TABLE III. Antibiotics Not Affecting Multiplication of L Cells in Suspension Culture.*

Amphotericin A	Fumagillin
" B	Gramicidin
Aspergillic acid	Griseofulvin
Bacitracin	Methymycin
Benzylpenicillin potassium	Neomycin B sulfate
Carbomycin	Nystatin
Chartreusin	5-Oxytetracycline•HCl
Chloramphenicol	Polymyxin B sulfate
7-Chlortetracycline • HCl	Subtilin
Citrinin	Tetracycline • HCl
Colistin	Tyrothricin
Cycloserine	Vancomycin
Dihydrostreptomycin sulfate	Vernamycin
Erythromycin	Viomycin sulfate

* Non-inhibitory when added to medium to give a concentration of 1 μg/ml.

periment to the next; this variation was reduced when several replicate tubes were started at each antibiotic concentration. Of the substances tested that inhibited growth the actinomycins and xanthomycin were significantly more cytotoxic than any of the others. Experiments with concentrates obtained from fermented media containing cytotoxic substances showed that the dose-response curves could be used in estimating the potency of the cytotoxic factors present.

Examination of the literature shows that the cytotoxicity of only a few antibiotics have been examined in tissue culture. Eagle and Foley(1) found that approximately 60 mμg per ml of actinomycin D were required to inhibit growth of 6 different cell lines of human and mouse origin. Whether the differences in activity of actinomycin D and the actinomycins listed in Table II is due to the chemistry or the sensitivity of the cell lines is unknown. Smith(8) has reported that approximately 100 mμg per ml of cycloheximide is required to inhibit growth of the KB cell line. This is somewhat more than the 33 mμg per ml that we need to inhibit the L cell line.

Summary. In a study of effects of antibiotics on multiplication of L cells in suspension culture, positive inhibition of multiplication was found when less than 1 μg/ml of any one of the following were added to the media: actinomycins B, B_V, or D_{IV}; cycloheximide; gliotoxin; hygromycin; mitomycin C; patulin; thiostrepton; and xanthomycin. Actinomycin B_V was the most active of the group tested as 1.5 mμg/ml was sufficient to cause a 50% reduction in growth of culture. While the shape of dose-response curves varied with different inhibitors, it was possible to use the linear portion as the basis of a method for quantitative estimation of concentration of inhibitor present. This method may be useful in determining the presence of cytotoxic metabolites formed in microbial fermentations.

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Influence of Fighting on Leukemia in Mice.* (25224)

PAUL LEMONDE

Inst. of Microbiology and Hygiene, University of Montreal, Montreal, Canada

It has been observed, in leukemic strains of mice, that lymphoid leukemia occurs less frequently and later in males(1,2). This observation has been confirmed in our subline of inbred AK mice, in which: 1) incidence of leukemia is 15% less in males; 2) average age at death from leukemia is 315 days in males and 281 in females, a highly significant difference. Such a difference of susceptibility is commonly attributed to sex hormones. Estro-

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TABLE I. Influence of Fighting on Leukemia in AK Mice.

Groups	No. of mice	Age at death (avg days)	Death from leukemia: No. of mice	Death from leukemia: Age at death (avg days)	Not leukemic (%)
Females in colony	437	284.3 ± 4.5*	388	281.3 ± 4.1*	5.5-11.2
Males " "	249	329.3 ± 7.9	182	315.4 ± 6.2	17.7-26.9
" non-fighting (isolated)	40	295.3 ± 13.8	33	286.8 ± 11.3	2.5-17.5
" fighting "beaten"	88	350.4 ± 13.4	66	331.8 ± 13.2	9.1-23.6
" " "beaters"	30	375.2 ± 21.3	21	329.6 ± 13.9	16.7-30.0

* Stand. error of mean.

gens promote leukemia, whereas testosterone inhibits the disease; conversely, castration may stimulate leukemia in males and decrease it in females(1,2). Since female hormones or lack of androgens increase leukemia, and male hormones or lack of estrogens diminish it, females are believed more susceptible because they possess estrogens. This interpretation, though logical, was not entirely satisfying. It appeared that some other explanation could be added, or substituted. In several strains of mice, including the AK, males fight, sometimes savagely, even killing one another. It was assumed that such fighting could influence leukemia. Hence, the following experiment was set up.

