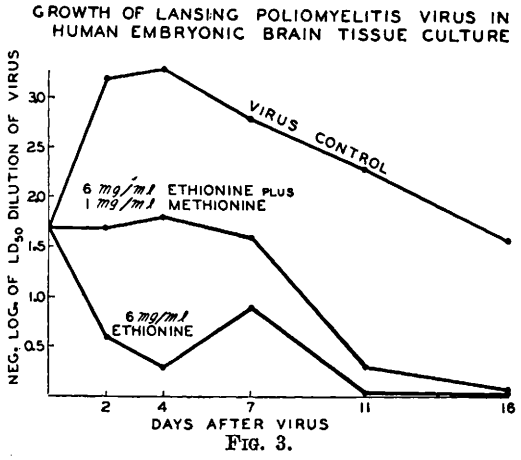

FIG. 3.

demonstrate true reversal of ethionine effect, methionine was added after several fluid changes to flasks already showing ethionine inhibition. No observable increase in virus growth took place but the significance of this experiment is rendered doubtful by the fact that cultures were in a state of deterioration at the time.

Attempts were made to substitute betaine and homocysteine for the methionine in the above experiment but the viral growth curve closely paralleled that with ethionine alone indicating that embryonic brain tissue is not able to synthesize methionine under these conditions from metabolites which in other biological systems are its immediate precursors (7).

Summary. 1. DL-ethionine inhibits the growth of Lansing strain of poliomyelitis virus in tissue cultures of human embryonic brain. 2. When the inhibitor is withheld from subsequent fluid changes virus growth is resumed indicating that the tissue itself is not irreversibly affected. 3. The effect of ethionine was shown not to be on the virus itself under conditions identical in all respects except for the absence of living tissue. 4. The inhibitory effect of ethionine was reduced by the addition of methionine to the cultures.

Conclusions. The Lansing strain of poliomyelitis virus requires L-methionine for its growth and multiplication in cultures of human embryonic brain tissue. Its growth can be materially inhibited with DL-ethionine, a structural analogue of the essential amino acid. The inhibitor acts by interfering with the biosynthesis of the poliomyelitis virus just as it suppresses the growth of influenza virus. This technic affords a procedure for studying the growth requirements of poliomyelitis virus and for testing materials which may influence its growth.

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Received June 4, 1951. P.S.E.B.M., 1951, v77.

A Non-Particulate, Dinitrocresol-Resistant, Glycolytic, Phosphorylating Mechanism Present in Malignant and Certain Normal Tissues. (18783)

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The present experiments deal with the respective abilities of cell-free fractions from normal and tumor tissues to derive energy for phosphorylation from oxidation of succinate or from glycolytic breakdown of hexose diphosphate. Cell-free, particulate systems from a number of normal tissues can apparently utilize up to 50 or 60% of the energy derived from oxidation of α-ketoglutarate, glutamate, or succinate for synthesis of phosphate compounds(1,2), which are in turn available for other cellular syntheses. These oxidative, phosphorylating, particulate systems have been shown by Loomis and Lipmann(3) and by Cross *et al.*(4) to be highly sensitive to the

1. Ochoa, S., *J. Biol. Chem.*, 1943, v151, 493.

2. Hunter, F. E., Jr., and Hixon, W. S., *J. Biol. Chem.*, 1949, v181, 73.

3. Loomis, W. F., and Lipmann, F., *J. Biol. Chem.*, 1948, v173, 807.

4. Cross, R. J., Taggart, J. V., Covo, G. A., and Green, D. E., *J. Biol. Chem.*, 1949, v177, 655.

action of dinitrophenol and other nitro- and halophenols.

