prednisone-treated non-obese subjects than in untreated non-obese or obese subjects. The mean post-infusion IRI concentration was only 190% of the mean value during infusion (comparable values of 291 and 374% in the untreated non-obese and obese subjects respectively).

Blood glucose values for the 3 groups of experimental subjects are shown in Table I. The comparable fall in blood glucose in non-obese and obese subjects despite hyperinsulinism in the latter underscores the role of insulin resistance in obesity.

Summary. Obesity is characterized by fasting hyperinsulinism and elevated IRI concentrations during and following an epinephrine infusion. Suppression of plasma IRI by epinephrine is normal in obese, nondiabetic subjects. The concept of sluggish alpha receptors cannot be invoked as a cause of the hyperinsulinism of obesity.

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Interferon and Murine Leukemia. — IV. Futher Studies on the Efficacy of Interferon Preparations Administered after Inoculation of Friend Virus. (32572)

I. GRESSER, R. FALCOFF, D. FONTAINE-BROUTY-BOYE,† F. ZAJDELA,† J. COPPEY, E. FALCOFF†

Laboratories of Viral Oncology and Pathophysiology, Institut de Recherches Scientifiques sur le Cancer, 94-Villejuif

It has been reported previously that continued interferon treatment initiated 48 hours *after* inoculation of Friend virus inhibited the development of splenomegaly in Swiss mice (1). Although an increased number of "Friend cells" may be present in the spleen at this time (48 hours), significant splenic enlargement is not observed(2,3). It was considered of interest therefore to determine the effect of interferon on the evolution of Friend disease when treatment was initiated at an even later stage. Thus, in one of the experiments reported herein, interferon treatment was begun one week after viral inoculation, at a time when splenomegaly had already developed in most mice. In addition, we present the results of investigations pertaining to the mechanism and specificity of interferon action in Friend disease. These studies include histologic examinations of the spleens of interferon treated and untreated mice, assays of several of these spleens for infective virus, and the determination of the efficacy of a highly purified preparation of interferon.

Methods and materials. The techniques employed in the assay of Friend virus; the preparation of concentrated crude mouse brain interferon and its assay have been previously described in detail(4). They may be summarized briefly as follows: 4- to 6-week-old Swiss mice were inoculated intraperitoneally (i.p.) with a 10-15% extract of Friend leukemic spleen and sacrificed 2-4 weeks later. Spleen weights provided criteria for infection and extent of disease(5). Interferon was extracted from the brains of mice infected

† Unité de Physiologie Cellulaire, I.N.S.E.R.M., Institut du Radium 91-Orsay.

‡ Groupe de Recherches sur les virus, Institut National de la Santé et de la Recherche Médicale, Hôpital Saint-Vincent de Paul, Paris.

with West Nile virus(6,7), concentrated 10-fold(4), and assayed by standard plaque reduction techniques utilizing monolayer cultures of mouse embryo fibroblasts or L cells inoculated with 50-100 p.f.u. of vesicular stomatitis virus (VSV).

Preparation of highly purified mouse brain interferon(8). Mouse brain interferon(6,7) was maintained at pH 2 for 18 hours and the pH was then raised to 5.9. 3 volumes of interferon were mixed for 16-19 hours (4°C with agitation) with 1 volume of gel CM Sephadex C_{50} (.01 M phosphate buffer pH 5.9). After filtering and washing several times with .01 M phosphate buffer pH 6.0, interferon was extracted from the resin by washing first with 0.1 M phosphate buffer at pH 7.3 and subsequently 3 times with 0.1 M phosphate buffer at pH 9.0. By these techniques 70-80% of the activity of the initial interferon preparation was recovered. Preparations of purified interferon were then concentrated 10-20-fold by pressure dialysis.

Histologic techniques. Spleens were fixed in buffered isotonic 10% aqueous formalin. Histologic sections were stained with a modified May-Grünwald-Giemsa which emphasized the cytoplasmic basophilia of the "Friend cells"(3).