Procedures. Forty male AK mice were isolated, each in a separate cage, before they began to fight, that is, when less than 65 days old. In a second group, males were placed 4-6 to a cage, to give them a chance to fight. When scuffling was noticed, this was watched closely and when a mouse showed traces of battle, was bitten and wounded, it was isolated. We took a larger number of animals in this group than in the first, for fear that some of them would be killed in fight and lost for the experiment; we ended with 88. It was more difficult to obtain a third group, *i.e.*, males that were not beaten, but beat and conquered the others. These are less numerous than their weaker companions, there is usually one per cage; they are hard to recognize, as they bear no trace of struggle: one has to watch for hours to be sure. Thirty were gathered. All mice were kept under observation until death.

Results. A difference was found between the animals that were isolated early and did not fight and those that fought. Unfighting males died of leukemia at 286.8 days, practically at the same age as females (Table I); moreover, the proportion of non-leukemic animals was low and comparable to that of females. There are 2 figures in the last column (Table I), because some individuals showed slight inconclusive signs of leukemia, or some had both leukemia and other diseases: although these mice died *with* leukemia, it could not be decided if they died *of* it. If they are reckoned as leukemic, we consider the first figure; if not, we consider the second. In the columns showing death from leukemia, they were regarded as not leukemic. Males that fought and were defeated died of leukemia at 331.8 days, a significant difference of 45 days over the non-fighting; the percentage of animals without leukemia was also higher in this group. As for the fighters that beat the others, their survival time was longer and the proportion of non-leukemics higher than in non-fighting mice. Their survival was also longer than that of beaten fighters, but the difference is not significant and, if only death from leukemia is considered, there is no difference; however, the percentage of non-leukemics is higher.

In the Table, mice termed "in colony" consist of animals that lived past the age of one month and, for the males, either did not fight or fought not enough to be separated as fighters. It is understood that females do not fight.

Comments. In brief, male AK mice that did not fight died of spontaneous leukemia at essentially the same age and in the same proportion as females, whereas males that fought had a lower and later incidence of leukemia. Therefore, if male mice in general become leukemic less often and later than females, it is not only because of sex hormones. Other causes contribute to this effect and fighting

may be one of them. The influence of fighting could be explained through the action of adrenal hormones. Damage suffered by beaten mice, wounds, sequels of the wounds, such as sterilization and urinary calculi (some mice are sterilized by bites and other lesions of scrotum and penis; some develop stones in the bladder, apparently as a result of interference with micturition), these and other injuries initiate ACTH discharge. This elicits a secretion of corticosterone or similar hormone, which, as is known, retards and decreases leukemia. This situation is comparable to what happens in other hard conditions, such as pregnancy or underfeeding. Pregnancy retards leukemia in AK mice and the same explanation has been proposed(3). In underfed mice, leukemia is also reduced(4,5).

Incidence of leukemia and survival time of fighters that conquered the others were expected to fall between those of non-fighters and of beaten mice, because they underwent the strain of battle, but suffered almost no wounds nor the frustration of being vanquished. Actually, their survival was about the same as that of the beaten. Incidence of leukemia was appreciably less; perhaps they are stronger than the others in many respects, not only in combat behavior, but also in resistance against disease; which is further intimated by the fact that their survival time in general, not only as regards leukemia, is the longest.

The figures representing age at death from all causes (Table I) may give the impression that the influence of fighting is exerted on life span in general and not concerned with leukemia in particular. Indeed, there is no definite answer to the question whether fighting acts specifically on leukemia or on any cause of death. However, it seems to have a special effect on leukemia, because differences in survival are significant when death from leukemia is considered, but not significant in non-leukemic animals.

It is possible that androgens have an influence. All male groups were composed of intact animals, with testes and therefore testosterone; hence, the differences do not appear to come from sex hormones. But fighting itself could increase androgen production. Testosterone stimulates the fighting instinct. Fighting in turn could stimulate hormone secretion, or action. Thus, by reciprocal causation, testosterone would be notably increased and could inhibit leukemia. We have no evidence for this at present.

Another idea would be that early isolated mice are not disfavored by escaping the hardships of battle, but by isolation itself. In some species, resistance against different noxious agents, including cancer(6), is higher in animals living together than in isolated individuals. It is difficult to investigate this in male mice. If put with males, they fight; with females, it is hard to decide whether any observed effect is due to escaping fight, to sexual activity, or to the company of the female. Experiments are under way with castrated mice to ascertain this point and to establish the influence of testosterone.

