TABLE II. Percentage Takes in Males and Females of the dba Strain of an Inoculated Transplantable Mammary Adenocarcinoma, dbrB.

	Males			Females		
	Total	Takes		Total	Takes	
Exp. No.	mice	No.	%	mice	No.	%
184	14	12	86	17	11	65
190	15	7	47	21	12	57
193	47	37	79	6	5	83
196	5	1	20	27	7	26
202	9	5	56	18	6	33
	90	62	69±3.3	89	41	46 ±3.57

examination of the gross tumor.

Results. A study of our data was carried out where there were less than 100% takes in the 2 strains of mice, *i.e.*, the Marsh and the dba strains. The data for 311 males and 258 females of the Marsh strain are shown in Table I. It is evident from this table that there is no consistent difference between the sexes in the individual experiments and no significant difference between the percentages when all animals are considered collectively.

On the other hand, the data of 90 males and 89 females of the dba strain, although showing no consistent sex influence for the individual experiments, do show a significant difference when considered collectively as shown in Table II.

Discussion. Thus, the growth and development of the dbrB tumor in the dba strain appeared to be related to the sex of the host, whereas no significant sex difference in the number of takes was observed in the Marsh strain when inoculated with a Marsh-Simpson tumor suspension. Experiments are in progress to examine further the possibility that the difference in tumor-take incidence observed for these adenocarcinomas might be explainable on an endocrine basis. From these observations, probably a sex difference in susceptibility to transplanted tumors may be present in some strains and absent in others.

Summary. A significant sex difference in incidence of tumor takes appeared when a known quantity of viable mammary adenocarcinoma cells in a dbrB tumor cell suspension was inoculated subcutaneously into dba mice, where males were more susceptible than females under similar conditions. This sex difference was not noted when a Marsh-Simpson mammary adenocarcinoma tumor cell suspension was inoculated into Marsh mice.

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Concentration of Lipid Phosphorus in Tumor and Carcass of Rats Growing Walker Carcinoma 256.* (19736)

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The progressive growth of Walker carcinoma 256 in albino rats has been shown(1-3), to cause a loss of lipid from the carcass, which loss of lipid, rather than failure of appetite, accounts for the caloric deficit produced in the animals(4). In view of the lipemia characteristic of rats growing Walker carcinoma 256 (2,3), and the high concentration of phospholipids in this tumor, a direct comparison of the phospholipid concentration in the tumor and the carcass of the host rat, and in the carcass of the host rat and of suitable control

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TABLE I. Concentration of Lipid Phosphorus in Walker Carcinoma 256 and in Normal, Control and Host Rats.

Group	No. rats	Lipid phosphorus, g per 100 g: % dry tissue (avg)	Stand. dev.	% fat-free dry tissue (avg)	Stand. dev.
I Young rats					
a—Normal	11	.156	.010	.210	.011
b—Rats plus tumor	11	.166	.012	.216	.015
II Young rats					
a—Normal	12	.124	.020	.190	.024
b—Rats plus tumor	5	.173	.022	.196	.022
III Adult rats					
a—Controls; non-tumor rats killed when tumors started to grow in rats of c—	7	.089	.003	.137	.012
b—Controls; non-tumor rats pair-fed to tumor rats of c—	7	.092	.010	.134	.018
c—Tumor rats	7				
Carcass		.122	.018	.137	.023
Tumor		.195	.036	.224	.043
Carcass plus tumor		.135	.018	.153	.023
IV Adult rats					
a—Controls; non-tumor rats killed when tumors started to grow in rats of c—	9	.095	.007	.153	.006
b—Controls; non-tumor rats killed with tumor rats of c—	6	.084	.007	.143	.010
c—Tumor rats	8				
Carcass		.106	.009	.156	.013
Tumor		.276	.039	.332	.047
Carcass plus tumor		.131	.016	.186	.020

rats is needed. This paper presents such a comparison for lipid phosphorus and for an alkali-resistant phospholipid, both of which are found to be concentrated in the tumor.

Materials and methods. Male albino rats and Walker carcinoma 256 grown subcutaneously, were used throughout the experiments. The rats of Groups I and II (Table I) were young; their body weights at the time of tumor transplantation averaged 74 g. The animals of Groups III and IV were adult rats from Dr. G. B. Mider's laboratory; the average body weights were 190 g and 230 g, respectively, at the time of tumor transplantation.

The rats of Groups I and II received *ad lib.* Diet 262 containing 21.3% coconut oil (5). In Group III, the rats without tumor were pair-fed with the tumor rats which ate *ad lib.* Data for the rats of this group, for the technic of pair-feeding, and for the diet, have been described previously(4). The loss of appetite which occurs during tumor growth was counteracted by force-feeding the animals of Group IV with the high-fat diet of Ingle (6) modified by the substitution of lactalbumin for casein, and the addition of 1 g of choline per kg of diet. Each rat in the group received 20 g of diet per day (50 cal per day) by stomach tube.