Warburg(5) showed that slices of tumor tissue exhibited a higher anaerobic and aerobic lactic acid production than most normal tissues. The Coris(6) demonstrated that high lactate production occurred also in tumors *in vivo*. From these observations arose the view that lactate production, by essentially anaerobic processes, could serve as a source of energy for function, *i.e.* growth of the tumor. Provisional support for this idea was provided by the repeated finding that certain oxidative enzymes, particularly succinic oxidase, were deficient in tumors(7), but comparatively little quantitative evidence as to the relative importance of the aerobic and anaerobic processes for energy utilization in tumors has been available. LePage and his associates (8-10) have studied anaerobic phosphorylation and glycolysis in malignant and other tissues and have shown that tumor and brain homogenates can give a positive phosphorus uptake under strictly anaerobic conditions. They did not apparently determine whether this anaerobic phosphorylation was affected by dinitrophenol.

Materials and methods. Liver and brain were obtained from normal albino rats weighing approximately 150-200 g. Walker carcinoma 256 was maintained by transplantation in rats of the same strain, weight, and age. The rat for each experiment was decapitated and the liver, brain, or tumor removed and placed immediately in 500 ml ice-cold KCl medium (0.12 M KCl buffered with 0.024 M glycylglycine to pH 7.2). All subsequent procedures were conducted at 4°C until introduction of the Warburg vessels into the bath for incubation at 25°C. After 5 minutes in the cold, buffered KCl medium, the tissues were removed, blotted to remove excess fluid, weighed, and homogenized with 2-3 volumes of the KCl medium. Liver or brain was hand ground in an ice-cold mortar with pestle for 2 minutes, half the KCl medium was added, the tissue reground for one minute, the rest of the KCl medium added, and the mixture reground for another minute. The resulting homogenate was brought up by addition of the KCl medium to a volume such that the materials from 250 mg original tissue were contained in 1 ml of the homogenate. The tumor, after removal of hemorrhagic and necrotic areas, was homogenized and handled in the same manner except that the Potter-Elvehjem homogenizer was used instead of the mortar. Each homogenate was filtered through cheesecloth to remove strands of tissue and inadequately dispersed materials. Separation into particulate and supernatant fractions was accomplished by centrifuging in certain experiments at 2,000 g for 15 minutes and in other experiments at 20,000 g for one-half hour, in all cases at a temperature of 4°C. In the experiments in which centrifuging was carried out at 20,000 g, the sedimented particles were made up to the original homogenate volume with the KCl medium and recentrifuged, the wash discarded, and the particulate material resuspended in the same volume of KCl medium. In the experiments in which centrifuging was carried out for 15 minutes at 2,000 g, a second centrifuging was carried out, the second wash discarded, and the particulate material resuspended in the same volume of KCl medium. In both cases the original supernates were used without further treatment.

The chilled Warburg flasks received additions in the following order: *Main compartment*: 1.3 ml of a solution to give the following final concentrations in a total volume of 3 ml; glycylglycine (pH 7.2), 0.025 M; substrate (succinate or pyruvate), 0.01 M; $MgCl_2$, 0.0066 M; KF, 0.0133 M; fructose, 0.0167 M; $KHCO_3$, 0.0066 M; nicotinamide, 0.04 M; diphosphopyridine nucleotide, 0.0002 M; adenosine triphosphate, 0.00033 M; hexose diphosphate (where present), 0.002 M; 0.1 ml yeast hexokinase; 1 ml homogenate,

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TABLE I. Phosphorus Uptake and Lactate Production of Walker 256 Rat Tumor under Aerobic and Anaerobic Conditions. Homogenate of Walker 256 rat tumors and particulate and supernate obtained from tumor homogenate by centrifuging at 20000 g for 30 min. Incubated 40 min. at 25°C. Substrate: hexose diphosphate and pyruvate. Each experiment is based on material derived from 250 mg original tissue and is the avg of 3 determinations. All results are expressed in micromoles.