Whatever the interpretation, spontaneous leukemia was delayed and diminished in males that fought. This fact must be remembered when differences are noted between male and female mice with respect to leukemia. In designing experiments with males, one should use only animals isolated early after weaning; otherwise, fighting may alter the results.

Summary. 1) In leukemic strains of mice, lymphoid leukemia is known to occur less often and later in males than females. However, in our AK mice, males that were isolated early in life and did not fight died of spontaneous leukemia at essentially the same age and in the same proportion as females, whereas fighting males died significantly later and in a lower percentage. Hence the difference between sexes is not explained by sex hormones only. An interpretation is proposed, based on adrenal corticosteroid secretion. 2) Fighting activity should be taken into account as a factor in pathogenesis of mouse leukemia and in the difference of incidence and survival time between male and female mice.

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Prevention of Peritoneal Free (Ascites) Tumor Cell Growth and Implantation by Pretreatment with Radioactive Yttrium.* (25225)

HORACE GOLDIE, SPENCER C. DISHER, CHARLES S. SMITH AND FRANK B. DAVIS
(With technical assistance of Esther White Turner)
Laboratory for Experimental Oncology, Meharry Medical College, Nashville, Tenn.

It is well known(1,2) that thoracic and abdominal malignant tumors extending into pleural or peritoneal cavity may induce there accumulation of an exudate containing numerous malignant, frequently mitotic cells released from the tissue. Some of these cells eventually implant into the cavity wall and give rise to secondary growth of "coelomic metastases"(3). Müller reported(4) "undoubtful therapeutic results" of intraperitoneally injected colloidal Au^{198} in 8 female patients with "carcinomatous peritonitis." We showed(5) that peritoneal carcinosis (later designated as "ascites tumor") of the mouse(6) regressed completely after a single injection of colloidal Au^{198}; we obtained analogous results by similar treatment of free tumor cell growth in the pleural exudate(7). Later experiments(8,9,10) suggested the use of radioisotopes for inactivation of tumor cells remaining in healthy tissues or spilled there after tumor removal. We recently investigated the possibility of preventing growth and implantation of cells separated spontaneously or by trauma from abdominal tumors and entering peritoneal cavity. Accordingly, we reversed the procedure of our earlier experiments by injecting a radioisotope intraperitoneally into mice before and not after tumor inoculation by the same route. Eventual effect on tumor cells was checked by comparison of the amount of effusion, its content of free tumor cells and the extent of implantation in experimental and control animals. The results are recorded below.

Material and methods. a) *Mice and tumor strain.* Swiss Albino mice (Albino Farms, Red Bank, N. J.) females of 24 to 27 g weight were used. Krebs-2 carcinoma was maintained as "ascites tumor" by several intraperitoneal transfers. b) *Tumor cell count in ascitic fluid.* Methods of isotope injection, of inoculation with requisite numbers of tumor cells, of exudate withdrawal from the cavity and of staining were described previously (5,7). Standard pattern of implantation in female mice (periuterine localization) was obtained(6). c) *Isotopes.* In preliminary experiments we have tested on parallel lines preparations of short-living and pure beta emitting isotopes: $CrP^{32}O_4$, $Y^{90}PO_4$, and $ZrP^{32}O_4$ obtained from Abbott Laboratories, Oak Ridge, Tenn. Since most consistent results were produced with $Y^{90}PO_4$, this isotope was selected for experiments presented below. Decayed (no radioactivity detected by standard counters) isotope was given to control mice for assaying any chemical effect of the compound independent from its radioactivity. d) *Amount of ascitic fluid.* Amount of exudate was estimated as proportionate to volume of fluid obtained by insertion of a glass capillary in peritoneal wall: +++ = 0.5 ml or more; ++ = <0.5 but >0.1 ml; + = <0.1 ml; 0 = no fluid. The reliability of this estimate was shown by comparison with amount of exudate measured at autopsy. e) *Recording spontaneous implantation.* Extent of implant growth of periuterine localization (6) was graded as follows: 0 = no macroscopic implants; + = small nodules (less than 2 mm in diameter); ++ = tumor (2-5 mm in longer diameter); +++ = growth

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