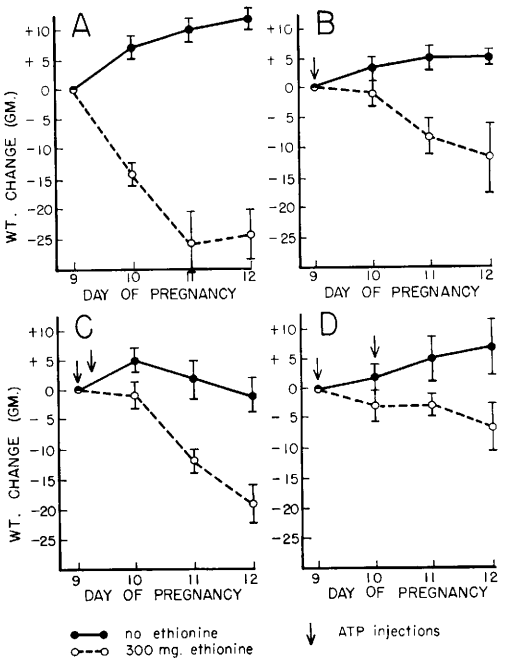

FIG. 1. Weight change in pregnant rats following injection of ethionine and/or ATP on day 9 of pregnancy. A, no ATP; B, single injection of 300 mg ATP; C, two 150 mg injections of ATP at 6 hr interval; D, two 300 mg injections of ATP at 24 hr interval. Mean ± standard deviation of mean.

Therefore, the observations to date on the effects of ATP or its metabolic products on pregnancy may indicate that the fetal and placental tissues handle or react to ATP in a manner different than other maternal tissues.

Further injections of ATP and ethionine will not be of value until we have determined the effects of ethionine and ATP on the cellular concentration of ATP within the fetus and placenta. These studies are now in progress.

The animals receiving ATP along with ethionine did not demonstrate the weight loss characteristic of ethionine treatment (Fig. 1). Since these animals showed greater resorption than those receiving ethionine only, this substantiates previous observations(6) that degree of weight loss associated with ethionine treatment cannot be correlated with degree of resorption. It may also be an indication that addition of ATP with ethionine may be of benefit to the maternal tissues and not affect or affect adversely the fetal tissues.

Summary. Administration of ATP with ethionine enhanced, rather than counteracted, the ethionine-induced resorption of the rat litter. ATP decreased the maternal weight loss caused by ethionine, thereby indicating a lack of correlation between maternal and fetal effects of the compounds.

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Correlation Between Kidney Muramidase* Activity and Plasma Iron Removal in Rats with Walker-256 Carcinosarcoma.† (29224)

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Muramidase activity in kidneys of rats with transplanted tumors is increased 2 to 12 times(1,2,3). Suu *et al*(4,5) found a similar large increase in kidney muramidase activity in irradiation chimeras. The highest level of enzyme activity was found in bone marrow, spleen and kidney. Because the

* Name for Lysozyme (E.C.3.2.1.17) recommended by Commission on Enzymes of International Union of Biochemistry.

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kidney muramidase activity increased at a time when hemopoietic regeneration was known to be occurring(6), Suu *et al* suggested that muramidase activity in kidney was related in some way to the proliferating grafted hemopoietic cells in the irradiation chimeras. The observation to be reported here, that there is a positive correlation between plasma iron turnover and kidney muramidase in tumor-bearing rats, is of interest in this connection.

Materials and methods. Male rats of the CFN strain, 230 to 340 g were individually caged and given commercial chow and tap water *ad lib.* Walker-256 carcinosarcoma tumor cells were injected into the femoral muscle mass of both legs. One microcurie of Fe^{59} (less than 0.05 μg of iron) was injected intravenously as ferrous citrate 4, 8, 12, and 16 days after implantation of the tumor. Blood samples were taken at intervals from 30 minutes to 2 hours after injection of the Fe^{59}. The activity in 0.1 ml of plasma was measured in a well-type scintillation counter. Counts, corrected for physical decay, were plotted on semilogarithmic paper to determine half-time (T ½) of plasma Fe^{59} clearance and the rate-constant of Fe^{59} removal was calculated (K = 0.693/T ½). Plasma volume was determined by extrapolation of the plasma Fe^{59} curve to zero time. The animals were killed by bleeding from the abdominal aorta. Four milliliters of plasma were taken for stable iron determination by the method of Bothwell and Mallett(7) except that 2, 4, 6-tripyridyl-s-triazine (TPTZ) was used for the colorimetric determination(8). Total iron removal from plasma, expressed as microgram per hour, was calculated by multiplying total plasma iron (stable iron concentration times plasma volume) by the rate-constant of Fe^{59} removal (K). Both kidneys were removed, weighed, and frozen until the muramidase activity was determined. For the enzyme determination, a 20% homogenate of the kidneys was made in phosphate buffer pH 7.3 (9). Muramidase was then extracted with 1% acetic acid in 95% ethanol. The extracts were pooled and lyophilized. The residue was dissolved in an appropriate volume of distilled water and the muramidase activity of these solutions determined by the spectrophotometric method of Shugar(10). The substrate was Worthington's *Micrococcus lysodacticus* cells, lot ML-C-606-4. The activity is expressed as milligram equivalents of crystalline Worthington egg white muramidase, lot No. 617, per gram of kidney tissue (fresh weight).

