dictory because the excretion studies, the evidence of mild cycasin toxicity in animals contaminated by it, and the ability of the bacteria to ferment salicin, all indicated that it contained at least moderate amounts of a glucosidase capable of splitting cycasin. Enzymatic studies, on the other hand, failed to reveal a detectable glucosidase. A 30- to 100-fold increase in substrate concentration, however, permitted the assay of glucosidase activity in the extracts. A determination of the K_m for cycasin and a calculation of the approximate concentration of cycasin in the alimentary canal of the experimental rats revealed that adequate concentrations of cycasin were probably present initially for some deglucosylation to occur.

Summary. The role of intestinal bacteria in converting the naturally occurring glucoside cycasin to its hepatotoxic and carcinogenic aglycone (methylazoxymethanol) has been studied. Germfree rats, in which cycasin is nontoxic, were monocontaminated with several strains of bacteria prior to being given cycasin. Levels of glucosidase activity in the bacteria were determined by the assay of cell free extracts, using cycasin as a substrate. The toxicity of cycasin in rats given the various bacteria and the amounts of unchanged cycasin excreted were consistent with the glucosidase assays. Intestinal microorganisms therefore convert cycasin to the toxic aglycone, and variations in the intestinal flora probably have a role in determining the toxicity of ingested cycasin.

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A Leukemogenic Filtrable Agent from Chemically-Induced Lymphoid Leukemia in C57BL Mice. (31827)

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Mice of the C57BL strain are refractory to spontaneous lymphatic leukemia, and highly susceptible to its induction by irradiation. There are no reports of successful attempts to induce lymphatic leukemia in adult C57BL mice by chemical carcinogens.

Although the problem of whether chemicals induce leukemia by activation of a latent virus, similarly to the induction mechanism thought to be involved in radiation-induced leukemias(1), has been investigated in avian leukosis(2) and murine leukemias(3), no definite conclusions have been reached.

The aim of the present work was to demonstrate the leukemogenic activity of 7,12-dimethylbenz(a)anthracene (DMBA) in C57BL adult mice, and to isolate a leukemogenic agent from these carcinogen-induced lymphomas.

Materials and methods. The carcinogen

TABLE I. Incidence of Lymphatic Leukemia in C57BL/6 Mice Inoculated with Filtrates from DMBA-Induced Lymphomas.

Filtrate series	No. of mice	Lymphomas No.	Lymphomas %	Age at leukemic death (days)
1	10	2	20	96[a]; 320
2	12	0	—	—
3	11	3	27	191[b]; 322[c]; 292
4	13	2	15	242; 301
First passage of series 1 [a]	10	2	20	234; 295
First passage of series 3 [b]	10	3	30	112; 130; 150
[c]	10	1	10	220
Control PBS	40	1	2.5	140

(a), (b), (c) = The donor mice for the filtrate passages of the original series.

DMBA was made up as a 1% solution in polyethylene glycol 400, and administered to the mice once weekly by stomach tubes prepared from polyethylene tubing of about 1 mm pore size. A dose of 0.1 ml of the DMBA solution was administered 10 times at weekly intervals.

Preparation and testing of cell-free filtrates. The carcinogen-induced thymic lymphomas were homogenized in 5 volumes of phosphate buffered saline (PBS), and the homogenate obtained was first centrifuged at 2,500 $\times$ g for 15 minutes, to remove cell fragments, and then 3 times for 15 minutes each, at 10,000 $\times g$; the final supernatant was passed thru a Millipore filter of 0.3 μ mean pore size. The leukemogenic activity of the filtrate was tested by injecting 0.05 ml into 5-7-day-old thymus grafts under the kidney capsule of thymectomized, irradiated hosts, as described earlier(4). In the control group, 0.05 ml PBS was injected instead of the filtrate.

Results. Thymic lymphoma induction with DMBA. Thymic and generalized lymphatic leukemias appeared in 21 of 36 mice(60%) treated with DMBA. The first death with thymic lymphoma occurred in a 190-day-old mouse, 143 days after the beginning of DMBA treatment, the mean latent period from the start of the DMBA feedings being 176 days. Mice that did not die from lymphatic leukemia were observed for one year, when they were killed and examined for leukemia development. No lymphatic leukemias were recorded in 35 mice fed with the polyethylene glycol 400 solvent and kept alive for one year.