Results. Effect of interferon preparations administered subcutaneously. Treatment initiated prior to viral inoculation. In previous experiments the development of ascites in mice inoculated i.p. with concentrated interferon preparations for several weeks prevented continuation of interferon treatment (4). It was desirable therefore to determine whether the subcutaneous route of inoculation might also prove effective. Accordingly, a concentrated interferon preparation (1:34,000/2 ml) was inoculated subcutaneously 24 and 4 hours prior to viral inoculation and twice daily thereafter for the 1-month duration of the experiment.

As can be seen from Table I subcutaneous interferon treatment inhibited the development of splenomegaly in Friend viral infected mice to a significant degree ($p = < .001$). It was of interest to note that the mean spleen weight of mice inoculated with normal mouse brain extract was greater than

Table I

EFFECT OF MOUSE BRAIN INTERFERON ADMINISTERED SUBCUTANEOUSLY ON DEVELOPMENT OF SPLENOMEGALY IN FRIEND DISEASE OF SWISS MICE

UNINOCULATED		INOCULATED WITH FRIEND VIRUS[1] TREATMENT[2]					
		NONE		NORMAL BRAIN		INTERFERON[3]	
No. mice	g. m. spleen wgt m ± se	No. mice	g. m. spleen wgt m ± se	No. mice	g. m. spleen wgt m ± se	No. mice	g. m. spleen wgt m ± se
10	178 mg 2.25 ± .03	24	729mg 2.86 ± .06	24	1208mg 3.08 ± .05	24	257mg 2.41 ± .02

Swiss mice 6 week old female, sacrificed 1 month after inoculation of virus.

[1] Virus, 10% extract of leukemic spleen, 0.2 ml i.p. (200 SD_{50}).

[2] Treatment with either normal brain extract or brain interferon was initiated 24 and 4 hours *prior* to inoculation of Friend virus and continued twice daily thereafter until termination of the experiment. Treatment consisted of twice daily injections of 0.2 ml subcutaneously.

[3] Titer of interferon employed 1:34,000/2 ml.

g.m. spleen wgt: geometric mean spleen weight.

m ± se: geometric mean in logarithms ± standard error.

that of untreated viral infected mice. This represented the sole instance in our experiments to date of a significant difference between the 2 viral control groups(1,4,9).

Treatment initiated after viral inoculation. 3 groups of Swiss mice were inoculated subcutaneously with concentrated mouse brain interferon (titer: 1:37,000/2 ml). In the 1st group, interferon was inoculated 24 hours after viral inoculation and twice daily thereafter thruout the 27 days of the experiment. In a 2nd group interferon was inoculated 24 hours after viral inoculation and twice daily for the ensuing 6 days. These mice were subsequently kept without treatment for the remainder of the experiment. In a 3rd group, interferon treatment was initiated *1 week after* viral inoculation and treatment was continued twice daily for the next 3 weeks of the experiment. At the time interferon treatment was initiated in this group (7th day) 13 of the 22 mice (59%) had enlarged spleens as determined by abdominal palpation.§ In contrast only 5 of 24 mice (21%) in each of the first 2 groups of mice

§ Another group of 16 untreated mice was sacrificed at this time (7 days) to confirm the presence of splenomegaly. The mean spleen weight of these mice was 356 mg as compared to 210 mg for uninfected mice in this experiment.

treated with interferon had palpable spleens at this time.

