a density equivalent to 6 to 7% sodium chloride solution. Spontaneous mammary adenocarcinoma in C3H mice read 7 to 8%.

In a limited experience with human material, the results seem similar to those in the experimental animal. A single specimen of carcinoma of the rectum obtained at operation read between 7 and 8. A retroperitoneal lipomyxosarcoma removed at autopsy floated in 3.5% sodium chloride solution (this was a grossly fatty tumor). In this case, and 3 cases of lymphosarcoma and one of myeloblastic leukemia examined postmortem, heart and muscle ranged from 7 to 8 or 8 to 9; kidney, 7 to 8 (6 to 7 in lipo-myxosarcoma and myelogenous leukemia); spleen, 8 to 9; liver, 9 to 10; lymphosarcoma read between 6 and 7. The organs of mice bearing Patterson lymphosarcoma (2 weeks after tumor implantation) were lighter than those of normal AKm mice (heart 8-9; liver 10-11; spleen 8-9; kidney 8-9; muscle 9-10). The liver and spleen were grossly enlarged and infiltrated with tumor in these mice.

Summary. 1. The specific gravity of tumor and normal tissues of mice and rats was determined by flotation in sodium chloride solutions of known concentrations.

2. The density of tumor tissue was always less than that of heart, liver, spleen, kidney, and muscle of the host.

3. Several human tumors were tested and exhibited the same property.

Received March 13, 1950. P.S.E.B.M., 1950, v73.

Effect of Infectious Myxoma Virus on Glycolysis of Chorioallantoic Membrane of Chick Embryo.* (17768)

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Little is known of the effect of viruses on the cell metabolism of the infected host. Most of the information originates from experiments on bacteriophage and from studies of the behavior of viruses in tissue culture. The chorioallantoic membrane of the developing chick embryo appeared to be a suitable model tissue for the study of cell metabolism in the presence of a virus for the following reasons: a) It can be infected readily with a great variety of viruses, and the membrane can be used conveniently for *in vitro* metabolic measurements. b) The development of chemical and morphological changes can be followed at any desired time intervals, thus permitting chronological correlations hitherto difficult to obtain on mammalian tissues.

The present paper summarizes some observations made on the glycolysis of chorioallantoic membranes of 11-day-old chick embryos which had been inoculated with myxoma virus. The measurement of respiration and glycolysis was carried out 48 hours after inoculation. The experiments reported here exemplify the usefulness of this technic (see point a), while time relationships between chemical and morphological effects (point b) will be reported elsewhere(1). Infectious myxoma virus as an infecting agent appeared particularly suitable for these studies, since this virus causes characteristic lesions on the membrane without killing the embryo itself(2-5). Moreover, the

* This investigation was supported in part by the Life Insurance Medical Research Fund and in part by a research grant from the National Cancer Institute, U. S. Public Health Service.

† With the assistance of Miss H. E. Sherrard.

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behavior of this tumor-producing virus is of intrinsic interest in relation to some aspects of tumor development.

