virus than is the demonstration of infectious leukemia virus in vivo by this technique.

The question whether the presence of MuLV antigen in the majority of these chemically induced lymphomas indicates a causal relationship between activation of leukemia virus and induction of lymphoma or if it merely indicates that the leukemia virus is a passenger preferentially localized in lymphomatous tissue requires further clarification. The detection of MuLV antigen in 58 percent of lymphomas induced by methylcholanthrene is comparable to the percentage of extracts of lymphomas induced by radiation in C57BL mice which had leukemogenic activity

Table 2. Incidences of complement-fixing antibody to MuLV in serial serum samples from C57BL mice treated with methylcholanthrene and trioctanoin (control). Samples with a titer of 1 : 10 were considered positive. Antibody-positive serums from the mice treated with methylcholanthrene ranged in titer from 1 : 10 to 1 : 80 with a geometric mean titer of 1 : 16. None of the serums from the mice treated with trioctanoin had an antibody titer greater than 1 : 10.

Wk	Inoculum		
	Methylcholanthrene		Trioctanoin
	10 μg	100 μg	
6	0/55 (0%)	1/67 (1.5%)	2/50 (4%)
12	7/53 (13%)	4/67 (6%)	0/50 (0%)
20	9/52 (17%)	21/64 (33%)	0/50 (0%)
37	5/51 (10%)	5/60 (8%)	0/50 (0%)
52	12/51 (23%)	13/57 (23%)	3/50 (6%)
62	12/38 (32%)	9/41 (22%)	6/47 (13%)

(*10*), and probably indicates the limitations of the sensitivity of this assay. Detection of MuLV antigen at 15 months of age in 13 percent of the grossly normal C57BL mice treated with methylcholanthrene suggests that the chemical may activate the latent leukemogenic virus prior to the development of overt lymphoma. The complement-fixing MuLV antigens have been demonstrated in nonlymphomatous spleens of other strains of mice, with the incidence approximating 100 percent in strains with high incidences of leukemia (*11*). To determine the temporal relation between the activation of latent leukemia virus and the development of overt lymphoma, mice in the groups inoculated with 10 μg of methylcholanthrene, 100 μg of methylcholanthrene, and trioctanoin alone were bled from the orbital sinus at 6, 12, 20, 37, 52, and 62 weeks after inoculation. The serums were tested for complement-fixing antibody to murine leukemia virus against a complement-fixing antigen prepared from subcutaneously transplanted lymphomas originally induced by radiation in C57BL/6 mice (*8*). This antigen reacts with rat antiserum (group reactive) to MuLV and is a sensitive and specific complement-fixing antigen for detection of antibody to indigenous leukemia virus in C57BL mice. Six to 33 percent of the serums obtained at week 12 and week 20 from mice treated with methylcholanthrene had antibody to murine leukemia virus (Table 2). Most of the serums had titers of 1 : 10 to 1 : 20. Since the lymphomas induced by methylcholanthrene did not occur until after week 20, the early and repeated detection of complement-fixing antibody to murine leukemia virus indicated the activation of leukemia virus prior to the development of overt lymphoma. Two of the 50 mice inoculated with trioctanoin showed antibody at 6 weeks, but antibody could not be detected again until 52 weeks.

In the mice inoculated with methylcholanthrene, 21 extracts contained MuLV antigen—11 lymphoma extracts and 10 extracts of normal spleen-thymus (Table 1). Of the 21 mice which yielded the antigen-positive extracts, 19 had previously demonstrable complement-fixing antibody. Of the 11 mice with antigen-positive lymphomas, all had antibody to murine leukemia virus in two or more of their serum samples.

Of 37 lymphomas induced by methylcholanthrene, urethan, or diethylnitrosamine, 21 contained mouse leukemia viral antigen, and lymphomas induced by methylcholanthrene were preceded by the development of antibody to murine leukemia virus in the serum. The finding of MuLV antigen and antibody in a few control mice over 1 year old suggests that the latent leukemia virus may occasionally become spontaneously activated. Treatment with methylcholanthrene accelerated and increased the magnitude of viral activation and the subsequent development of lymphoma.

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Synthetic Juvenile Hormone: Induction of Sex Pheromone Production in Ips confusus

Abstract. *Topical application of 25, 50, or 100 micrograms of 10,11-epoxyfarnesenic acid methyl ester in peanut oil induced male* Ips confusus *to produce sex pheromone in the hindgut Malpighian tubule region. Twenty-four hours after treatment of male beetles with 100 micrograms of hormone, their hindgut Malpighian tubule extract was more attractive to female beetles in a laboratory bioassay than was extract from males producing pheromone naturally in ponderosa pine logs.*

Within 4 to 6 hours after entering host logs (*1*), male *Ips confusus* (Coleoptera: Scolytidae) begin to produce three sex pheromone compounds (*2*) in the hindgut Malpighian tubule region (*3*). There is then a rapid degeneration of the flight muscles, and the insects approach reproductive maturity (*4*). Degeneration of the flight muscles can be induced in both sexes by topical application of a synthetic juvenile hormone (*5*), a fact which suggests that other events associated with reproduction, such as sex pheromone production, might be similarly induced. In cockroaches, sex pheromone production is dependent on the presence of the corpora allata (*6*), and, in male locusts, the corpora allata control the production of a maturation pheromone (*7*). We report that synthetic juvenile hormone (*8*) (10,11-epoxyfarnesenic acid methyl ester) (EFA) induces male *I. confusus* to produce sex pheromone.

We used beetles which had emerged from a colony maintained in ponderosa pine logs in cages. Groups of 12 to 20 male beetles were treated (i) with EFA in peanut oil applied topically to the abdominal venter; (ii) with peanut oil applied in the same manner; or (iii) with introduction into fresh pine logs to induce normal pheromone production (*1*). Topical applications were made with capillary pipettes, and insects so treated were held on moist lichen in closed glass jars at room temperature. After a specified time, usually 24 hours, all insects were removed from the jars or excised from the host logs. Their hindguts with Malpighian tubules were dissected out and extracted in benzene (one gut to 0.025 ml); the extract was bioassayed with groups of 10 to 16 female beetles in an open stage, multiple airstream olfactometer (*1*). Each extract attracting more than 20 percent of the test females was serially diluted and bioassayed until a nonattracting stimulus was attained.