may contribute to the delay of onset of atelectasis.

Summary. THAM, 0.3 M, protected mice from the CNS symptoms of oxygen toxicity at 4 atm. This was shown by the prolonged time required before onset of seizure activity. The same concentration of THAM did not protect at 6 atm. Addition of low concentrations of KCl and NaCl appeared to reverse this protection. A mixture of THAM, 0.6 M, and 0.15 M KCl is lethal to mice.

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Effect of Oxamate on Oxidation of Specifically Labeled Glucose by Ehrlich Ascites Carcinoma Cells.* (28066)

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Oxamic acid, a compound structurally related to pyruvic acid, has been demonstrated to be a potent inhibitor of lactic dehydrogenases derived from beef heart muscle(1), from rabbit skeletal muscle(2), and from several tumors(2). With Ehrlich ascites tumor cells under aerobic conditions, oxamic acid inhibits uptake of glucose, inhibits glycolysis of glucose to lactic acid, stimulates consumption of oxygen with glucose substrate, has no significant effect on endogenous respiration, and restores the glucose-inhibited respiration (Crabtree effect) to the endogenous rate. The concentrations of oxamic acid required for reversal of the Crabtree effect parallel approximately those needed for inhibition of glycolysis(2).

A stimulation in expired $C^{14}O_2$ with Ehrlich ascites carcinoma cells occurs when the substrate glucose-U-C^{14} concentration is reduced below 0.0025 M. This phenomenon has been ascribed to a release of the Crabtree effect(3); at the same glucose concentrations consumption of oxygen is also increased(4). We have noted (unpublished data) a profound stimulation of expired $C^{14}O_2$ in presence of oxamate (0.01 M, 0.03 M, 0.06 M) with whole Ehrlich ascites carcinoma cells incubated aerobically with glucose-U-C^{14} (0.01 M) for 2 hours. To assess the participation of the hexose monophosphate shunt and the Krebs tricarboxylic acid cycle in this oxamate induced stimulation, the fates of glucose-C^{14} substrates labeled in the 1 position and in the 6 position were studied.

Materials and methods. For all experiments, oxamic acid was converted to the sodium salt. The glucose substrate (0.01 M) was labeled with either glucose-1-C^{14} or glucose-6-C^{14} purchased from commercial sources.

The Ehrlich ascites carcinoma cells were harvested after 7 to 10 days of culture in the

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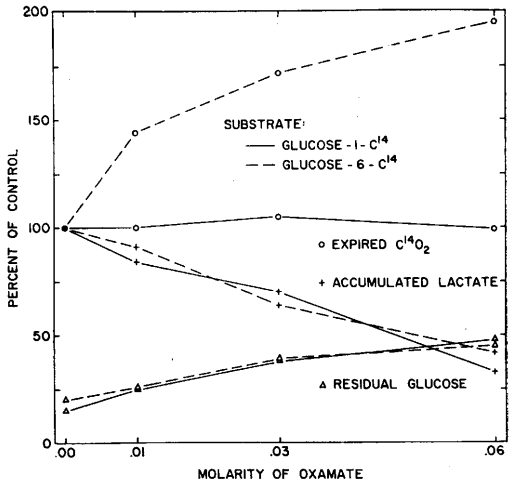

FIG. 1. Effect of oxamate concentration on glucose-C^{14} oxidation by Ehrlich ascites carcinoma cells. Experimental flasks incubated for 2 hr in absence of oxamate taken as control for expired $C^{14}O_2$ and accumulated lactate; 0.01 M glucose taken as control for residual glucose. In absence of oxamate, equivalents of 0.27 mg of glucose-1-C^{14} and of 0.1 mg of glucose-6-C^{14} were converted to $C^{14}O_2$ (calculated on the basis of complete oxidation of glucose molecule).

peritoneal cavities of male Swiss white mice. These cells were washed several times with calcium-free Krebs-Ringer phosphate solution (pH 7.4) at room temperature by short and gentle centrifugations to remove body fluids and erythrocytes, and the whole cells finally suspended in 3 volumes of Ringer solution.

Incubations were carried out at 37°C in the presence of air in 50 ml Erlenmeyer flasks in a Dubnoff metabolic shaking incubator. A total fluid volume of 2 ml, of which 1 ml was an aliquot of the washed cell suspension, was employed. The amount of respiration was determined by expired $C^{14}O_2$ trapped in alkaline center wells, precipitated with carrier as $BaCO_3$, and counted with a gas flow counter. Residual glucose was determined by the colorimetric glucose oxidase method. Glycolysis was indicated by accumulated lactate measured by the colorimetric method of Barker and Summerson(5).