Experimental. In the present experiments the inoculum consisted of a suspension of chorioallantoic membranes infected with myxoma virus which had undergone 39 consecutive passages in the embryonated egg. The infected membranes were ground in a Pyrex grinder to a 10% suspension in 0.85% NaCl. This suspension was divided in ampoules each containing 1 ml of the material and then lyophilized. By this method a uniform infective dose could be secured in successive experiments. Just before use the contents of the ampoules were reconstituted with 1 ml of sterile distilled water and diluted ten-fold with 0.85% NaCl. A sample of 0.3 ml of this final dilution (10^{-2} concentration by weight containing approximately 100 egg infective doses of the virus) was dropped on the chorioallantoic membrane, the egg rotated to insure even virus distribution, then incubated at 35°C for 48 hours. Control eggs were inoculated with a similarly prepared suspension of lyophilized normal chorioallantoic membrane or with distilled water. After incubation the eggs were opened and the central part of the membrane cut out and immediately dropped into ice cold Krebs-Henseleit solution(6). The membranes were washed in this solution several times, then blotted dry on a filter paper, cut into 3-4 mm long strips, weighed on a torsion balance, and transferred to ice chilled Warburg respirometer flasks. The wet weight of the membranes per flask varied between 90-125 mg. Each respirometer flask contained Krebs-Henseleit solution (with 10 mg/ml glucose) and 0.2 ml 20% KOH in the center well. The volume was made up to 3 ml with Krebs-Henseleit solution, the flasks were gassed with 100% O_2 for 5 minutes, and O_2 consumption measured for 60 minutes at 37°C. The values of O_2 consumption were calculated in terms of Qo_2 from the amount of O_2 absorbed in one hour. The aerobic lactic acid formation was also determined. Lactic acid analyses were carried out according to Barker and Summerson(7). In each experiment the dry weight of the membranes was measured and found to vary between 4.8 and 6.9%. The conversion of hexose diphosphate to 3-phosphoglyceric acid was determined manometrically according to Kennedy and Lehninger(8). Zymohexase activity was measured by reading the optical density of the dinitrophenylhydrazone chromogen in the spectrophotometer at 540 mμ using the test system of Sibley and Lehninger(9). For these enzyme assays 0.9% KCl homogenates or centrifuged (6,300 x g for 15 minutes) extracts were used. Since the mechanism of glucose fermentation and oxidation of the chorioallantoic membrane was hitherto unknown, an aerobic fermentation experiment was carried out on a larger scale. One gram each of normal and infected membranes was incubated with 500 mg glucose in 10 ml Krebs-Henseleit solution (containing 20 mg P in the form of KH_2PO_4 and $MgSO_4$ in final conc. 0.005 M) for 5 hours in 100% O_2 atmosphere at 36.8° C. The reaction was stopped with 10 ml cold 10% trichloroacetic acid, and the membranes were then homogenized and extracted. Extraction was carried out 3 times with 5% trichloroacetic acid, the final volume made up to 50 ml. The esterification of inorganic P, the labile P ($\triangle_7$ P) of the Ba insoluble fraction, and the alkali labile P of the alcohol precipitable fraction were determined as outlined by Umbreit *et al.*(10). Phosphorus analyses were made according to Gomori(11), keto acids determined according to Friedemann and Haugen(12). Since the dry weight of the membranes did not differ significantly (normal 5.1%, infected 4.8%),

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TABLE I.
Effect of Infectious Myxoma Virus on O_2 Consumption and Aerobic Lactic Acid Formation of Chorioallantoic Membrane of the Chick Embryo.

No. of exp.	Q_{O_2}		^{q}Lactic acid	
	Normal	Infected	Normal	Infected
1	13.2	14.8	19.2	32.0
	12.4	11.0	14.7	28.6
2	13.8	14.7	18.5	40.1
	15.8	10.8	13.6	38.0
3	14.9	18.2	15.5	48.4
	15.2	17.0	14.0	39.2
4	19.7	16.6	15.8	50.0
	18.1	21.0	16.8	49.0
m	15.3	15.5	16.0	40.6
δ	2.6	3.66	2.1	7.35
ϵ	$\pm$0.92	$\pm$1.28	$\pm$0.74	$\pm$1.92
d	0.24		12	

m = mean value.
d = difference from mean ($\pm$).
n = number of measurements.
Q_{O_2} = microliters of O_2 per mg dry wt per hr.
^{q}Lactic acid = micrograms of lactic acid per mg dry wt per hr.

$$\delta = \sqrt{\frac{\Sigma d^2}{n-1}} \qquad \epsilon = \frac{\delta}{\sqrt{n}} \qquad d = \frac{m_1 - m_2}{\sqrt{\epsilon_7^2 + \epsilon_2^2}}$$

the results of the incubation experiments were expressed in terms of 1 g of fresh weight.

Results and discussion. The oxygen consumption and aerobic lactic acid formation of normal and infected membranes are summarized in Table I. The metabolism of 2 separate samples of each membrane was compared in 4 series of experiments. Simple statistical evaluation of the data revealed an increased aerobic lactate formation from glucose by the infected membranes as compared to the normals. The metabolism of control membranes, *i.e.*, those inoculated with 0.3 ml of suspension of lyophilized chorioallantoic membrane containing no virus, was identical with the normal membranes. The marked increase in glycolysis exhibited by the infected membranes, as contrasted with these controls, indicates that this metabolic effect is due to the presence of the virus.

