4. Siri, W., *Isotopic Tracers and Nuclear Radiations*, 1949, 267.
5. Boycott, A. E., *J. Path. Bact.*, 1911, v16, 269.
6. Geiling, E. M. K., and Green, H. H., PROC. SOC. EXP. BIOL. AND MED., 1921, v18, 191.
7. Jencks, Z., *Am. J. Physiol.*, 1922, v59, 240.
8. Cartland, G. F., and Koch, F. C., *Am. J. Physiol.*, 1928, v87, 249.
9. Harne, O. G., Lutz, J. F., and Davis, C. L., *Bull. School Med. Univ. Maryland*, 1938, v23, 143.

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Effect of Ultracentrifugal Fractions of Small Intestinal Tissue upon Transplanted Lymphosarcoma.*† (19221)

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(Introduced by J. F. Mead.)
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The literature contains numerous reports of attempts to isolate from various normal tissues substances which are inhibitory to the growth of malignant tumors. The majority of these reports indicate highly variable results. Since it is generally known that malignant tumors rarely originate in the small intestine, the present work has been concerned primarily with efforts to derive inhibitory material from this organ. Previous work with this tissue has involved chemical extraction of material from the whole organ(1). In the present studies, ultracentrifugal fractionation has been employed. Similar methods have been used successfully in the separation of hemolytic and antihemolytic substances from liver(2).

Results of preliminary investigations based on centrifugal fractionation are reported in this communication.

Materials and methods. Small intestinal tissue of normal mice or rats was frozen on dry ice, then thawed and ground with sand. A saline suspension of this homogenate was fractionated in the following manner. A fraction corresponding to the mitochondrial-nuclear fraction of normal tissue(3) was separated by centrifugation for 10 to 20 minutes at 4000 x g (Fraction 4). The supernate from this was further centrifuged for 45 to 60 minutes at 90000 x g to yield a microsomal fraction (Fraction 90). The fractions were prepared for use by resuspending in saline. Fractions 4 and 90 and the supernatant from the latter were tested for inhibitory action on lymphosarcoma (Gardner). Lymphosarcoma cells in saline suspension were incubated *in vitro* at 5° C for 10 to 20 minutes with an equal volume of the tissue fraction suspensions described above. Two-tenths ml of these tissue fraction-tumor cell incubates was inoculated subcutaneously in CBA mice (Strong). Controls received saline-tumor cell inoculations.

Results. Results are summarized in Tables I and II. The technic employed yielded 100% takes of the tumor in 124 control mice. Tumors developed to palpable size in 4 to 7

TABLE I. Results of Pre-Inoculation Treatment of Lymphosarcoma Cell Suspensions with Mouse Tissue Fractions. Subcutaneous Injection of Tumor Cells and Tissue Fractions in CBA Mice.

Tissue fraction		No. cases	Survivals	Deaths
Intestine:	Fraction 90	49	47	2
	" 4	13	9	4
	Supernatant*	51	16	35
Liver:	Fraction 90	8	0	8
	" 4	8	0	8
	Supernatant	8	0	8
Spleen:	Fraction 90	6	0	6
	Supernatant	6	0	6
Muscle:	Fraction 90	6	0	6
	Supernatant	6	0	6
Controls:	Tumor + saline	65	0	65

* Supernatant fraction in all cases was the fluid portion remaining after precipitation of Fraction 90.

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TABLE II. Results of Pre-Inoculation Treatment of Lymphosarcoma Cell Suspensions with Rat and Rabbit Small Intestinal Fractions. Subcutaneous injection of tumor cells and tissue fractions in CBA mice.

Tissue fraction		No. cases	Survivals	Deaths
Rat intestine:	Fraction 90	20	19	1
	Supernatant	20	0	20
	Controls	11	0	11
Rabbit "	Fraction 90	26	0	26
	" 4	6	0	6
	Supernatant	16	0	16
	Controls	48	0	48

days and death ensued in 17 to 27 days after inoculation. Death in all instances was due to uncontrolled lymphosarcoma. The treated mice have survived as long as 180 days after inoculation in good condition and with no evidence of tumors.

From the data it is evident that the maximum inhibition occurs in animals inoculated with tumor cell suspensions incubated with Fraction 90. Instances of survival in animals given tumor cell suspensions exposed to Fraction 4 or to the supernatant from Fraction 90 occurred in early experiments. Data from later experiments suggest that these survivals were due to overlapping during the fractionation procedure. Improved centrifugation technics in subsequent experiments have eliminated such results.

Corresponding fractions prepared in identical fashion to the above from other normal tissues (spleen, muscle, liver) showed no inhibitory properties. Fractions prepared from rabbit small intestine were ineffective, though this may be due to technical insufficiencies rather than to the absence of inhibitor.

In order to establish further that the inhibitory activity is due to specific substances in Fraction 90 rather than to bacterial contamination, proteolytic enzymes, mechanical or irritating effects or pH differences, appropriate experiments have been conducted to rule out these factors. Fraction 90 appears to be non-toxic since large intraperitoneal doses yielded no deleterious effects.

A limited number of experiments using Sarcoma 180 as the stock tumor indicated a response similar to that found with Gardner lymphosarcoma.

The data reported suggest that a non-toxic substance inhibitory to the development of lymphosarcoma is present in mouse and rat small intestine and that this material is recoverable by ultracentrifugal fractionation.

Further experiments are in progress to determine the nature of the inhibitory substance and to prepare it in a manner that will permit treatment of animals bearing established tumors.

1. Ladd, R. L., Goldstine, M. T., and Burns, O. R., *Growth*, 1950, v14, 265.

2. Tyler, D. B., *Science*, 1950, v112, 456.

3. Schneider, W. C. and Hogeboom, G. H., *Cancer Research*, 1951, v11, 1.

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Stabilizing Action of Glycerine on Hemagglutination of Egg-Adapted Mumps, Newcastle Disease and Influenza Viruses. (19222)

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It has long been recognized that glycerine either destroys or inhibits certain bacterial contaminants and it has been extensively used as a bacteriostatic and preservative agent in virus infected tissues since its introduction by Copeman(1) for the purification of calf lymph vaccine. Levaditi and Harvier(2) reported that herpes, vaccinia, rabies, and poliomyelitis viruses remained virulent for weeks and even months *in vitro* when placed in pure glycerine or glycerine diluted with an equal volume of normal saline and stored at 4°C. Their findings were confirmed by Doerr and others(3,4). According to Rivers(5) the preservative ac-