When the mice were sacrificed 27 days after viral inoculation, it was apparent (Table II) that interferon treatment initiated either

Table II

EFFECT OF MOUSE BRAIN INTERFERON ADMINISTERED SUBCUTANEOUSLY ON DEVELOPMENT OF SPLENOMEGALY IN FRIEND DISEASE: TREATMENT INITIATED AT VARYING INTERVALS AFTER VIRAL INOCULATION

UNINOCULATED		INOCULATED WITH FRIEND VIRUS[1]							
		TREATMENT[2]							
		NONE		NORMAL MOUSE BRAIN Days +1 to +27		INTERFERON[3] Days +1 to +7		Days +1 to +27	Days +7 to +27
No mice	g.m. spl. wgt m ± se	No mice	g.m. spl. wgt m ± se	No mice	g.m. spl. wgt m ± se	No mice	g.m. spl. wgt m ± se	No mice	g.m. spl. wgt m ± se	No mice	g.m. spl. wgt m ± se
10	210mg	24	1265mg	23	1128mg	24	1125mg	24	615mg	22	693mg
	2.32 ± .03		3.10 ± .04		3.05 ± .03		3.05 ± .05		2.78 ± .05		2.84 ± .05

Swiss mice, 5- and 6-week-old female, sacrificed 27 days after inoculation of virus.

(1) Virus, 10% extract of leukemic spleen, 0.2 ml i.p.

(2) Treatment in various groups as follows: *Normal mouse brain*: Treatment initiated 24 hours after inoculation of virus and continued (0.3 ml subcutaneously) twice daily for 27 days. *Interferon*: Days +1 to +27: treatment initiated 24 hours after inoculation of virus and continued (0.3 ml subcutaneously) twice daily for 27 days. Days +1 to +7: treatment initiated 24 hours after inoculation of virus and continued (0.3 ml subcutaneously) twice daily for the next 6 days. Thereafter mice received no treatment. Days +7 to +27: treatment initiated 7 days after inoculation of virus and continued (0.3 ml subcutaneously) twice daily for the next 20 days.

(3) Titer of interferon employed 1:37,000/2 ml.

g.m. spleen wgt: geometric mean spleen weight.

m ± se: geometric mean in logarithms ± standard error.

1 or 7 days after viral inoculation and continued daily thereafter was equally effective in retarding splenomegaly when compared to the 2 viral control groups ($p = <.001$). Although 1 week of interferon treatment initially inhibited splenomegaly, as judged by abdominal palpation, it did not alter the ultimate splenomegalic response when those mice were left untreated for the ensuing 3 weeks.

Presence of infective virus in the spleens of mice treated with normal brain extract or interferon. At the termination of the experiment summarized in Table I, 3 moderately enlarged spleens and the 2 smallest spleens of mice treated with normal brain extract were assayed individually for infective Friend virus. Significant amounts of virus were recovered from the 3 enlarged spleens, but not from the 2 small spleens¶ (Table III).

Table III

TITER OF FRIEND VIRUS IN REPRESENTATIVE SPLEENS OF SWISS MICE AFTER TREATMENT FOR ONE MONTH WITH NORMAL MOUSE BRAIN EXTRACT OR MOUSE BRAIN INTERFERON

NORMAL BRAIN		INTERFERON	
Wgt of spleen (mg) tested	Titer[1]/0.2ml 10% extract	Wgt of spleen (mg) tested	Titer[1]/0.2ml 10% extract
1380mg	200 SD_{50}	423mg	<1 SD_{50}
1250mg	40 SD_{50}	395mg	3 SD_{50}
1180mg	>500 SD_{50}	360mg	<1 SD_{50}
340mg	<1 SD_{50}	260mg	2 SD_{50}
170mg	<1 SD_{50}	160mg	<1 SD_{50}

(1) Titered in 4-6 week old Swiss mice and expressed as spleen $dose_{50}$ (SD_{50}). Spleen weights above 300 mg were considered as indicative of disease.

The 3 largest spleens and 2 smaller spleens from the interferon treated group of mice were also assayed individually for their content of infective virus. Only small amounts of virus were recovered from each spleen (Table III). Thus viral infected mice treated with interferon had smaller spleens than untreated mice, and less virus was recovered from small spleens than from large spleens (this was so for both groups).