Preparation	P uptake		Lactate formed		O_2 uptake in air
	In air	In 95% N_2–5% CO_2	In air	In 95% N_2–5% CO_2	
Homogenate	11.1	6.6	9.9	7.7	2.5
Particles	3.3	1.9	4.8	3	1.1
Supernate	6.5	6.2	7.3	6.1	2

TABLE II. Avg Phosphorus Uptake, Lactate Formation, and Oxygen Uptake of Homogenates of Walker 256 Rat Tumor, Rat Brain, and Rat Liver and Particulates and Supernates of the Same Obtained from the Homogenates by Centrifuging at 20000 g for 30 Min. Incubated 40 min. at 25°C. Substrate: hexose diphosphate and pyruvate. Each experiment is based on material derived from 250 mg original tissue and is the avg of 2 or 3 determinations. All results are expressed in micromoles.

Source of material	No. of exp.	Measurement made	Homogenate	Particles	Supernate
Walker 256 rat tumor	6	P uptake	14.6	1.6	8.9
		Lactate formed	9.5	2.7	7.3
		O_2 uptake	2.5	1.1	1.6
Normal rat brain	7	P uptake	17.8	7.8	12.3
		Lactate formed	5.3	2.1	6.8
		O_2 uptake	7.4	4.6	2.2
Normal rat liver	4	P uptake	18.1	11.8	6.9
		Lactate formed	2.8	.5	4.9
		O_2 uptake	10.4	8.1	4.1

particulate system, or supernate fraction (each derived from 250 mg original tissue); 0.3 ml water or 4,6-dinitro-o-cresol (DNC) solution to give the final concentrations shown in the tables. The DNC solution was added after the tissue fraction in order to utilize the well-known effect of substrate saturation preserving optimal activity of the enzyme as long as possible. *Side arm*: 0.3 ml 0.05 M Sorensen phosphate, pH 7.2; consequently with the additional phosphate introduced with the experimental materials the total amount of phosphate phosphorus initially present in each flask was approximately 500-600 μg. *Center cup*: 0.3 ml 5 N NaOH for aerobic experiments; unused for anaerobic experiments. The flasks, prepared as described, were attached to Warburg manometers, equilibrated in the bath for 3 minutes at 25°C, the taps were closed, phosphate tipped from the side arm, and the zero time reading for oxygen consumption taken. The flasks were shaken for 40 minutes, then removed to an ice bath. The flask contents were deproteinized by addition of an equal volume of ice-cold 20% trichloroacetic acid. Determinations of phosphate by the Fiske-SubbaRow and of lactate by the LePage method were made on the protein-free filtrates from the incubated and also from correspond-

TABLE III. Phosphorus Uptake, Lactate Formation, and Oxygen Uptake with Succinate as Substrate in Homogenates from Walker 256 Tumor, Rat Brain, and Rat Liver and Particles and Supernates Obtained by Centrifuging the Homogenates for 30 Min. at 20000 g. Gas phase: air. Each experiment represents material derived from 250 mg original tissue. Incubation time 40 min. at 25°C. All results are expressed in micromoles.

Source of material	Measurement made	Homogenate	Particles	Supernate
Walker 256 rat tumor	P uptake	9.2	3.2	.2
	Lactate formed	1.3	0	−.5
	O_2 uptake	3.9	2.5	1.8
Normal rat brain	P uptake	16.1	8	1.3
	Lactate formed	1.4	.8	0
	O_2 uptake	8.8	6.6	.7
Normal rat liver	P uptake	17.8	15.1	2.9
	Lactate formed	.8	.4	0
	O_2 uptake	14.3	9.7	3.7

TABLE IV. Effect of Dinitrocresol on (1) The Oxidative, Particulate, Phosphorylating Systems (2) The Water-Soluble, Glycolytic, Phosphorylating Systems Obtained by Centrifuging the Homogenates of Walker 256 Rat Tumor, Rat Brain, and Rat Liver at 20000 g for 30 Min. Each experiment represents material derived from 250 mg original tissue. Substrates: succinate or pyruvate plus hexose diphosphate. Gas phase: air. Incubation time 40 min. at 25°C. All results expressed in micromoles.

