3. Van Dyke, D. C., Garcia, J. F., Lawrence, J. H., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 541.
4. Winkert, J., Gordon, A. S., Medici, P. T., Piliero, S. J., Luhby, A. L., Tannenbaum, M., *ibid.*, 1958, v97, 191.
5. Brecher, G., *Am. J. Clin. Path.*, 1949, v19, 895.
6. Mode, E., *Elements of Statistics*, 2nd ed., Prentice, Hall, New York, 1951.
7. Lowy, P. H., Keighley, G., Borsook, H., Graybiel, A., *Blood*, 1959, v14, 262.
8. Fried, W., Plzak, L. F., Jacobson, L. O., Goldwasser, E., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 237.
9. White, W., Gurney, C., Goldwasser, E., Jacobson, L. O., *Recent Progress in Hormone Research*, 1960, v16, 261.

Received July 1, 1960. P.S.E.B.M., 1960, v104.

Effect of Dietary Lipids on Growth of Transplantable Neoplasms in Mice.* (25964)

CAROLYN H. LINGEMAN, EDWARD B. SMITH AND PARKER R. BEAMER
Department of Pathology, Indiana University School of Medicine, Indianapolis

The effects of total restriction of diet on incidence and growth of neoplasms in experimental animals have been summarized by Tannenbaum(1) and by Cowdry(2). Neoplasms inhibited by dietary restriction are spontaneous mammary tumors, induced skin tumors, induced sarcomas, primary lung tumors, and leukemias. Spontaneous tumors are affected more than are artificially induced tumors.

Diets with a high content of fat have enhanced development of certain spontaneous and artificially induced neoplasms in rats and in mice(3), including stilbesterol-induced mammary carcinoma in rats, chemically induced hepatic carcinoma in rats, and carcinoma of the skin in mice produced by tars and polycyclic hydrocarbons. The fatty acid composition and degree of saturation of the fats used in most of these experiments varied and showed no consistent effect, but varied with individual neoplasms.

This report deals with a comparison of the effects of diets containing various lipids upon incidence and rate of growth of L1210 lymphatic leukemia in DBA/2 mice and the adenocarcinoma 755 in C57 Bl/6 mice(4). These transplantable tumors were selected because their characteristics are consistent and predictable. The dietary fats were selected to compare the effects of fats with a high content of unsaturated fatty acids with those with a lower content of unsaturated fatty acids.

Materials and methods. L1210 leukemia in DBA/2 mice. Two hundred fifty adult DBA/2 mice, 125 males and 125 females, were divided into 5 dietary groups of 50 mice each, (25 males and 25 females). They were approximately 4 months of age when special diets were substituted for *Purina Laboratory Chow*. The artificial diets were identical in composition except for fat content (Table I). The basic material, "fat-free" diet powder†, consisted of vitamin-free casein (21.1%), alphacel "cellulose" (16.45%), sucrose (58.45%), salt mixture (4%), and vitamins. Diet A (group A mice) consisted of the basic powder, Vit. B_{12} (3 μg/100 g diet), folic acid (0.2 mg/100 g diet), and ascorbic acid (0.1 g/100 g diet). Diets B, C, and D (groups B, C, and D) consisted of the basic diet powder and added vitamins plus 11% (by weight) of corn oil,‡ hydrogenated vegetable oils,§ or lard.‖ The characteristics of these various fats are shown in Table II.

The mice were maintained on the special

* Supported by grant from U.S.P.H.S.

† Nutritional Biochemicals Corp., Cleveland.

‡ Mazola, Corn Products Co., Argo, Ill. furnished through the courtesy of Mr. Neal E. Artz.

§ Shortening J (similar to Crisco), Procter and Gamble Co., Cincinnati, furnished through the courtesy of Mr. W. H. Meyer.

‖ Reliable Packing Co., Chicago, Ill., through the courtesy of Mr. Hugo E. Wistreich, who also supplied a chemical assay of this product.

TABLE I. Composition of Synthetic Diets.

	"Fat-free" base			Fat			% total calories, final diet mixture			Total calories/100 g final diet mixture
	Protein (casein)	Carbohydrate (sucrose)	Cellulose and salts	Corn oil	Hydrogenated vegetable oils	Lard	Fat	Carbohydrate	Protein	
	g/100 g diet									
A	21.1	58.5	20.5					73.5	26.5	358
B	18.8	52.3	18.3	10.5			22.9	56.5	20.5	415
C	18.8	52.3	18.3		10.5		22.9	56.5	20.5	415
D	18.8	52.3	18.3			10.5	22.9	56.5	20.5	415

diets, *ad lib.*, and tap water, for 7 months prior to inoculation with L1210 leukemia. During this preliminary period, mice from each group expired from various causes. Several mice of group A (minimal fat diet) developed signs of "essential fatty acid deficiency" (5), in most instances promptly relieved by addition of minimal amounts of corn oil to the diets.