Histology of the spleens of viral infected mice treated with interferon. At the termination of the experiment summarized in Table I, splenic imprints and histologic sections of the spleens of all mice were examined.

The "Friend cell"(10) is a large cell with deeply basophilic cytoplasm considered to be similar to a proerythroblast(3). This type of cell was occasionally observed singly or in small clumps in erythropoietic foci in the spleens of uninfected control mice (Fig. 1). In Friend viral infected *untreated* mice numerous large foci of Friend cells were observed thruout the splenic red pulp. It was apparent that in some instances these foci had merged since large areas of the red pulp were replaced by sheets of these cells (Fig. 2). In contrast, the number and size of the foci of Friend cells were markedly reduced in the spleens of *interferon treated* mice, and these foci were discrete leaving most of the red pulp intact (Fig. 3). Numerous cells at different stages of erythroid maturation were noted in close

¶ As previously noted(4), 10-15% of Friend viral infected Swiss mice in our experiments do not develop significant splenomegaly. Moreover on histologic examination of the spleen only a few foci of Friend cells are observed. It was not unexpected therefore that little or no infective virus was recovered from the two small spleens.

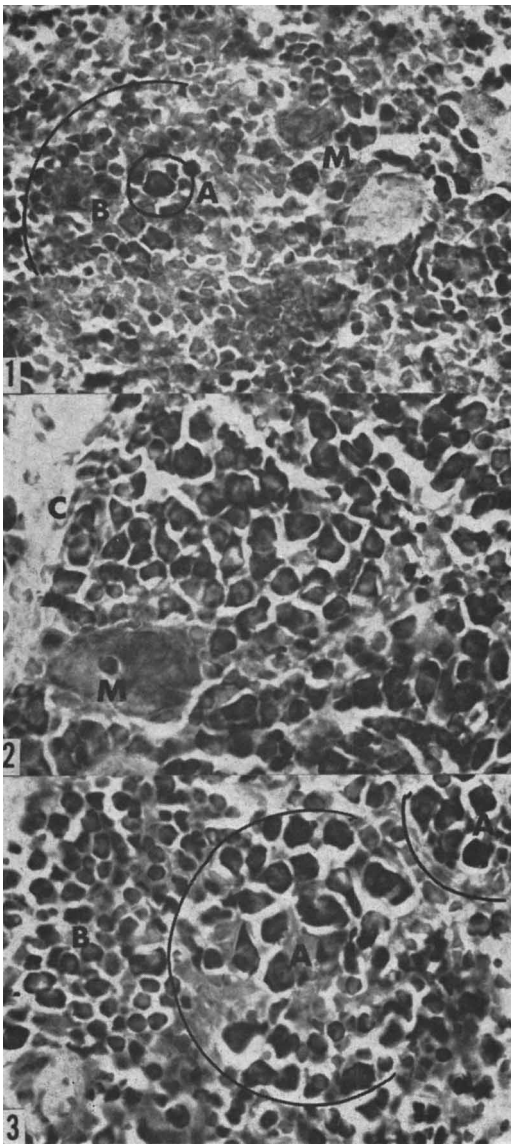

FIG. 1. Splenic red pulp of uninoculated Swiss mouse. Note large cell with hyperbasophilic cytoplasm (A) and focus erythropoiesis (B). M = focus of megakaryocyte maturation (modified May-Grünwald Giemsa stain-oil immersion).

FIG. 2. Spleen of Swiss mouse 1 month after inoculation of Friend virus. Note red pulp almost entirely replaced by large hyperbasophil cells characteristic of Friend disease. C = Connective tissue. M = megakaryocyte. (modified May-Grünwald Giensa stain-oil immersion).

FIG. 3. Spleen of Swiss mouse 1 month after inoculation of Friend virus. Treated with interferon. Note 2 small foci of large hyperbasophil cells (A), surrounded by a zone of erythropoietic maturation (B), composed essentially of basophilic normoblasts, (modified May-Grünwald Giemsa stain-oil immersion).

association with these foci of Friend cells in the spleens of mice treated with interferon (Fig. 3). These areas of erythroid maturation were more extensive than in the spleens of untreated viral infected mice (see also 11).