	Conc. of DNC	Substrate: Succinate — P uptake	Lactate formed	O_2 uptake	Substrate: Pyruvate + HDP — P uptake	Lactate formed	O_2 uptake
			Walker 256 rat tumor				
Particles	0	0	0	1.8	2.9	3.8	1.3
	0	−.1	−.2	2.2	3	4.2	1.4
	10^{-5} M	−.9	.1	1.8	2.5	3.9	.7
	10^{-4} M	−.8	0	1.7	1.9	3.7	.9
	10^{-3} M	−1.1	.1	1.5			
Supernate	0	.2	.2	.9	8.6	7.9	1.5
	0	−.2	−.3	.7	8.2	7.6	1.2
	10^{-5} M	.4	.4	1.3	8.5	7.7	1.3
	10^{-4} M	.2	−.4	1	8.9	8.1	1.1
	10^{-3} M				10.5	8.5	1.1
			Normal rat brain				
Particles	0	12.9	.6	7.6	7.4	1.4	4.6
	0	13.6	2.2	7.2			
	10^{-5} M	0	.7	4.6	1.2	1.6	5.5
	10^{-4} M	0	1	4	1	1.7	4
	10^{-3} M	0	1.3	2.2	.6	1.5	3
Supernate	0	1.4	−.5	2.1	11.6	7.6	2.1
	0				10.2	7.1	2.1
	10^{-5} M	.7	−.3	1.8	13.1	8.3	1.1
	10^{-4} M	.5	−.4	2	12.3	8	2.2
	10^{-3} M	1.1	−.7	1.6	13.5	8.4	1.9
			Normal rat liver				
Particles	0	13.1	.6	9.6	9.2	1.1	8.8
	0	13.2	.5	9.8			
	10^{-5} M	−.6	.8	13.5	−.4	1.5	10.4
	10^{-4} M	−1.2	.8	12.1	−.5	1.4	5.7
	10^{-3} M	−.8	.7	7.4	−.7	1.3	3.9
Supernate	0	2.3	.2	1.9	7.8	7.8	2.4
	0				7.6	7.9	2.3
	10^{-5} M	2	−.1	1.9	8	8.5	2
	10^{-4} M	2.8	0	1.9	7.6	6.6	3.5
	10^{-3} M	2.4	.3	1.4	7.4	5.4	1.6

ing zero time control samples. All results in the tables represent gain or loss during the incubation period.

In preliminary measurements it was found that the aerobic phosphate uptake by liver particles prepared and tested by this procedure with succinate as substrate was very rapid; the average values for P uptake in micromoles, for oxygen uptake in microatoms, and for the P/O ratio were, respectively: 5 minute incubation, 10.2, 4.58, and 2.22; 10 minutes, 14.4, 9.04, 1.59; 20 minutes, 15.2, 14.74, 1.02; 40 minutes, 15.0, 25.22, 0.59. Since a 40-minute incubation period was used for all tabulated measurements, the P/O ratios are obviously lower than the optimal values obtainable over shorter periods. No measurements of the time course of the P uptake associated with lactate formation have been made. It should also be noted that phosphorus uptake, oxygen consumption, and lactic acid production are minimal values, since a certain amount of material has of necessity been lost in the various steps involved in the preparation of the fractions and no allowance has been made for such loss.

Results. The observations will be considered under two main headings.

1. *Phosphorylation associated with particle and supernatant fractions.* In the first experiment to be reported phosphorus uptake and lactic acid production of tumor homogenate, particles, and supernate were determined under aerobic and anaerobic conditions (Table

TABLE V. Effect of Dinitrocresol on Phosphorus Uptake, Lactate Formation, and Oxygen Uptake of Homogenates of Rat Liver, Rat Brain, and Walker 256 Rat Tumor and Supernates of the Same Obtained from Homogenates by Centrifuging at 2000 g* for 15 Min. Incubation 40 min. at 25°C. Substrate: hexose diphosphate and pyruvate. Each experiment is based on material derived from 250 mg original tissue. All results expressed in micromoles.