Results and discussion. Fig. 1 shows the results of a typical experiment of 2 hour incubation in which the oxidation to $C^{14}O_2$ of glucose-1-C^{14} and of glucose-6-C^{14} are compared; amounts of residual glucose and of accumulated lactate are also shown. With increase in concentration of sodium oxamate the expired $C^{14}O_2$ is strikingly increased with glucose-6-C^{14} substrate while the output remains essentially unchanged with glucose-1-C^{14} substrate; thus, the stimulation seems to be associated only with oxidation *via* the Krebs tricarboxylic acid cycle. Simultaneously, the amount of residual glucose increases (glucose uptake decreases) and amount of accumulated lactate decreases. Three separate experiments with incubations of 0.5 hour resulted in changes similar to those occurring with a 2 hour incubation period (Fig. 1).

Glycolysis, with the subsequent accumulation of lactic acid, is an important energy source of tumor cells. This lactic acid accumulation can be inhibited by oxamate, a potent inhibitor of the enzyme lactic dehydrogenase. As a result of this block more pyruvic acid enters the Krebs tricarboxylic acid cycle; even though glucose uptake is retarded, a greater percentage of it is oxidized all the way to CO_2. Oxamate has little or no effect on that part of the glucose oxidation by Ehrlich ascites carcinoma cells occurring by way of the hexose monophosphate shunt; that part going by way of the Krebs cycle is strikingly stimulated.

The effects of oxamate on the complete oxidation of glucose-1-C^{14} substrate and of glucose-6-C^{14} substrate by intact Ehrlich ascites carcinoma cells are compatible with the findings of Papaconstantinou and Colowick(2) that oxamate inhibits the conversion of pyruvate to lactate by the enzyme lactic dehydrogenase.

Summary. The oxidation of specifically labeled glucose (0.01 M) by whole Ehrlich ascites carcinoma cells in the presence of sodium oxamate (0.01 M, 0.03 M, 0.06 M) was investigated. With increase in concentration of oxamate, the expired $C^{14}O_2$ is strikingly increased with glucose-6-C^{14} substrate and essentially unchanged with glucose-1-C^{14} substrate. Simultaneously, the amounts of residual glucose increase and of accumulated lactate decrease.

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Mast Cell Response to Cestode Infection. (28067)

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Mast cells participate, to some degree, in inflammation, neoplasia, wound healing, and anaphylaxis(1). These cells, by their sudden release of large amounts of histamine induce hyperemia and infiltration of tissue by blood elements at the site of an inflammation(1). This report describes the appearance of mast cells in the circulating blood, and their presence in the wall of a cestode liver cyst, as a response to invasion by a tapeworm, probably *Hydatigena taeniaeformis*.*

Materials and methods. Male Sprague-Dawley rats, 3-4 months of age and 200-300 g weight, were used. Five rats with infections of the larval cestode (*Cysticercus fasciolaris*, larval stage of *H. taeniaeformis*) and 5 non-infected rats were studied.

Liver, spleen, lung, heart, stomach, jejunum, kidney, and testis were prepared histologically by the paraffin technic (5 μ sections) and examined by bright field at 1250 diameters. Stains used were hematoxylin and eosin, toluidine blue (0.05% in 70% ethanol at pH 7.5 for 1 minute, with alcoholic extraction by 95% ethanol for 15 minutes)(2), and periodic acid-Schiff (P.A.S.)(3). Blood films were taken by cardiac puncture and stained with Wright's stain. Measurements were made by optical micrometer. The method of Floderus(4) was used to count mast cell populations. In particular, mast cell counts of liver were made on representative cross-sections of several entire lobes.

Results. Erythrocytes with Howell-Jolly bodies and globular mast cells were found in cardiac blood. There were 10-12 mast cells in each film. Mast cells were distinctly different from basophils in structure and in staining properties.

The mast cells were 30-35 μ in diameter, with a light blue, spheroid nucleus about 8 μ in size; and contained numerous large cytoplasmic granules. Whereas, basophils were approximately 15 μ in diameter, with a deep blue, polymorphic nucleus less than 8 μ in size; cytoplasmic granules were fewer and smaller than those of the mast cells.

In each of the infected rats, there was a well-developed, unruptured cyst in the upper right lobe of the liver, impinging on, but not penetrating, the diaphragm. The encysted parasite had a structure similar to that of a unilocular hydatid cyst. The cysts were 5-6 mm in diameter and the infections were estimated to be of 2-4 months duration. The cyst wall (Fig. 1) was 90 μ thick and was composed of 3 layers: 1) an outer cellular layer *ca* 35 μ thick, containing endothelial cells, eosinophils, and fibroblasts, which was vascularized by blood capillaries; 2) an inner fibrous layer *ca* 50 μ thick containing many types of cells; and, 3) a germinal layer *ca* 3 μ thick was produced by the parasite, within the first 2 layers and lining the cyst lumen. The interior of the cyst contained the developing body of the parasite.

Mast cells could be located easily by comparing serial sections stained alternately in toluidine blue and hematoxylin and eosin. Mast cells could be observed with H & E alone, but because they stained so deeply with hematoxylin their granular nature was

* Identified by Dr. L. A. Stauber.