Further evidence that the inhibitory effects were attributable to interferon. Since various biologic effects observed with crude interferon preparations have often not been observed with relatively purified interferon preparations (12), it was considered of interest to determine a) whether crude interferon preparations were toxic for mice or induced alterations which might prove responsible for inhibition of splenomegaly, and b) whether highly purified preparations of interferon would in fact inhibit splenomegaly.

Absence of toxicity. Twelve 6-week-old female Swiss mice were inoculated subcutaneously twice daily with 0.2 ml of concentrated interferon (titer : 1:50,000/2 ml) and 12 mice were inoculated with 0.2 ml of concentrated normal mouse brain extract. 12 mice were kept as uninoculated control animals. Total body weight was determined weekly for each mouse. Mice were sacrificed at 17 and 24 days.

No significant difference was observed between the total body weight or the weights of the spleen, liver, left kidney and heart of mice in the 3 groups. Furthermore there was no significant difference in peripheral blood leucocyte count, reticulocyte count or hematocrit. No abnormalities were observed on histologic examination of the liver and spleen of these mice.

Efficacy of highly purified interferon. Fig. 4

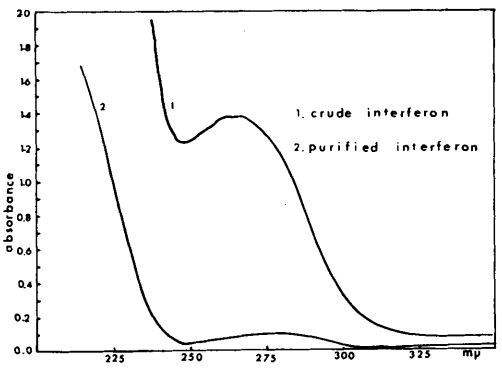

FIG. 4. Ultraviolet spectrophotometric absorption of crude and highly purified interferon.

illustrates the marked difference in the ultraviolet spectrophotometric absorption between a highly purified concentrated interferon preparation (methods and materials), and the standard crude concentrated interferon preparation. The viral inhibitory titers of the 2 interferons however were comparable (purified interferon 1:24,000/2 ml; 37,8 units interferon/1 μg protein:crude interferon 1:34,000/2 ml; 5 units interferon/1 μg protein).

To compare the effect of these interferon preparations in Friend disease mice were inoculated intraperitoneally, 24 and 4 hours prior to viral inoculation and twice daily for 7 days thereafter by this route. For the ensuing 9 days mice were inoculated subcutaneously twice daily.

When mice were sacrificed 16 days after viral inoculation it was apparent (Table IV)

Table IV

EFFECT OF CRUDE AND HIGHLY PURIFIED MOUSE BRAIN INTERFERON ON DEVELOPMENT OF SPLENOMEGALY IN FRIEND DISEASE OF SWISS MICE

UNINOCULATED		INOCULATED WITH FRIEND VIRUS [1] TREATMENT [2]							
		None		Highly-purified normal brain		Crude [3] interferon		Highly-purified [4] interferon	
N° mice	spleen wgt [5] (m ± se)	N° mice	spleen wgt (m ± se)	N° mice	spleen wgt (m ± se)	N° mice	spleen wgt (m ± se)	N° mice	spleen wgt (m ± se)
10	165mg (2.22 ± .03)	20	500mg (2.70 ± .06)	21	540mg (2.73 ± .04)	20	395mg (2.60 ± .05)	21	385mg (2.58 ± .05)

Swiss mice 5-week-old male, sacrificed 16 days after inoculation of virus.

[1] Virus, 10% extract of leukemic spleen, 0.2 ml, i.p.