Preparation	Conc. of DNC	Normal rat liver: P uptake	Normal rat liver: Lactate formed	Normal rat liver: O_2 uptake	Normal rat brain: P uptake	Normal rat brain: Lactate formed	Normal rat brain: O_2 uptake	Walker tumor 256: P uptake	Walker tumor 256: Lactate formed	Walker tumor 256: O_2 uptake
Homogenate	0	19.2	4.5	9.8	18.4	7.8	7.6	10.9	9.6	3
	2×10^{-6} M	18.2	4.1	10.2	14.8	8	7.9	9.1	9	2.5
	8×10^{-6} M	9	4.7	10.3	11	8	8.6	8.8	10	2.1
	3.2×10^{-5} M	5.8	4.9	15.1	8.8	7.7	8.6	8.6	9.3	2.3
	5.12×10^{-4} M	4.6	4.5	13.3	8.3	7.9	6.2	9.7	11.1	2.3
Supernate	0	5.5	7.9	8.2	12.8	8.7	3.5	7.7	9.3	1.1
	2×10^{-6} M	6	8.5	7.3	12.9	8.1	3.2	8.1	9.3	.9
	8×10^{-6} M	5.8	8.6	7.9	15.4	9.7	3.3	8.2	9.2	1
	3.2×10^{-5} M	5.8	8.8	8.5	14.6	11.6	3.3	8.7	10.6	1.2
	5.12×10^{-4} M	6.1	7.7	9	15	11.3	3.4	9.1	9.8	1

* Centrifuging at 2000 g instead of 20000 g was used in this and the following experiment, as it had been found that when equal amounts of a given tumor homogenate were centrifuged (1) at 2000 g and (2) at 20000 g, the phosphorus uptakes of particles, after washing, were 1.2 and 1.3 micromoles and of supernates 9.9 and 10.5 micromoles, respectively, and that both supernates were unaffected by dinitrocresol. The separation of particles may have been facilitated by a slight jelly formation.

TABLE VI. Phosphorus Uptake and Lactate Production by Homogenate of Walker 256 Rat Tumor and Supernate Derived Therefrom by Centrifuging at 2000 g for 15 Min. Measurements were made under aerobic and anaerobic conditions with and without addition of dinitrocresol. Each experiment represents material derived from 250 mg original tissue. Incubation time 40 min. at 25°C. All results expressed in micromoles.

Preparation	Conc. of DNC	P uptake: In air	P uptake: In 95% N_2–5% CO_2	Lactate formed: In air	Lactate formed: In 95% N_2–5% CO_2	O_2 uptake in air
Homogenate	0	10.9	7.3	9.6	8.9	3
	3.2×10^{-5} M	8.6	7.2	9.3	8.4	2.3
	5.12×10^{-4} M	9.7	9	11.1	10.8	2.3
Supernate	0	7.7	7.2	9.3	8.2	1.1
	3.2×10^{-5} M	8.7	7.1	10.6	8.6	1.2
	5.12×10^{-4} M	9.1	8.1	9.8	8.3	1

I). The observation of Schneider and LePage that phosphorylation occurred under anaerobic conditions in the homogenate, particles, and supernate of tumor material was confirmed. In the aerobic series the sum of the phosphorus uptakes by particles and by supernate was 88% of the phosphorus uptake by the whole homogenate, in the anaerobic series 117%. Under anaerobic conditions the homogenate can account for 60%, and the combined fractions for 73%, of the total phosphorus uptake by the homogenate under *aerobic* conditions. Further, about 60% of the phosphorus uptake by the homogenate under aerobic conditions is accounted for by the anaerobic, water-soluble, glycolytic activities of the supernate fractions, which are, as might be expected, the same, within the range of experimental error, under both aerobic and anaerobic conditions.

In the second experiment to be reported the average capacities of homogenates and of individual fractions of homogenates of tumor, brain, and liver to effect phosphorylation, to form lactate, and to consume oxygen under aerobic conditions were determined (Table II). The phosphorus uptake of the homogenates from each of the three tissues is not greatly different: 14.6 micromoles for tumor, 17.8 for brain, and 18.1 for liver. However, that of the particles differs greatly: 1.6 micromoles for tumor, 7.8 for brain, and 11.8 for

liver. For homogenate, particles, and supernate, the oxygen uptake is lowest with the tumor material, considerably higher with brain, and highest of all with liver. The lactate formation, on the contrary, is highest with the tumor, intermediate with brain, and lowest with liver.