The rats of Group I were killed by diethyl ether 2 weeks after tumor transplantation when the tumors constituted from 0.26 to 8.8% of the total body weight. Group II was killed by diethyl ether 3 weeks or more after transplantation, when the tumors comprised from 22 to 52% of the total body weight. The whole rat plus weighed tumor was ground and dried in the frozen state (lyophilized)(3). The rats of Group IV were decapitated and

the blood collected in saturated citrate. Nine animals without tumor were killed 7 days after transplantation of tumor in the experimental group when the tumor had started to grow. Six rats without tumor were killed when the rats with tumor were killed, at 4 and 5 weeks after transplantation; at this time the tumors were 20% of the body weight, except for one which was only 7%. Lyophilized dried carcass and tumor tissues were prepared as described(3).

The methods used for extraction and determination of the total lipid of the tissues(3) and of the blood plasma(2) have been described previously. In this study the concentration of lipid phosphorus rather than of phospholipid was determined in order to eliminate the use of an oxidative factor for a mixture of phospholipids, since a single factor might be considerably in error in the case of tumor where the phospholipids may differ qualitatively from those in the host rat. Since chloroform-methanol was used for extraction in addition to alcohol-ether, our values represent virtually all the lipid phosphorus of the tissues; (the phospholipids of both wet and dry tissues are incompletely extracted by alcohol-ether alone)(7,8). Lipid phosphorus was determined in duplicate on the solutions of total lipid by the method of Kuttner and Lichtenstein(9). On samples from Group IV, an alkali-resistant phospholipid was isolated from the fatty acid fraction as the lead salt(10) and the amount determined oxidatively.

Results. The values for lipid phosphorus as percentage of the dry tissue and of the fat-free dry tissue, respectively, are given in Table I. That lipid phosphorus concentrates in Walker carcinoma 256, is evident from the results for the adult rats of Groups III and IV where tumor and carcass were analyzed separately. The values for tumor are significantly higher ($P = <.01$ in both groups) than those for carcass of the rat with tumor, whether they are expressed as percentage of dry tissue or of fat-free dry tissue. The difference between tumor and carcass was not the result of a decrease in the lipid phosphorus concentration in the carcass, since the values for carcass are the same or slightly higher than the values for rats killed either when the tumor started to grow or when the tumor rats were killed. The greater concentration of lipid phosphorus in the tumor is also shown by the fact that values are significantly higher ($P = <.01$) in the combined carcass plus tumor than in the non-tumor animals of either of the control groups. The tumor-bearing rats given 50 cal per day of a high-fat diet by gavage (Group IV) had a significantly greater ($P = <.01$) concentration of lipid phosphorus in their tumors than did similar rats which consumed diet *ad lib.* (Group IIIc).

Lipid phosphorus was also concentrated in the tumors of the young rats of Groups I and II since values for the rat plus tumor (percentage dry tissue) are significantly higher (Group I—$P = >.02, <.05$; Group II—$P = <.01$) than values for the normal rats. It is interesting to note that the values for lipid phosphorus of the normal young rats of these groups are higher than the values for the normal adult rats of Groups III and IV.

Although little is known regarding the comparative concentration in tumor and rat of the different kinds of phospholipid which contribute to the high lipid phosphorus concentration of Walker 256, the concentration of a phospholipid fraction which is highly resistant to alkali has been determined for the animals of Group IV and is presented in Table II. The concentration was significantly greater ($P = <.01$) in the tumor than in the carcass of the tumor rat, or in the rats of either of the control groups.

Discussion. The high concentration of phospholipid in tumors as compared with various other tissues of the body has been known for almost half a century. The magnitude of the concentration has now been determined by this comparison between the tumor and the rest of the rat's body. The greater concentration of lipid phosphorus in the tumor than in the carcass was not caused by loss of appetite during tumor growth; the rat plus tumor had a greater concentration of lipid phosphorus than normal rats that received the same diet *ad lib.*, or the same amount and kind of diet by pair-feeding or by gavage, as the respective tumor-bearing rats. The high concentration of lipid phosphorus (phospholipids)

TABLE II. Concentration of Alkali-Resistant Phospholipid in Walker Carcinoma 256 and in Control and Host Rats.

Group	No. rats	Phospholipid, g per 100 g: % dry tissue (avg)	Stand. dev.	% fat-free dry tissue (avg)	Stand. dev.
IV Adult rats					
a—Controls; non-tumor rats killed when tumors started to grow in rats of c—	9	.097	.043	.156	.022
b—Controls; non-tumor rats killed with tumor rats of c—	6	.097	.025	.166	.053
c—Tumor rats	8				
Carcass		.120	.035	.178	.054
Tumor		.373	.052	.448	.065

in the tumor is probably concerned with the enhanced metabolism of fat which may account for the caloric deficit of the animal, and which is undoubtedly related to the lipemia that accompanies growth of this tumor. Evidence for this assumption is not entirely lacking.