[2] Treatment with either normal mouse brain extract or mouse brain interferons initiated 24 and 4 hours prior to inoculation of Friend virus and continued twice daily (0.2 ml) intraperitoneally for seven days and subcutaneously for the ensuing 9 days.

[3] Titer of crude interferon 1:32,000/2 ml.

[4] Titer of purified interferon 1:24,000/2 ml.

[5] Spleen weight: geometric mean spleen weight. m ± se: geometric mean in logarithms ± standard error.

that highly purified and crude interferon were equally effective in retarding splenomegaly ($P = .05$ compared to viral controls).

Representative spleens of mice that had been treated with either purified normal mouse brain or purified mouse brain interferon were assayed individually for their content of infective virus. 3 spleens (170 mg, 960 mg and 1250 mg) from mice inoculated with normal brain extract were assayed and the viral titers (per 0.2 ml of a 10% extract) were 64 SD_{50}, 640 SD_{50} and 100 SD_{50} respectively. 3 spleens (120 mg, 190 mg, 780 mg) of mice inoculated with purified interferon were also assayed and the viral titers obtained were 2 SD_{50}, 1 SD_{50} and 400 SD_{50} respectively. Thus, as in the experiments with crude interferon outlined above (Table III) less Friend virus was recovered from the spleens of mice treated with purified interferon than from the spleens of mice treated with purified normal mouse brain.

Discussion. Previous experiments demonstrated the necessity of continuing interferon treatment after viral inoculation in order to inhibit the development of splenomegaly in Friend disease(4,9). Pretreatment of mice with interferon was not essential, since initiation of interferon treatment 48 hours after viral inoculation proved effective(1). The experiments reported herein confirmed and extended these observations. Initiation of interferon treatment even 1 week after viral inoculation, at a time when splenic enlargement had already developed in most mice, still exerted a significant inhibitory effect on the further development of splenomegaly. Interferon treatment for *only* the 1st week after viral inoculation was inadequate.

It was apparent from histologic examination, that splenic size was related to the extent of Friend cell involvement. There were large foci of Friend cells in the spleens of untreated mice and the red pulp was often completely replaced by sheets of these cells. In comparison, foci of Friend cells were reduced both in size and in number in the spleens of interferon treated mice. Likewise the amount of Friend virus recovered per unit weight of spleen appeared roughly proportional to splenic size and the extent of Friend cell involvement. Thus, virus was easily recovered from several large spleens of mice inoculated with normal mouse brain, whereas the small spleens of interferon treated mice and the occasional small spleens of untreated mice contained only small amounts of infective virus. It seems reasonable to suggest that these findings were causally related, *i.e.*, fewer foci of Friend cells were present in the spleens of interferon treated mice because continued interferon treatment inhibited viral multipli-

cation. It was not unexpected, therefore, that only continued interferon administration proved effective in our experiments. Interferon treatment for only a few days after viral inoculation ultimately proved ineffective since viral multiplication probably ensued unchecked when interferon was withheld with the resulting development of splenomegaly.

If this interpretation is valid it suggests that in Friend disease viral multiplication thruout the course of the disease is responsible at least in part for the progressive increase in the number of malignant cells. In this respect Friend disease appears to differ from several other oncogenic virus-animal host systems in which cellular transformation although related to the initial viral dose does not seem to depend on continued multiplication of infectious virus(13). This apparent difference between these 2 systems seems of some importance to us, since the evolution of other malignant processes may also depend in part on continued viral multiplication and may therefore be attenuated or retarded by continued antiviral therapy.