It is clear that a substantial fraction of the phosphorylation in the tumor is associated with glycolytic processes and this is confirmed by the phosphate uptake found under anaerobic conditions previously reported. These results offer quantitative experimental support for the assumption widely held for over twenty years that the energy made available by lactate production in tumors can be used for synthetic processes, in this case production of phosphate intermediates which can lead to synthesis of organic phosphate esters.

In liver and brain the sum of the phosphorus uptakes of the particles and supernates was equal to or in excess of that of the whole homogenate. In the case of the Walker tumor the phosphorus uptake of the particles and supernates in relation to that of the homogenates varied greatly, possibly due in part to the varying state of development of the tumor tissues employed.

In this series of experiments the average phosphorus uptake of the tumor supernate was approximately 61% and that of the particles plus supernate 72% of that of the homogenate. This discrepancy is due in part to loss of the glycolytic phosphorylating mechanism in the wash liquors, which has been found to be as much as 30% of that present in the supernate. When a tumor homogenate was centrifuged, the phosphorus uptake and lactate production of the recombined, unwashed particles and supernate were over 90% of those of the original homogenate, from which it may be concluded that no serious injury of the phosphorylating mechanism results from centrifugation. Some loss of phosphate ester, due possibly to phosphatase action, undoubtedly occurs in connection with the tumor particles, which in certain experiments results in a negative phosphorus uptake, which never occurs with liver or brain particles. Considerable difficulty has been experienced in securing an adequate amount of tumor material to study the phosphorylating mechanism of tumor particles as compared with those of liver and brain particles. It is proposed to make this the subject of a future investigation. With brain and liver, the combined lactate production of the individual particle and supernate fractions was considerably larger than that of the homogenate. This is interpreted as a Pasteur effect associated with oxidations carried out by the particles.

When succinate is used as substrate, without addition of hexose diphosphate, the oxygen uptake of the particles is substantially higher than when pyruvate plus HDP is employed (Table III), being least with the tumor material, intermediate with brain, and highest with liver. The oxygen uptakes of the homogenates are also in the same order and in similar proportions. It is important to note that the endogenous lactate formation without added hexose diphosphate is apparently very low with all the tissues, as shown by the lactate values with succinate as substrate. In confirmation of earlier investigators, the aerobic phosphorylation by the tumor or brain homogenates with succinate as substrate is higher than that of the sum of the individual fractions (Table III).

2. *Action of dinitrocresol on phosphorylation of washed particles and phosphorylation of supernates.* The initial stimulus for these experiments was the observation that phosphorylation by tumor hemogenates under aerobic conditions was resistant to or only slightly affected by concentrations of dinitrocresol (DNC) which completely suppressed aerobic phosphorylation of particles derived from liver and other normal tissues. In the experiments reported in Table IV the effects of various concentrations of dinitrocresol upon phosphorylation, lactate production, and oxygen uptake by washed particles of rat tumor, brain, and liver are compared with those exerted by the same concentrations of reagent upon carefully centrifuged, water-clear supernates derived from the same tissues. Since the purpose of this experiment was to determine the effect of DNC on particles freed as far as possible from supernate and on supernates completely freed of

particles and since it appeared desirable to use two different substrates (succinate, which might be expected to give optimum results for an oxidative phosphorylating system, and pyruvate plus HDP, which might be expected to give optimum results for an anaerobic, glycolytic, phosphorylating mechanism), no attempt was made in this experimental series to make simultaneous observations on the homogenates from which the particles and supernates were derived.