Seven months after the beginning of the special diets, half of the surviving mice in each group received, intraperitoneally, 0.1 ml of a 1:100 dilution (in 0.25% methyl cellulose in 0.85% NaCl) of ascitic fluid that had been freshly aspirated from the peritoneal cavity of a DBA/2 mouse with L1210 leukemia (approximately 71,000,000 tumor cells per mouse). The mice which were not inoculated served as controls.

The mice in all groups, both inoculated and uninoculated, continued to receive the special diets, *ad lib.*, and tap water, until spontaneous deaths occurred. Daily total body weights of all inoculated mice and periodic body weights of uninoculated control mice were made.

All of the L1210-inoculated mice expired within 12 days following inoculation (Fig. 1). Fluid was withdrawn from the peritoneal cavity of these mice, smeared on slides and stained with Wright's stain. Histologic sections were made of organs from all mice.

Adenocarcinoma 755 in C57 Bl/6 mice. Eighty male C57 Bl/6 mice (age 4 months) were divided into 4 dietary groups of 20 each. Groups A, B, and C received diets identical in composition except for the fat content and tap water, *ad lib.* Group A received diet A, (fat-free), group B received diet B (11% corn oil), and group C received diet D (11% lard). Group D (controls) received *Purina Laboratory Chow.* The composition of these diets was identical to those described in the preceding section.

Five days after beginning of the special diets, 10 mice in each group received a subcutaneous implant of fresh adenocarcinoma 755 which had been removed from the donor mouse and chopped into uniform pieces 1 to 2 mm in diameter. To eliminate variation in viability of the tumor cells, implants were given alternately to the mice in each group. The 40 control mice received no tumor.

All mice, including the uninoculated controls, continued to receive the special diets. The inoculated mice were examined daily for tumors, which, in the majority, appeared within 8 or 9 days as a subcutaneous nodule

TABLE II. Characteristics of Fats.

		Predominant constituent fatty acids in %								
		Saturated					Unsaturated			
Fat	Iodine No.	Myristic	Palmitic	Palmatitic	Stearic	Approx. total	Oleic	Linoleic	Linolenic	Approx. total
Corn oil	125	.2	9.9		2.9	13.0	30.1	56.2		86.3
Hydrogenated vegetable oil product*	76	Saturated fatty acids consist primarily of palmitic and stearic acids.				24.5	65	10	.5	75.5
Lard	59 (approx.)	1.9	29.5	3.1	16.5	51.0	37.0	9.4		46.4

* Shortening J consists primarily of soybean and cottonseed oils.

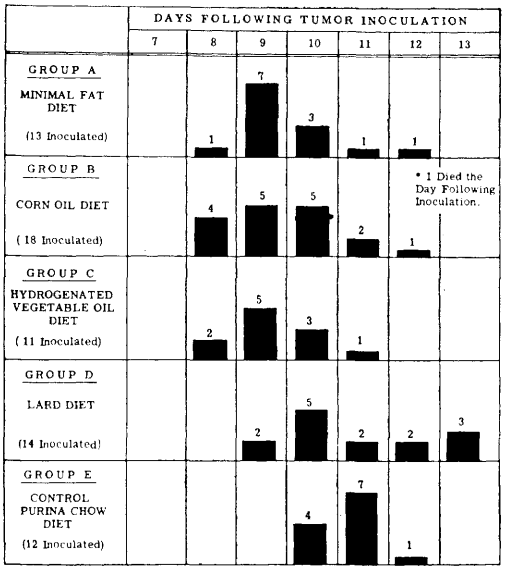

FIG. 1. Survival of DBA/2 mice after inoculation on day 1 with L1210 leukemia. Numbers over vertical bars represent No. of animals.

near site of inoculation. Total body weights of all mice were determined on alternate days. The tumors were measured daily by means of a vernier caliper and consisted of the product of the greatest dimension and a dimension at a 90 degree plane from the greatest. Several tumors grew rapidly resulting in death of the hosts. Because by the 21st day, a total of 6 mice had expired, surviving mice of all groups, including uninoculated controls, were sacrificed by deep chloroform anesthesia. The tumors were carefully removed and weighed in the fresh state. Because metastases were found in only 2 mice, they were not included in the final tumor weights. Histologic sections were made of the organs and tumors from all mice.

Results. L1210 leukemia. Examination of smears of the peritoneal fluid and histologic sections revealed that all inoculated mice had developed classical lymphatic leukemia. There was no significant difference in distribution of leukemic infiltration in any of the dietary groups. All mice had slight to moderate infiltration of the liver; more than one-half had definite involvement of the bone marrow (sternum), and adrenal glands. Insignificant numbers of mice in various groups had infiltration of the lungs, epicardium, kidneys, skin, and gastrointestinal tract.

None of the uninoculated control mice developed signs of leukemia during the interim between inoculation of L1210 leukemia and death of the inoculated mice, nor did any die during this period. A few are living after 17 months of maintenance on the special diets; some have ultimately developed spontaneous lymphatic leukemia or other neoplasms. The results of the effects of the various diets upon development of the spontaneous neoplasms will be reported later.

