The mouse ascites tumor has been employed widely in the study of tumor metabolism (7, 8, 10, 11) and in the evaluation of carcinolytic agents (14). It has been observed independently in two laboratories (10, 11) that respiration in air was inhibited by glucose at somewhat elevated levels of substrate. This paper presents a biochemical explanation for this inhibition. A preliminary report of this study has appeared (2).

MATERIALS AND METHODS

The tumor was propagated in C57BL mice by the intraperitoneal injection of 0.2 ml. of cell suspension. For respiration studies the cells were washed 5 times with 10 volumes of cold phosphate saline (NaCl, 0.115 M; KCl, 0.004 M; MgCl₂, 0.005 M; Na₂HPO₄·NaH₂PO₄, 0.02 M, pH 7.4) to remove plasma and red cells (11) and resuspended in this buffer to give a cell to volume ratio of 0.18–0.20. In studies in which inorganic phosphate was titrated, a buffer previously described (12) was employed. Respiration studies were made in Warburg flasks under air with 0.4 ml. of cell suspension containing 0.08 ml. packed cells (3.4×10⁸ cells) per flask. Anaerobic glycolysis was studied by CO₂ evolution from a bicarbonate-reinforced buffer under an atmosphere of 95 per cent N₂, 5 per cent CO₂, to maintain the pH at 7.4. In addition, glucose disappearance was evaluated by the Nelson procedure (13) and lactate appearance by the method of Barker and Summerson (1). The various substrates were added, each at a concentration of 5 mM/l, and coenzyme supplements were deionized of heavy metals where necessary, neutralized, and made to volume in phosphate saline at pH 7.4. Most experiments were conducted for 4 hours at 38°C. L(+) Lactate was synthesized as described previously (4, 6).

RESULTS

The effect of hexoses on the respiration of the ascites tumor is shown in Chart 1, a for glucose, 1, b for fructose and 1, c for mannose. At a concentration of phosphate of 0.05 m/g/l., levels of glucose of less than 80 mg. per cent stimulated respiration, and this might in part be attributed to the oxidation of the lactate formed from the glucose. As was reported previously, this tumor produces pure L(+) lactate from glucose (3), and it is shown in Chart 1 that the tumor did indeed oxidize L(+) lactate in preference to D(−)-lactate. Subsequent studies on the lactate metabolism of this tumor have been reported (5). Chart 3 demonstrates that respiration was still inhibited by glucose when the substrate was not tipped in until 1 hour after zero time, and Chart 4 shows that glucose not only inhibited the respiration involving unidentified endogenous substrates but also inhibited the oxidation of L(+) lactate, a preferred substrate.

Aerobic and anaerobic glycolysis were also studied. Glucose disappearance and lactate appearance were followed over a period of 4 hours (a) by CO₂ production from a bicarbonate buffer in the Warburg flask and (b) by chemical determinations on the media after incubation under air and under nitrogen.

Chart 5 demonstrates that glucose at a concentration of 107 mg. per cent was completely utilized by the end of the 1st hour when incubated under nitrogen with the ascites tumor. Under air, however, the rate of glycolysis was depressed somewhat, but the glucose was almost completely utilized after 2 hours. The product of glycolysis is lactic acid, and the rate of lactate production under these experimental conditions is shown in Chart 6. Here it is seen that, under nitrogen, most of the lactate had appeared after the 1st hour, at which time the rate decreased markedly, and a
CHART 1.—The inhibition of respiration by hexoses at different concentrations. (a) glucose, (b) fructose, (c) mannose.

CHART 2.—The stimulation of oxygen consumption by L(+) and D(−) lactate.

CHART 3.—The inhibitory effect of glucose on respiration persisted when the glucose was added 1 hour after zero time.
Chart 4.—The inhibition of the oxidation of lactate by glucose.

Chart 5.—The rate of disappearance of glucose in air and under nitrogen.

Chart 6.—The rate of production of lactate in air and under nitrogen.

Chart 7.—Carbon dioxide production as a measure of anaerobic glycolysis.
slightly greater maximum was attained after 2 hours. Under air, the rate of lactate production was much less and was a straight line function with a maximum value at 2 hours. Chart 7 presents the production of CO₂ as a measure of anaerobic glycolysis, and it supports Chart 5 by demonstrating the completion of glycolysis under nitrogen by the end of the 1st hour.

