affect the relative efficiency of feed utilization as indicated by starter utilization.

Summary. 1. Feeding of 0.5 g of potassium penicillin per 100 lb of milk replacement to Holstein bull calves resulted in a decreased rate of gain in body weight and growth in height at the withers in this experiment. 2. Feeding of 0.1 g, 0.3 g, 0.9 g, and 2.7 g of procaine penicillin also resulted in decreased growth rates in terms of mean gains in body weight and mean increase of height at the withers in this trial.

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Further Studies on Effects of a Small Intestinal Microsomal Fraction Upon Transplantable Tumors.*† (20212)

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In a previous paper(1), we reported results of exposing Gardner lymphosarcoma cell suspensions to a microsomal fraction, prepared by ultracentrifugation, from small intestinal tissue of mice and rats. The active fraction was sedimented at 90,000 x g. Animals inoculated with a mixture of lymphosarcoma cells and intestinal fraction exhibited either delayed tumor development or failure of tumors to appear within 180 days following inoculation. All control animals, given tumor cell suspensions in saline, developed tumors and died within 28 days.

Results of further work are reported in this paper, including data on effects of the fraction upon two other tumors, the Ehrlich ascites tumor and Yoshida rat ascites sarcoma. Data on the general chemical nature of the fraction are presented also.

Experimental procedure. CBA mice (Strong) were employed as hosts for lymphosarcoma and the Ehrlich ascites tumor. Tissue for subcutaneous inoculation experiments with lymphosarcoma was obtained by briefly comminuting minced tumor in 5 volumes of saline and filtering through gauze. Such suspensions

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TABLE I. Effect of Pre-inoculation Exposure of Tumor Cell Suspensions to Microsomal Sediment and to Organic Solvent Extracts of Inhibitory Material.

Treatment of microsomal fraction	No. of animals	Survivals	Deaths*
Cold ethanol extraction†	8	6	2
Ethanol extraction, room temperature†	27	23	4
Petroleum ether extraction from ethanol†	35	28	7
Controls, inoculum in buffer, pH 7.4†	40	0	40
Crude sediment in buffer, pH 7.4‡	30	30	0
Controls, inoculum in buffer, pH 7.4‡	30	0	30

* All deaths from uncontrolled tumor.
† Test tumor, lymphosarcoma.
‡ Test tumor, Ehrlich ascites. ½ of the animals in experimental and control groups received subcutaneous inoculations; ½ received intraperitoneal inoculations.

were mixed with equal volumes of the crude sediment in saline or buffer solution or with extracted material prepared by treating the ultracentrifugal sediment with various fat solvents. The mixtures were incubated for 5 to 10 minutes (lymphosarcoma) or 60 minutes (Ehrlich tumor) at 37.5°C and injected subcutaneously in amounts of 0.2 ml. Ehrlich tumor cells were washed 3 times in Krebs-Ringer phosphate buffer, pH 7.4, before exposure to the fraction and subsequent intraperitoneal inoculation. Cell suspensions of Ehrlich ascites tumor were prepared from 7-day-old intraperitoneal tumors.

Results. Examination of Table I indicates that a 100% positive response was elicited in control animals inoculated with either of the tumors in saline or buffer. No animals so inoculated failed to develop and eventually die from uncontrolled tumor. The small intestines of both normal and tumor-bearing animals have yielded actively inhibitory fractions, but because of the relatively crude nature of the fraction and solvent-extracted materials, quantitative studies have not been possible. The presence of inhibitory activity in fractions from intestines of tumor-bearing animals has been demonstrated by means of inoculation and respiration studies.

a. *Chemical studies.* The active material is insoluble in 0.02M sodium phosphate solution from pH 7.4 to 8.6, in glycerol and in 5% bile solution. It has been extracted from the crude ultracentrifugal sediment by 95% ethanol at room temperature and at 5°C. (Table I), and can be precipitated from alcoholic solution by the addition of 2 volumes of saline. The inhibitor is soluble in petroleum ether and in isooctane. Active fractions were prepared by treating precipitates from ethanol solution with these solvents or by direct fractionation from alcoholic solution. In both instances, evaporation of the solvent under nitrogen yielded a yellowish oily substance. The oily material, shaken vigorously with saline or buffer, formed a fairly stable emulsion which was incubated with tumor cell suspensions prior to injection into experimental animals. Further stabilization of the emulsion was achieved in certain instances by the addition of small amounts of corn oil, which has been shown to be entirely inactive with respect to any tumor-inhibiting properties. Preliminary studies on identification have shown that the active material is soluble in acetone at −10°C and not precipitated by digitonin. Further studies are now in progress using chromatographic methods.