Adenocarcinoma 755. There was no significant difference in the mean time of appearance of tumors in any of the 4 dietary groups (Table III). By the 9th day after tumor implantation, one-half of the mice in each group had grossly palpable tumors; by the 21st day all inoculated mice in all groups had detectable tumors. *None of the uninoculated controls developed tumors.* All of the 6 mice (one in group A, 2 in group B and 3 in group D) which had expired by the 21st day had large tumors. Fig. 2 shows rate of growth of the tumors in each dietary group expressed as the product of 2 dimensions as described above.

Tumors of mice of all groups grew at approximately the same rate until the 14th day when those of group B mice began to lag slightly. The fastest rate of tumor growth occurred in groups A and D. Tumor weights compared favorably with measurements calculated in this manner (Table IV). The largest tumors occurred in group D (controls, regular diet) and the smallest in group B (artificial diet with corn oil). Some of the tumors were not weighed because portions had

TABLE III. Time of Appearance of Grossly Palpable Tumors.*

Dietary groups of mice	Days after implantation			
	10	13	15	22
A	5	8	8	10
B	5	9	10	10
C	5	8	8	10
D	6	8	8	9†

* Figures in vertical columns represent total No. of mice with grossly palpable tumors on any day.
† One mouse expired on day of tumor implantation.

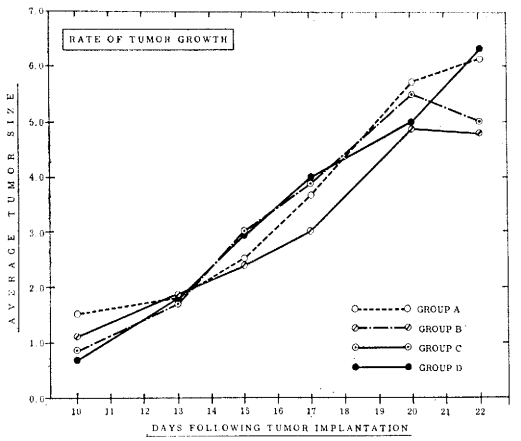

FIG. 2. Rate of growth of adenocarcinoma 755 in C57 Bl/6 mice. Tumor size is expressed by product of 2 dimensions (in cm), as explained in text.

become necrotic and in several instances had been partially eaten by other mice.

Although the mice of group A received no fat during the 4 weeks of the experiment, none developed overt signs of "essential fatty acid deficiency"(5), nor did the lack of fat significantly affect development or rate of growth of the tumors. Incidence and rate of growth of tumors in mice of all dietary groups were almost identical to those of the mice maintained on *Purina Laboratory Chow* (approximately 5% fat). Average total body weights of the uninoculated control mice on diets with 11% (by weight) of fat were not more than one or 2 grams higher than those of the mice receiving *Purina Laboratory Chow*. The mice which developed tumors appeared to gain weight, but when final tumor weights were subtracted from total body weights, it was found that they actually lost weight as the tumors developed.

TABLE IV. Comparison of Weights and "Surface Areas" of Tumors on Day of Death.

Dietary groups of mice	Wt of tumors (g)		"Surface areas" of tumors*	
	Avg	Range	Avg	Range
A	5.03	.91-8.64	4.8	.1-9.4
B	3.70	1.35-5.90	4.8	3.1-7.8
C	4.73	.18-9.20	5.0	.5-8.5
D	6.29	4.46-7.99	6.3	5.4-8.7

* Calculated by method described in text.

Histologic sections of inoculated mice revealed that all had typical adenocarcinomas. Metastatic tumors were found in only one mouse of group A and one of group C, in both cases to the liver and abdominal lymph nodes. *Uninoculated mice did not develop neoplasms of any type.*

Summary. The incidence of susceptibility of inbred mice to 2 transplantable neoplasms, L1210 leukemia and adenocarcinoma 755, was not influenced by variation in the dietary fats. Diets deficient in fat likewise had neither inhibiting nor stimulating activity in the pathogenesis of the 2 neoplasms.

The authors are indebted to Dr. Clyde Culbertson and Dr. Irving Johnson, Eli Lilly and Co., for help in supplying mice and transplantable tumors, and to Mr. James F. Glore, Dept. of Illustration at the Medical Center, for preparation of graphs. The capable technical assistance of Pearl Driver, Betty Corya, Shirley Stockslager, and Donna King is also appreciated.

1. Tannenbaum, A., *Ann. N. Y. Acad. Sci.,* 1947, v49, 5.

2. Cowdry, E. V., *Cancer Cells,* W. B. Saunders Co., Philadelphia, 1955, 400.

3. Haven, F. L., Bloor, W. R., *Advances in Cancer Research,* v4, Academic Press, 1956, 237-314.

4. Dunham, L. J., Stewart, H. L., *J. Nat. Cancer Inst.,* 1954, v13, 1299.

5. Decker, A. B., Fillerup, D. L., Mead, J. F., *J. Nutrition,* 1950, v41, 507.

Received June 30, 1960. P.S.E.B.M., 1960, v104.