It has been reported that Friend and Rauscher diseases are influenced by numerous factors : hormones(14,15); blood transfusion (14); erythropoietin(16); a variety of chemical compounds(17); clam extracts(18); statolon(19) and host weight loss(20). Thus it was possible that inhibition of splenomegaly in Friend disease by interferon was due to a factor(s) other than interferon in the crude preparations or was mediated by a mechanism as yet undetermined. Several observations, previously summarized(4,21) had suggested that interferon itself was responsible for these inhibitory effects. Further evidence for this assumption however stemmed from the experiments presented here: a) There was no indication that concentrated crude interferon inoculated subcutaneously was toxic for mice or induced any changes which might have been responsible for the inhibition of splenomegaly, b) A highly purified preparation of mouse brain interferon was as effective as a crude interferon preparation of equivalent titer. Although this "highly purified interferon" was still chemically impure, its specific activity per microgram of protein was comparable to the "purified interferons" reported by others(22,23). It seems reasonable to us therefore to attribute the inhibitory effects in our experiments to interferon.

Summary. Daily subcutaneous administration of concentrated preparations of crude or highly purified mouse brain interferon inhibited the multiplication of Friend virus and the development of splenomegaly in Swiss mice. Initiation of a continued interferon treatment 1 week after viral inoculation, at a time when splenic enlargement had already developed, still exerted a significant inhibitory effect on further development of splenomegaly. Interferon administration for only the 1st week after viral infection was ultimately ineffective. On histologic examination, far fewer foci of Friend cells were observed in the spleens of interferon treated mice than in the spleens of untreated mice. It was suggested that the inhibition of splenomegaly observed after repeated interferon administration was due to a continued inhibition of viral multiplication. Further evidence was presented that interferon itself was responsible for the inhibitory effects observed.

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‖ It should be emphasized that a direct action of interferon on the proliferation of viral infected transformed cells has not been excluded. This problem is currently being investigated.

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Uptake and Utilization of 2-Phosphoenolpyruvate (PEP) by Malignant Cells. (32573)

M. GUMINSKA* AND J. KIELER

The Fibiger Laboratory,† Kgs. Lyngby, Denmark

Although various carbohydrates are taken up by cells after a preliminary phosphorylation in the cell membrane(1,2), the latter is usually considered impermeable to phosphate esters(1,3), and it is generally thought that only the presence of phosphatases in the membranes(4) makes the uptake of phosphorylated compounds possible.

During our studies of the metabolism of cancer cells the question arose whether intact neoplastic cells are able to take up and metabolize 2-phosphoenolpyruvate (PEP) added to the medium. PEP is a direct precursor of pyruvate (PA) in the Embden-Meyerhof glycolytic pathway. It holds a key position in carbohydrate metabolism at a branching point, where glycolysis is coupled to the citric acid cycle(5), and it deserves, therefore, the attention of the experimental oncologist. The purpose of the present work was to elucidate the question whether intact malignant cells are able to take up and utilize 2-phosphoenolpyruvate.

Material and methods. The cells studied in the present investigation were the following:

1. A near-diploid line of the Ehrlich's ascites tumor (ELD), which was carried in inbred mice of the StA and the DBA 2 strain.

2. Yoshida's ascites tumor carried in inbred Wistar rats.

3. Earle's strain L-929 fibroblasts propagated *in vitro* in a medium (Fig. 1 a) composed of 20 per cent horse serum, and 80 per cent Tyrode's solution fortified with yeast extract to yield a final concentration of 50 mg%. The final glucose concentration was 5 mM. Penicillin and Streptomycin were added at concentrations of 250 i.u. and 25 micrograms per ml respectively. The medium was changed 3 times weekly, and each time the cultures were gassed with a mixture containing 5 per cent CO_2, 20 per cent O_2, and 75 per cent N_2. Cultures selected for biochemical studies received new medium 24 hours before the experiment. The cells were removed mechanically from the culture flasks without the use of trypsin.

Extensive studies to be reported elsewhere showed that although the L-929 fibroblasts do not produce any tumors in adult animals they cause progressive and metastasizing tumor growth in newborn C_3H mice. Thus, they

* Permanent Address: Department of Physiological Chemistry, Medical Academy, Cracow, Poland.

† Under the Danish Cancer Society.