Ultraviolet and visible spectroscopic studies on preparations of the active material in isooctane showed the presence of multiple unsaturated bonds. Whether these were present in the active fraction or in associated fats remains to be determined. However, since these studies suggested that rapid oxidation and conjugation of fatty acids were occurring, it seemed desirable to determine whether the observed effects of the inhibitory fraction were due to fat peroxides. Accordingly, experiments were undertaken in which peroxidized corn oil and peroxidized methyl linoleate replaced the usual intestinal fraction. Results demonstrated clearly that these peroxides were without inhibitory activity. Consistent with these findings on peroxides were observations made on stability. The activity of the inhibitor declines fairly rapidly with time, and samples stored at −10°C may become inactive in 10 to 14 days. This would suggest that the activity is due to a natural labile lipid and not to a breakdown or oxidation product.

TABLE II. Oxygen Consumption Studies Demonstrating Effect of Microsomal Fraction from Small Intestine upon Slices of Lymphosarcoma and upon Certain Normal Tissues of the Mouse and Rat.

Tissue	No. of cases	Exp. treatment	Q_{O_2} Mean	S.D.	t
Mouse liver	20	C*	8.92 ± .93		
	20	M*	9.17 ± .99		.82
Mouse kidney	25	C	14.37 ± 1.44		
	21	M	15.36 ± 1.37		2.38
Mouse spleen	50	C	9.81 ± 2.04		
	46	M	8.90 ± 2.08		2.16
Rat thymus	20	C	9.32 ± 1.47		
	18	M	7.19 ± 1.37		4.63
Lymphosarcoma	20	C	12.56 ± 2.19		
	30	M	7.12 ± 1.85		9.14

* C = controls; M = microsomal fraction.

b. *Respirometry studies.* Respirometry has been a highly satisfactory means for comparing the effects of the inhibitor on tumor and normal tissues. Incubations in which slices were employed were carried out in Krebs-Ringer phosphate buffer, pH 7.4; those in which cell suspensions were used were done in Krebs-Ringer phosphate buffer of the same pH fortified with 0.15% glucose and 0.01 M sodium succinate. Temperature was maintained at 37.5 ± 0.05°C.

Data for oxygen consumption values of certain normal tissues of mice and rats and for the Gardner lymphosarcoma are summarized in Table II. From these data it is apparent that with the exception of thymus and spleen, the fraction does not affect respiration of normal tissues studied, and that the effect of the fraction upon lymphosarcoma slices is much more profound than that observed for thymus and spleen. Further comparative studies of tumor cell and bone marrow cell suspensions are in progress. The use of bone marrow cell suspensions eliminates certain difficulties inherent in interpreting results of experiments with surviving tissue slices in the presence of the insoluble fraction. Results of bone marrow experiments will be reported later.

Fig. 1 demonstrates the effect of the crude microsomal fraction on oxygen consumption of the Ehrlich ascites tumor and Yoshida sarcoma. The initial stimulation of respiration of the Ehrlich tumor following addition of the fraction is characteristic of these cells in the presence of the crude inhibitor. The stimulation is less marked when the fraction has been partially purified by solvent extraction.

Discussion. The extensive list of agents (Dyer, 2) investigated for cancer-inhibiting properties contains a relatively small number of lipid substances. Isolation from normal intestine of a lipid material with *in vitro* inhibitory effects against 3 transplantable tumors is therefore of interest.

Certain features of this material deserve emphasis. It is apparently a moderately labile lipid and is insoluble in aqueous media. The latter suggests that the inhibitor is an insoluble cellular component rather than a hormone-like compound soluble in body fluids. Its action appears to be localized *in vitro* where direct contact is required for action. This insolubility may also account for its failure to control development of malignancy *in vivo,* even though it has been shown to be present in the small intestines of tumorous animals.