Leukemogenic activity of filtrates from DMBA-induced lymphoid tumors. Four series of filtrates were prepared from DMBA-induced leukemias—mainly from the enlarged thymuses of the mice. The activity of each filtrate was tested in 10-13 mice, by injecting the filtrate directly into the subcapsular thymic implant in thymectomized, irradiated hosts. The leukemogenic activity of the tested series is summarized in Table I. Only typical lymphocytic lymphosarcomas, similar to those arising after irradiation of this strain, were classified as lymphomas. No leukemogenic activity was found in one of the series of filtrates tested. In the other 3 series, 15-27% of the mice developed lymphomas, usually of a generalized type: the thymic implant was greatly enlarged, always leukemic, and leukemic infiltration was also generally noted in the liver, spleen and lymph nodes. Three first passages of a leukemogenic filtrable agent, prepared from the leukemias induced with the original filtrates, revealed a similar leukemogenic activity of 10-30% (Table I). The incidence of spontaneous lymphomas in this strain is 1%. The control groups of thymectomized, irradiated mice regrafted with an isologous newborn thymus under the kidney capsule, which received an injection of 0.05 ml PBS into the graft, showed a 2.5% lymphoma incidence (Table I).

Discussion. Adult mice of the C57BL strain, though highly susceptible to thymic lymphoma induction by irradiation(5), were found to be refractory to leukemia induction by methylcholanthrene(6) and urethane(7), though urethane was effective when administered to newborn mice(8). The present experiments show that DMBA is capable of inducing thymic lymphomas in adult C57BL mice, the effectiveness of DMBA as a leukemogenic agent appearing to be similar to the radiation effects obtained in our studies:—Four weekly doses of 170 r wholebody exposure induced lymphatic leukemia in 60% of the treated mice at a 240-day average latent period, as compared to 60% at a 176-day average latent period in the DMBA treated mice.

Multiple agents—carcinogens, X-rays and estrogens—are capable of inducing lymphomas of thymic origin(3), and leukemogenic, filtrable agents have been obtained from lymphomas induced by these different agents.

Radiation-induced lymphomas have been transmitted by cell-free filtrates to newborn mice(9,10), and some recent experiments(4) have demonstrated the "release" of a transmissible leukemogenic agent from irradiated, non-leukemic tissues in C57BL mice shortly after termination of the irradiation.

The isolation of a filtrable agent from estrogen-induced lymphomas in Rf mice has also been reported recently(11).

Attempts to isolate a filtrable agent from chemically-induced lymphomas in mice have been unsuccessful except for the report by Toth(12), who describes the isolation of a leukemogenic agent from DMBA-induced lymphomas in Swiss mice. The present finding of a leukemogenic agent isolated from DMBA-induced lymphomas in C57BL mice coincides with the observation by Toth. (The possibility of some DMBA from the initial treatment being present in the filtrate must be taken into consideration, although this appears unlikely, in view of further successful serial passages having been obtained from the original filtrate.)

The question whether all the different leukemogenic agents induce lymphomas by activation of a latent virus is still debatable. The isolation of a leukemogenic agent from radiation and chemically-induced lymphomas in C57BL mice with a low spontaneous incidence of lymphatic leukemia, encourages the assumption that different leukemogenic agents may act by "activating" a latent virus present in postnatal life in these animals.

Summary. Thymic lymphomas were induced in 60% of C57BL mice fed with 7,12-dimethylbenz(a)anthracene dissolved in polyethylene glycol 400, the mean latent period from the start of the DMBA feedings being 176 days. Three series of cell-free filtrates prepared from the DMBA-induced lymphomas were leukemogenic, producing 15-27% lymphomas in the tested mice. Serial cell-free passages of the original filtrate-induced lymphomas revealed a similar leukemogenic activity of 10-30%.

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Influence of a Fat-Enriched Meal on Human Serum (L-Phenylalanine-Sensitive) "Intestinal" Alkaline Phosphatase.* (31828)

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One of the isoenzymes present in the mixture of alkaline phosphatases in serum has been demonstrated to have the properties of alkaline phosphatase of the striated border of the villi of intestinal mucosa(1-5). This intestinal alkaline phosphatase isoen-

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