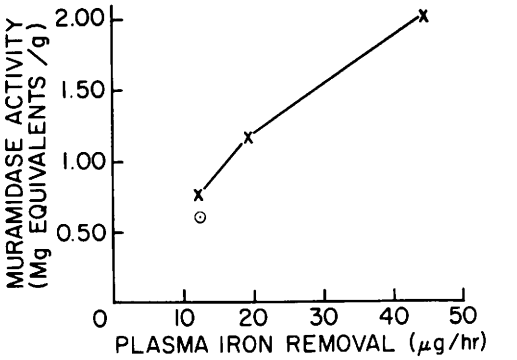

FIG. 1. Kidney muramidase against rate of plasma iron removal in tumor-bearing rats. ⊙ = control animals; × = tumor-bearing rats, 4, 8, and 16 days after implantation. The iron data are averages of separate determinations on 6 rats at each point. The muramidase data are averages of 4 determinations on the pooled extracts from kidneys of 6 rats for each point.

Results and discussion. Fig. 1 shows the muramidase activity plotted against the rate of iron removal from plasma. The muramidase level in kidney 4 days after implantation of tumor is slightly elevated (0.75 mg equivalents per gram) with no increase in rate of removal of iron. At 16 days both are about 4 times the control value (2.00 and 45 vs. 0.60 and 12). The 12-day muramidase extracts were unfortunately lost; iron removal was 30.4 μg per hour. The levels of muramidase activity are similar to those reported by Cappuccino *et al*(2) for rats with this tumor.

Since plasma iron removal has been shown to be correlated with erythropoiesis(11), the increase in amount of iron removed from plasma is taken to indicate an increasing rate of erythropoiesis in our rats with Walker-256 tumor; an assumption further supported by the great enlargement of their spleens due to hemopoiesis(16). Price *et al*(12) have shown with Fe^{59}- and Cr^{51}-labeled red blood cells that increase in erythropoiesis occurs in

rats with lymphosarcoma R 2788. Other reports from their laboratory(13-15) have shown that large numbers of red cells are extravasated into a variety of transplanted tumor in rats and that this is sufficient to account for most of the red cells lost from the circulation. This extravasation of blood into the tumor probably induced the increased red-cell formation.

The reason for the increase in muramidase activity in kidney of rats with tumors is not understood, but Perri(17) has shown that endogenous and exogenous muramidase is removed from the blood stream and excreted, in part at least, by the kidneys. Suu *et al*(5) found that bone marrow muramidase level was the highest of all the tissues measured and that the peak in muramidase activity in kidney of irradiation chimeras occurred at a time when hemopoietic regeneration was known to be proceeding at a maximal rate (6). The observation reported here that increased muramidase activity of kidney is correlated with increased plasma iron removal is further evidence that the endogenous level of muramidase in kidney is somehow related to proliferative activity in hemopoietic tissue.

The level of muramidase in kidney is increased when the reticuloendothelial system is stimulated(18). Radioactive colloidal gold has been shown to concentrate in the regions of active hemopoiesis in bone marrow of humans and this uptake of colloidal gold is presumably a function of the reticuloendothelial system(19,20). Possibly there is some relation between rate of hemopoiesis and activity of the reticuloendothelial system and through this relation muramidase levels are increased in animals with an activated hemopoietic system.

Summary. Muramidase activity in kidney increases progressively for 16 days after implantation of Walker-256 carcinosarcoma into rats. A similar progressive increase in rate of iron removal from plasma was found in these rats. At 16 days both muramidase activity and iron removal were increased 4 times above control values. A relation between kidney muramidase and hemopoietic activity is suggested.

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