Summary. 1. By ultracentrifugation at 90,000 x g, a particulate fraction inhibitory to growth of transplantable lymphosarcoma,

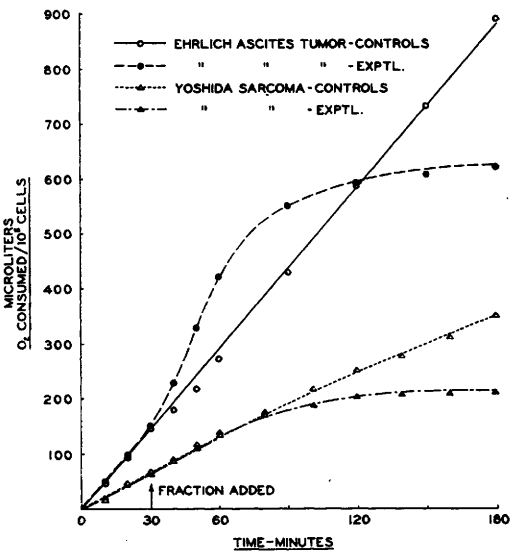

FIG. 1. Effect of crude microsomal fraction upon oxygen consumption of cell suspensions of the Ehrlich ascites tumor and Yoshida sarcoma. The fraction, suspended in phosphate buffer, was tipped into the reaction vessels 30 min. after the beginning of the experiment.

Ehrlich ascites tumor and Yoshida sarcoma has been obtained from small intestines of mice and rats. 2. Inhibitory properties of the fraction have been demonstrated by pre-inoculation exposure of tumor cell suspensions to the inhibitory material. 3. The intestinal fraction markedly suppresses oxygen consumption of tumor slices, slightly reduces the respiration of normal lymphoid tissues, and has no effect on respiration of other normal tissues tested; an initial respiratory stimulation was observed with ascites tumor, followed by marked depression. 4. The active component of the intestinal fraction appears to be an ethanol and petroleum ether-soluble lipid.

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Effect of Stress on Diurnal Fluctuations in Eosinophils of the Laboratory Mouse.* (20213)

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Diurnal fluctuations in the number of circulating eosinophils have been described in several strains of mice(1), and in humans(2,3). Stress, and the adrenal and adrenocorticotrophic hormones cause a decrease in the number of circulating eosinophils in various animals(4,5). The purpose of this paper is to show the effect of sustained cold on the diurnal fluctuations of eosinophils in mice.

Methods and materials. In the first experiment (A) 20 normal male mice of the Jax C-57 Black Strain, obtained from Jackson Memorial Laboratory, were used. The animals were from 6 to 7 weeks old at the beginning of the experiment. They were fed a Rockland Mouse Diet, and food and water were available to the animals at all times. The mice were kept in battery jars which contained a layer of wood chips, 3 to 4 mice being placed in each jar. All of the mice were kept at room temperature for 10 days after arrival to insure full recovery from the rigors of shipment. They were then divided into two groups each containing 10 animals. One group was kept in a cold room at a temperature of 3°C except for the short periods necessary to make eosinophil counts, while the other group, which served as a control, was kept at room temperature. Eosinophil counts were made twice a week for 9 weeks using the direct count method described by Speirs and Meyer (4). Speirs-Levy eosinophil counting slides were used. A day count was made every Sunday between 1:30 and 4:00 P.M. and a night count every Thursday between 6:00 and 9:00 P.M. The interval between counts was thought long enough to permit recovery from any effects on the number of eosinophils resulting from the handling of the animals and the taking of blood. The counts were always made on the control group first and the experimental group second so that the record for each group was standard as to time of day. The actual number of cells per chamber is used as the measure of eosinophil levels of the two groups rather than the number of cells per cubic millimeter of blood since the former shows satisfactorily the relation between the eosinophil levels of the two groups, and for statistical purposes is more conservative. The cells per cu mm of blood may be obtained by multiplying the number of cells per chamber by 10. In a sec-

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