Decrease of Respiration by Glucose (Crabtree Effect) in Rous Sarcoma of Chorioallantoic Membrane.* (24565)

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Crabtree's observation(1) that addition of glucose to respiring tumor slices produced a reproducible decrease in respiration has been confirmed in Ehrlich ascites tumor(2) and studied in reconstructed systems(3,4). It is also reported to occur in the developing retina but not in adult retina(5). The following report presents data demonstrating this effect of glucose metabolism in a virus-induced tumor.

Materials and methods. Virus. A standard lot of Rous sarcoma virus(6) (CT 669) was obtained from Dr. W. Ray Bryan of National Cancer Inst. and stored in dry ice chest until used. The virus inoculum was 0.2 ml of a 10^{-2} dilution of CT 669 in sterile physiological saline containing 2% inactivated horse serum as a stabilizer of viral infectivity. After thawing a vial of standard preparation of virus, the dilution was made and kept in ice water bath and not held longer than 1 hour from time of thawing of virus preparation until inoculation onto the chorioallantoic membranes. Fertile chicken eggs obtained from a local hatchery were incubated at 39°C for 9 days. False air sacs were made as described by Beveridge and Burnet(7). Volumes of 0.2 ml of virus or diluent as control were inoculated onto the dropped chorioallantoic membrane and the eggs were closed with a patch of Scotch tape. The eggs were further incubated at 39°C, candled daily and dead eggs were discarded. After 9 days' incubation coalescing tumors were harvested from infected eggs and the chorioallantoic membranes were harvested from saline inoculated control eggs after peeling off the remaining shell membrane. *Preparation of tissue for Warburg.* Tumor tissue and normal chorioallantois were excised aseptically from 18-day-old eggs. The tissue was minced finely enough to be drawn into a 1 ml serological pipette, washed 3 times each in 10 to 20 volumes of Hanks' balanced salt solution without glucose (BSS) by centrifuging at 500 rpm for 3 minutes and resuspended as final 20% suspension by volume in BSS. One ml of tissue suspension was added to the reaction chamber of Warburg vessels, of standard size—about 15 ml. The direct method of Warburg was used to determine oxygen uptake of normal chorioallantois and virus-induced chorioallantoic membrane tumors. To 1 ml of tissue suspension in the reaction chamber was added 1 ml of either BSS, BSS containing 0.2% glucose, or BSS containing 0.2% sodium pyruvate. Respiration studies were carried out at 37°C. The dry weights of tissue were determined from contents of the reaction chamber after removal and drying overnight at 80°C on weighed watch glasses and subtraction of the dry weight of 2 ml of the appropriate comparative medium. Tissue per flask averaged about 20 mg dry weight.

Results. Data shown in Table I are from duplicate sets of experiments. Other experiments repeated at intervals of several months have given similar results. Q_{O_2} values determined at 2 and 4 hours show an observable depression in rate of oxygen uptake of the sarcomatous chorioallantoic tissue when 0.2% glucose is present. With glucose as substrate normal chorioallantoic membrane showed little difference in rate of oxygen uptake from endogenous levels. A pH drop from 7.4 to 6.6 with Rous sarcoma and 6.8 with normal chorioallantois with glucose as substrate was observed at 4 hours. When 0.2% pyruvate was present as substrate there was a rise in Q_{O_2} values in tumor tissue and normal tissue over the endogenous levels.

* This work was aided by research grant of Nat. Cancer Inst., U.S.P.H.S., and by funds from Eugene Higgins Trust. The authors gratefully acknowledge the technical assistance of Mr. A. R. Scala and Miss Sigrid Weeks.

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TABLE I. Comparative Rates of Respiration of Normal Chorioallantois and Rous Sarcoma Virus-Induced Chorioallantoic Tumor.

	μl O_2/mg dry wt/hr					
	BSS		BSS glucose		BSS pyruvate	
Tissue*	2 hr	4 hr	2 hr	4 hr	2 hr	4 hr
RSV tumor	3.5	3.6	1.0	1.0	5.5	6.0
	2.3	2.8	.4	.7	2.5	3.0
		pH 7.5		pH 6.6		pH 7.8
Chorioallantoic membrane	2.2	1.9	2.7	1.9	6.5	8.5
	2.5	2.0	2.5	2.5	7.5	7.8
		pH 7.5		pH 6.8		pH 7.8

* All tissue was chorioallantoic membrane from 18-day-old embryonated eggs. BSS: Balanced salt solution; with .2% glucose; with .2% sodium pyruvate. pH, avg at 4 hr.