The question arises as to whether or not the virus itself has glycolytic enzyme activity. This appears unlikely, based on the observation that the virus titer and increase in glycolysis do not correspond, and the increase in the rate of glycolysis persists, even increases, after the virus titer has decreased to a lower level. This finding will be reported elsewhere(1). The mechanism of increased glycolysis was further demonstrated by comparing the rate of conversion of the Harden-Young ester to 3-phosphoglyceric acid by homogenates of normal and infected membranes. (See graph.) Since the rate of fermentation of Harden-Young ester to triose phosphate was greater in the infected membranes, the conclusion seems warranted that the activation of a glycolytic enzyme occurred in the chorioallantoic membranes inoculated with virus. It was found, furthermore, that in 0.9% KCl extracts of infected membranes the zymohexase activity was markedly increased over the enzyme activity of the extract of normal membranes. The magnitude of the zymohexase activation is shown by the following typical experiment. The optical density of the triose chromogen (at 540 mμ) formed during 15 minutes' incubation at 37°C was 0.290 for an

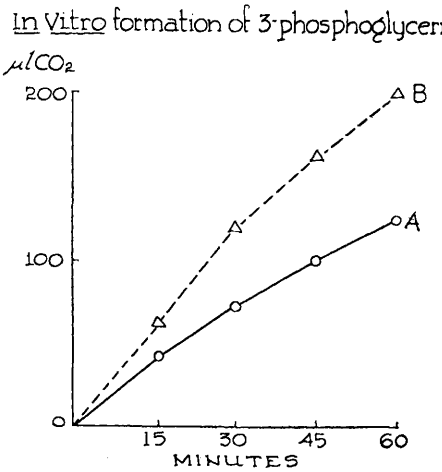

FIG. 1.

In vitro formation of 3-phosphoglyceric acid from hexosediphosphate by 0.9% KCl homogenates of normal (A) and myxoma-infected chorioallantoic membranes of the chick embryo (B). Each flask contained: Tissue homogenate (corresponding to 200 mg membrane); 0.03 *M* HDP; 0.002 *M* $Na_2HA_8O_4$; 0.02 *M* NaF; 0.02 Na Pyruvate; 0.02 *M* Nicotinamide; 0.048 *M* Na HCO_3. Reaction started after 15 minutes incubation by the addition of 100 γ DPN from the side arm. Total volume of reactants 3 ml. Gas = 95% N_2 + 5% CO_2. Temperature = 37°C.

extract made from 20 mg of normal chorioallantoic membrane in contrast to 0.480 in the case of an equivalent extract of infected membrane. Further data on zymohexase activation will be presented elsewhere. These measurements were corroborated by the analytical results obtained from the fermentation experiment, using 1 g each of pooled normal and of pooled infected membranes. The formation of triose phosphates (determined as alkali labile P of the alcohol precipitable fraction), keto acids (expressed in terms of pyruvic acid), and lactic acid from glucose was increased in the fermentation mixture containing the infected membranes. Results of this typical experiment are summarized in Table II. The esterification of inorganic P occurred to the same extent in both fermentation experiments. During 5 hours' incubation 5.3 mg of the 20 mg of added inorganic P were esterified, 3.1 mg were taken up in the "phosphocreatine" fraction (difference between inorganic and Ca precipitable P), and 2.2 mg in other fractions. In both experiments 0.7 mg pyrophosphate P (presumably adenylpyrophosphates) were formed (as determined by the 7 minute P of the Ba insoluble fraction). While the pathway of carbohydrate metabolism of the chorioallantoic membrane is at present unknown, our data support the view that glucose is metabolized by way of phosphorylation.

TABLE II.
Formation of Triose Phosphates, Keto- and Lactic Acids During Glucose Fermentation.

Metabolite	Normal membrane, mg	Myxoma infected membrane, mg
Triose phosphates	.017	.033
Keto acids (in terms of pyruvic acid)	.023	.051
Lactic acid	.575	2.025

Results expressed in terms of mg formed by 1 g of membrane in the incubation mixture during 5 hr.

Interestingly enough Racker(13) found that certain neurotropic viruses inhibit brain glycolysis, which raises the question as to whether or not the activation of glycolysis is a specific effect of the myxoma virus. Experiments with other viruses are in progress in this laboratory. Barron(14) found that rabbit myxoma tissue, similar to other neoplastic tissues, has a high aerobic glycolysis. From the experimental evidence presented it appears likely that myxoma virus increases the glycolysis of the chorioallantoic membrane by activation of a glycolytic enzyme (zymohexase). It may be that a similar mechanism is responsible for the increased rate of glycolysis in rabbit myxoma tissue.

Summary. The lactic acid formation from glucose is increased in the chorioallantoic membrane of the chick embryo after inoculation with infectious myxoma virus. This increase must be due to an activation of glycolysis since respiration at this stage is not impaired.

The authors are indebted to Dr. Lewis Thomas for his encouragement and helpful criticism offered in the course of these experiments.

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Received March 10, 1950. P.S.E.B.M., 1950, v73.