In confirmation of earlier work on particles from mammalian tissues(4,5) and from marine eggs(11), phosphorylation associated with oxidation of succinate by brain or liver particles was found to be completely suppressed by 10^{-5} M dinitrocresol. Aerobic phosphorus uptake by brain particles was markedly inhibited and by liver particles completely suppressed by 10^{-5} M DNC with hexose diphosphate and pyruvate as substrate. The lactate production in all these cases was low and the oxygen consumption relatively high. In the case of the Walker 256 rat tumor the particles showed no phosphorus uptake or lactate production with succinate. This was confirmed by a repeat experiment, but, as shown in Table III, tumor particles may give a moderate phosphorus uptake with succinate.

These differences may, as previously indicated, depend on differences in the state of the tumor and differences in the phosphate ester decomposing effect, which appears to be associated with tumor particles, and possibly also to the unavoidable inclusion of a certain amount of normal tissue. However, with pyruvate and HDP as substrate the tumor particles show a moderate uptake, which is only partially suppressed by DNC, and this phosphorus uptake appears to be associated with lactate formation substantially in excess of that observed in the corresponding experiments with brain and liver particles. The supernates with succinate show little or no phosphorus uptake or lactate production. However, the supernates of all 3 tissues show a very substantial phosphorus uptake when pyruvate plus HDP is employed as substrate. This phosphorus uptake is either unaffected or slightly increased by DNC, the highest phosphorus uptake in the case of tumor and brain supernates being associated with the highest concentration of DNC. The production of lactate is also substantial and virtually unaffected by DNC.

It would appear from these results that DNC at a concentration of 10^{-5} M completely blocks oxidative phosphorylation of brain and liver particles with succinate as substrate and also completely blocks the phosphorus uptake of liver particles with pyruvate plus HDP as substrate. However, when pyruvate plus HDP is used as substrate, 10^{-5} M DNC appears to reduce phosphorylation by brain particles to one-sixth and that by tumor particles to five-sixths of that observed without the addition of DNC. This observation lends support to the suggestion resulting from the observations on aerobic and anaerobic phosphorylation by tumor particles that a substantial amount of the water-soluble, anaerobic, glycolytic mechanism remains associated with the tumor particles, even after extensive washing by centrifugation, and contributes to the phosphorylating effects exerted by these particles.

In the experiments reported in Table V the effects of various concentrations of dinitrocresol upon phosphorylation, lactate production, and oxygen uptake by homogenates of rat tumor, brain, and liver are compared with those exerted on supernates obtained by centrifuging the homogenates in question. The DNC exerts a maximum effect on the liver homogenate, which at the highest concentration is reduced to 24%, an intermediate effect on the brain homogenate, which is reduced to 45%, and the minimum effect on the tumor homogenate, which is reduced to 90%, or 80% if a lower concentration of DNC is selected. The lactate production in micromoles, which appears to be unaffected by DNC, is in a similar order: 23% of the phosphate uptake in micromoles for liver homogenate, 42% for brain, and 88% for tumor homogenate, thus supporting the conclusion that the extent to which the phosphorylation

11. Clowes, G. H. A., Keltch, A. K., Strittmatter, C. F., and Walters, C. P., *J. Gen. Physiol.*, 1950, v33, 555.

of a given homogenate survives the action of DNC affords an approximate index of the proportion of the anaerobic, glycolytic, phosphorylating mechanism functioning in the system in question. The oxygen uptake, which is in the reverse order, conforms with this concept. The phosphorylation of the supernates is found in all cases to be either unaffected or increased by the action of the DNC.

At this point reference should be made to an individual experiment in which the relation between the phosphorus uptake of a tumor homogenate and the supernate derived therefrom differed from those observed in the experiments reported above. A Walker tumor homogenate prepared in exactly the same manner as that employed in the experiment reported in Table V showed a phosphorus uptake of 13.4 with reduction under the influence of the concentrations of DNC employed in Table IV to 10.6, 10.6, 10.3, and 9.8, respectively. However, the supernate prepared from this homogenate in exactly the same manner as that employed in the experiments reported in Table V showed a phosphorus uptake of 17.1 and under the influence of the four concentrations of DNC values of 16.8, 16.1, 16.8, and 16.2, respectively. These values were associated with a very high lactate formation and low oxygen uptake. Obviously, in this case the phosphorus uptake of particles plus supernate would have exceeded that of homogenate, as with brain or liver.