The greater concentration of lipid phosphorus in the tumors of rats force-fed a high-fat diet (Group IVc) may have resulted from the necessity for metabolizing excessive amounts of fat. The dietary procedure produced a marked lipemia in the 8 tumor-bearing rats; 7 of them had values for total lipid in the blood plasma ranging from 552 to 1372 mg %, while one had a value of 5350 mg %. The 15 non-tumor rats of this group averaged 423 mg % with a standard deviation of 59. In addition to the excessive lipemia caused by the forced feeding, the concentration of total lipid in the tumors increased by about 30% as compared with the concentration in the tumors of Group III. This increase in total lipid is fully accounted for by increased phospholipid as estimated from the increase in concentration of lipid phosphorus.

When expressed as percentage of the fat-free dry tissue, the increased concentration of lipid phosphorus in rat plus tumor was apparent in adult rats but not in young rats even in those of Group II where tumors comprised over 20% of the total body weight. Tumor tissue is a young tissue with a high lipid phosphorus concentration similar in magnitude to that of young rats. In the tumor the high value for lipid phosphorus was maintained even when the tumor was grown on adult rats of lower lipid phosphorus concentration. The greater growth potential of this tumor in young than in adult rats might conceivably be correlated with the respective lipid phosphorus concentrations of the rats.

The concentrations of most of the component phospholipids which account for the greater concentration of lipid phosphorus in the tumor remain to be determined. However, we have shown that the tumor is rich in a phospholipid which is stable to alkali. Similar (unpublished) data for the concentration of this material were obtained in tumor rats that ate *ad lib.* 2 diets(3) of lower fat content. From the stability toward alkali, the nitrogen and phosphorus contents, the solubility in various organic solvents, the occurrence, and the general behavior of the substance, we assume tentatively that it is a crude acetal phospholipid.

The maintenance by the rat growing Walker carcinoma 256 of a lipid phosphorus concentration, or of the concentration of a particular phospholipid in his tumor, far in excess of that in his carcass, may contribute in large measure to the stress which causes many of the adverse effects of the tumor on the host rat.

Summary. In rats growing Walker carcinoma 256, 1) the concentration of lipid phosphorus was greater in the tumor than in the carcass; 2) the concentration of lipid phosphorus was greater in the rat plus tumor than in pair-fed or force-fed rats without tumor that consumed the same amount and kind of diet as the tumor rats; 3) the concen-

trations of lipid phosphorus in young rats and in the tumor were similar, and greater than in adult rats; 4) the concentration of an alkali-resistant phospholipid was greater in the tumor than in the carcass.

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Multiple Effects of Fluoroacetate on Pyruvate Metabolism *in vitro*. (19737)

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The injection of fluoroacetate into rats has been shown to result in the accumulation of large amounts of citrate in certain organs (1-5). No accumulation of citrate was found (3) in livers of fluoroacetate-treated rats; these rats were of the same sex and strain as those whose livers had previously been shown to produce large amounts of citrate *in vitro* (6).† A possible explanation for this discrepancy was furnished by studies(7,8) showing that an interruption in the Krebs cycle in the case of liver results in the diversion of C_2 fragments away from the Krebs cycle and into acetoacetate formation, and it might have been assumed that the results could be explained solely on this basis. However, when *in vitro* studies were carried out to test this simple hypothesis, new results were obtained, some of which appear to be unexplainable in terms of the inhibition of citrate removal alone.

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† The rats used by Potter and Busch(3) were normal males. Subsequent work in this laboratory and in that of Dr. K. P. DuBois makes it clear that hormonal and other physiological factors can alter the capacity of the liver to accumulate citrate following the injection of fluoroacetate. Fed female rats accumulated citrate in their livers following the injection of fluoroacetate, and male rats could be shown to do likewise under special conditions.

Experimental. Chemicals. Sodium monofluoroacetate was supplied by Dr. K. P. DuBois of the University of Chicago Toxicity Laboratory; adenosine triphosphate (ATP) was prepared by Dr. G. A. LePage and associates.

Homogenates. Male rats (weighing 200-300 g) obtained from the Holtzmann-Rolfsmeyer Rat Co. were used in this study. After the rats were decapitated, the kidneys were quickly removed and placed in ice-cold isotonic KCl solution; after weighing, they were placed in glass homogenizers(9) containing 9 volumes of ice-cold isotonic KCl solution and homogenized in the cold; the same procedure was followed with liver homogenates, but the homogenizers contained 5¼ volumes of KCl solution to give a concentration of 16% liver by weight.

Flask contents. a) *Reaction mixtures.* Each Warburg flask contained 1.0 ml of a basal medium composed of 0.01 M $MgCl_2$, 0.2 M KCl, and 0.01 M K phosphate buffer adjusted to pH 7.2(6) as well as 30 μM potassium pyruvate, 3 μM potassium ATP and sufficient 0.2 M or 0.02 M sodium monofluoroacetate‡ to produce the desired concentration. All substrates were added as potassium salts neutralized to pH 7.2-7.4. In all experiments other than those involving phosphate studies, an additional 0.1 ml of 0.1