Attempts to eliminate the inhibition with phosphorylated hexoses and trioses of the glycolysis series failed. Little lactate was formed during these incubations, however, indicating, as was expected, that the phosphorylated compounds did not enter the cells and were not metabolized. Similarly, the addition of intermediates of the tricarboxylic acid cycle such as citrate, a-ketoglutarate, and succinate had no inhibitory action at a level of 54 mg. per cent when the concentration of inorganic phosphate in the medium was increased from 0.005 M to 0.030 M. A typical experiment indicating this influence is shown in Table 1.

However, there was a marked release of the inhibition produced by glucose at a level of 54 mg. per cent when the concentration of inorganic phosphate in the medium was increased from 0.005 M to 0.030 M. A typical experiment indicating this influence is shown in Table 1.

DISCUSSION

It is of interest that glucose, mannose, and fructose all inhibit the respiration of the ascites tumor about equally, and further, that the inhibition occurs within the physiological range of glucose concentration. Although the glucose content of ascitic fluid is less than that of blood,¹ it is still of interest that levels of glucose as low as 50 mg. per cent will inhibit respiration under the conditions described.

It is significant at this point that the inhibition by glucose at a concentration of 107 mg. per cent disappeared at 2–3 hours, at which time the rate of respiration accelerated to a rate greater than the endogenous and more similar to that with L(+)-lactate. In fact, at this level of glucose, the total oxygen utilized at 4 hours was often greater than that of the endogenous system with no added substrate. This is indicated in Chart 1. It is also significant that the inflection in the curve of inhibition occurred at the time that glucose had disappeared and the maximum amount of lactate had been produced. It has already been demonstrated that the stimulation of respiration by lactate does not occur in the presence of glucose, Chart 4, and, therefore, the inhibition must be due directly to the influence of the enzymic reactions of glycolysis.

Evidently, therefore, the glycolysis enzymes can compete effectively with the tricarboxylic acid cycle enzymes for some metabolite. The data presented in Table 1 demonstrate that the inhibition is largely eliminated by increasing the concentration of inorganic phosphate to 55 mM/l. It is reasonable to infer, therefore, that the rate of diffusion of phosphate ion into the ascites tumor cell is not adequate to support both the metabolic processes of the cell, i.e., glycolysis, oxidations, and others, plus the added requirement for phosphate in carrying the glucose into the cell. Indeed,

¹ J. Jehl and R. W. McKee, unpublished experiments.

### Table 1

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<th>Inorganic Phosphate (μM/l)</th>
<th>Glucose (mg. per cent)</th>
<th>Oxygen Consumption (μl/2 hours)</th>
<th>ΔO₂</th>
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a very similar situation has been reported with kidney cyclophorase preparations (9). In the latter case, the mandatory phosphate requirement for the aerobic oxidation of α-ketoglutarate, succinate, malate, oxalacetate, citrate, and glutamate was demonstrated by the addition of hexokinase and fructose to the kidney preparation. Upon this addition, the oxygen consumption due to these substrates was depressed from 40 to 70 per cent, depending upon the substrate. This was a direct demonstration that the phosphorylation of a hexose competed for phosphate sufficiently well as to make it limiting for oxidative metabolism.

Many laboratories have since confirmed Warburg's original observation that tumors, as a group, have a high rate of glycolysis and exhibit a low "Pasteur effect" (15). The mouse ascites tumor not only has these characteristics but, in addition, demonstrates a positive preference for glycolysis when glucose is present in the environment, by inhibiting respiration. The superior affinity of the glycolysis enzymes for the available inorganic phosphate may be, in part, the explanation for the low "Pasteur effect" in these tissues.

SUMMARY

1. The respiration of the mouse ascites tumor was inhibited equally by glucose, fructose, and mannose at concentrations above 30 mg. per cent at a phosphate concentration of 20 mm/l. The inhibition was released in 2–3 hours, after which interval respiration was accelerated. Below 30 mg. per cent, respiration was stimulated. Studies of aerobic glycolysis demonstrated that the glucose had disappeared and that the appearance of lactate was maximal just before the release of the inhibition.

2. These hexoses inhibited not only endogenous respiration but also that obtained from the oxidation of L(+)-lactate and other metabolites. L(+)-lactate was oxidized by the tumor in preference to n(−)-lactate.

3. Attempts to prevent the inhibition by the addition of accelerators, coenzymes, and various metabolites were unsuccessful. Raising the concentration of inorganic phosphate to 55 mm/l, however, reduced the inhibition markedly. These observations are discussed in terms of a competition by the glycolysis enzymes with the oxidative enzymes for phosphate.

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REFERENCES

The Inhibition of Respiration by Glucose, Fructose, and Mannose in the Ehrlich Mouse Ascites Tumor

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