In the concluding experiment reported in Table VI, observations were made on the phosphorus uptake and lactate formation of a tumor homogenate and a supernate derived therefrom under aerobic and anaerobic conditions similar to those reported in Table I, but in addition the effect exerted by two concentrations of DNC on the phosphorus uptake and lactate formation was determined. The phosphorus uptakes of the homogenate and supernate under anaerobic conditions and of the supernate under aerobic conditions were found, as in the experiment previously reported, to be the same within the range of experimental error and their average was 66 per cent of the phosphorus uptake of the homogenate under aerobic conditions. The effect of the dinitrocresol was to slightly reduce the phosphorus uptake of the homogenate under aerobic conditions and to increase the phosphorus uptake of the supernate under aerobic conditions and of the homogenate under anaerobic conditions, with the result that the phosphorus uptake in all 3 cases was the same within the range of experimental error. That of the supernate under anaerobic conditions was increased, but not to the same extent as the homogenate under anaerobic conditions.

In conclusion reference should be made to the observations of LePage and Schneider (10) on anaerobic phosphorylation of a homogenate of a Flexner-Jobling carcinoma and fractions derived therefrom in comparison with a normal liver homogenate and similar fractions. The tumor homogenate showed under these anaerobic conditions a high phosphorus uptake, while the liver homogenate gave only 6% of this amount and may therefore be considered to be negative. The tumor supernate appeared to account for about 60% and the nuclei for 33% of the phosphorus uptake of the homogenate under these anaerobic conditions. The mitochondria and microsomes showed a negative phosphorus uptake, which supports the conclusion that a negative phosphorus uptake of tumor particles may be due to the fact that under certain experimental conditions the decomposition of phosphate ester may exceed its synthesis.

LePage and Schneider's observations regarding the phosphorus uptake of tumor homogenates, nuclei, and supernates under anaerobic conditions give support to the suggestion that a part of the so-called aerobic phosphorylation induced by tumor particles may be attributable to the anaerobic glycolytic mechanism remaining associated with the tumor particles, even after the major part of this water-soluble, glycolytic mechanism has been removed by washing and centrifuging. This question will be made the subject of a future investigation.

Summary. 1. In liver and brain there are two distinct mechanisms for phosphorylation, which may be separated by centrifugation of the tissue homogenates: (1) particulate elements dependent on oxidation of succinate

or other substrates of the tricarboxylic acid cycle and (2) water-soluble or at least water-borne systems glycolyzing hexose diphosphate. In the Walker 256 tumor the major part of the phosphorylating effect appears to be induced by the water-soluble, hexose diphosphate glycolyzing system, which functions equally well under aerobic and anaerobic conditions. 2. While the oxidative phosphorylation carried out by liver and brain particles with succinate or α-ketoglutarate is readily blocked by extremely dilute solutions of dinitrocresol, the water-borne, glycolytic, phosphorylating system which passes into the supernate on centrifuging the homogenate is not suppressed by dinitrocresol, even when used at concentrations from 100- to 500-fold those which completely suppress aerobic phosphorylation of washed liver particles. The phosphorus uptake of the tumor particles, which in any case is substantially less than that of liver and brain particles, is only partially suppressed under anaerobic conditions. Furthermore, a substantial part of the activity appears to be maintained at concentrations of DNC which completely block phosphorus uptake by liver particles. The phosphorus uptake of the tumor supernate is, like that of the liver and brain supernates, entirely unaffected by concentrations of DNC 100- to 500-fold those required to suppress the oxidative phosphorylation of liver particles. 3. The peculiar behavior of tumor particles under aerobic conditions and also when exposed to certain concentrations of DNC suggests the possibility that at least a part of the phosphorus uptake of tumor particles may be attributable to the anaerobic phosphorylating mechanism, which, even after extensive washing, remains attached to or associated with the tumor particles.

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