Discussion. A comparison of Qo_2 values of virus-induced chorioallantoic tumors with normal chorioallantois shows that presence of glucose results in marked depression in respiration of tumor tissue, but not of normal chorioallantois. Aerobic glycolysis as indicated by lowering of pH of medium appeared to be somewhat greater in tumor tissue. Smith and Kun(8) in their studies with Rous sarcoma virus infection of the chorioallantois at 48 hours found increased anaerobic hexose diphosphate dismutation in homogenates as compared with those of normal chorioallantois but their Qo_2 values were 12 and 14 respectively for whole membranes with glucose as substrate. They did not consider this difference as an effect on respiration and they did not give data on endogenous Qo_2. Since very little tumor growth is observable at 48 hours the discrepancies between their results and ours are attributable to differences in age and maturity of the tumors. Our studies compare respiration of minced tumors taken 9 days following inoculation of virus onto the chorioallantois of 9-day-old embryos (total 18 days) with normal chorioallantois taken from eggs of the same age injected with saline. Depression of respiration by glucose was also found in 7-day tumors. Smith and Kun studied the effect of myxoma virus on respiration of chorioallantoic tissue for 12 to 288 hours after infection and observed very little difference between normal and infected whole membranes in the presence of glucose. However, the Qo_2 values of the infected tissues tended to be slightly lower than the controls between 48 and 288 hours.

Usually there is a rise in Qo_2 values over the endogenous rate when 0.2% pyruvate is present with normal or tumor tissue, however, repeated determinations have occasionally failed to show an increase. These variations with pyruvate as substrate may possibly be explained by differences in the stored reserves of endogenous substrate of tissues and the effect of maintaining the tissues in BSS until setting up a determination. Tissues are kept in BSS 1 to 2 hours before a Warburg determination.

It should be noted that with Ehrlich ascites cells(9) concentrations of glucose much lower than those used in our experiments do not inhibit respiration but maintain it above the endogenous level for some time, although higher concentrations are depressive. We have not yet tested the effect of various glucose concentrations with Rous sarcoma.

Summary. Qo_2 values of Rous sarcoma virus-induced chorioallantoic tumors show a depression in oxygen uptake when glucose at 0.1% final concentration is added as substrate. Respiration of normal chorioallantoic membrane was not depressed by addition of glucose. The reaction of the medium with glucose dropped from pH 7.4 to 6.8 with normal tissue and to 6.6 with tumor tissue during 4 hours. Pyruvate as substrate gave increased oxygen uptake with both tumor tissue and normal tissue. These data demonstrate in a virus-induced tumor a metabolic alteration associated with glucose metabolism comparable to that observed in tumors not demonstrated to be of viral etiology.

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Received October 10, 1958. P.S.E.B.M., 1959, v100.

Nutritive Substances and Reconstitution in Tubularia. (24566)

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Experiments of Barth(1) and of Rose and Rose(9) showed that substances circulate in the tubularian coelenteron which influence hydranth reconstitution. Barth(1,2,3) postulated that these substances are nutritive and that dominance of distal over proximal end is a result of the competition of the two for a postulated substance which he calls "S." His data can be interpreted either as the result of stimulatory or of inhibitory substances. The latter possibility was suggested by the experiments of Goldin(4,5) in which inhibition was produced by increasing hydrogen ions in the surrounding medium. In addition, Miller(6, 7,8) found that a decrease in pH of similar magnitude as found inhibitory by Goldin, occurred in the coelenteric fluid of stems which failed to reconstitute when placed in glass tubes. The experiments described below were designed to test the effects of reducing the amount of possible nutritive substances which might be available to the distal end of the stem during reconstitution.*

Material and methods. For the first experiments, Tubularia were collected from the Oceanographic Dock at Woods Hole. For later series they came from the U.S. Engineers Dock at Sagamore, Mass. The apparatus for maintaining a constant flow of seawater through the stems consisted of a battery jar 27 cm diameter, with wooden cover containing 21 holes in 2 concentric rings (Fig. 1). Filtered seawater was introduced through a glass tube inserted in the middle hole. In the other 20 holes were placed eye-droppers with tips drawn out to a diameter of less than ½ mm. These were inserted into proximal ends of 10 mm long segments of Tubularia stems. To insure uniformity the distal end of each stem was cut transversely 5 mm below the hydranth and to prevent possible mistakes in orientation the proximal cut was oblique (Fig. 2). After insertion each stem was checked to make certain that the eye-dropper was not occluded by tissue and that a free flow of seawater took place. Controls were given no further treatment and the eye-droppers gradually filled with seawater to the same level as that in the jar. A glass tube with 11 nipples and bent to fit the battery jar was placed on the cover. One nipple was connected through a water trap to an aspirator. The other 10 were connected to short lengths of glass tubing inserted into the eye-droppers as far as the constriction (Fig. 1). Flow of aspirated air was controlled by screw clamps (not shown in Fig.). By this means a difference of about 4 cm in the water level inside and outside of eye-droppers of experimental stems was maintained (Fig. 2). This produced a gentle flow of seawater which, usually, was sufficient to prevent closure of the ends of the stems but was not strong enough to flush the coenosarc out of the perisarc. Patency of the experimental stems was determined at intervals by stopping the aspirator for one-half hour and observing the rise of water in the eye-droppers. Stems which

* Biol. Bull., 1950, v99, 361.