in the sera of the immunized mice against homologous intact or disrupted tumor cells or cell supernates. The loss of immunogenicity of tumor cell vaccine upon cell disruption and the absence of humoral antibodies and passive transfer leads to the conclusion that immunity produced by irradiated, killed tumor cell vaccine is primarily cell mediated.

The authors wish to thank Miss Mabel Halloran and Mr. Lawrence Feingold for technical assistance, Dr. K. Sugiura for the ascites tumors and Micro-Biological Associates for the adenocarcinoma 755.

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Received Sept. 5, 1967. P.S.E.B.M., 1968, Vol. 127.

The Effect of Lactate Infusion on Serum Uric Acid* (32610)

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In 1923 Gibson and Doisy(1) showed that the ingestion of sodium lactate was associated with decreased renal excretion of uric acid and a slight elevation of blood uric acid. Clausen (2) identified lactic acidosis two years later in a group of children with dehydration and circulatory insufficiency. Both observations remained relatively obscure till the present time.

In recent years there has been intensive interest in studies related to blood lactate, per se, due to the recognition of spontaneous lactic acidosis by Huckabee(3,4). Studies by Yü *et al.* (5) have generated much interest in the relationship of blood lactate to the renal

* Supported by Grants HE-00052 from the National Institutes of Health and U-1562 from the Health Research Council of the City of New York.

excretion of uric acid. They reported that infusion of sodium lactate decreased the renal clearance of uric acid in individuals with gout but infusion of bicarbonate or acetate or the ingestion of citrate had no such effect. They found no change in blood uric acid during the infusion of lactate. Lieber *et al.* (6) studied the effect of alcohol on blood lactate and uric acid in alcoholic subjects. In one alcoholic individual who ingested sodium lactate for 6 hours the blood uric acid slowly rose over the period of lactate ingestion. To date, however, there are no published studies of normal humans dealing with the effect of controlled (i.e., parenteral) administration of lactate on serum uric acid.

It is the purpose of this report to show that with the decreased renal clearance of uric acid in response to lactate infusion there is a concomitant rise in serum uric acid in normal subjects. This change in serum uric acid occurs rapidly and is apparent 30 min after the start of the infusion.

Methods and Materials. Normal volunteers, one female and three males, between the ages of 26 and 36 fasted after midnight. They voided after arising and discarded the urine. Several hundred milliliters of water were ingested to facilitate urine flow for clearance studies during the experiment. A urine sample was obtained approximately 30 min before the infusion was started and as frequently as possible during the course of the experiment.

At zero-time blood was drawn without the aid of a tourniquet. Blood for uric acid determination was collected in plain tubes and allowed to clot. Blood for lactate was allowed to flow directly into weighed tubes which contained 6% perchloric acid; the amount of blood collected was determined by weighing the tubes and perchloric acid was added to give a ratio of 7.2 ml perchloric acid to 1 gm of blood. The volunteer was then given 1000 ml of 1/6 *M* racemic sodium lactate intravenously at a constant rate over a period of approximately 60 min.

Bloods were collected as described above at 30, 60, 120, and 180 minutes for determination of blood lactate and serum uric acid. Blood L-(+)-lactate was determined enzymatically with lactic dehydrogenase (7) and serum and urine uric acid were determined enzymatically with uricase (8). Serial uric acid determinations on the same sample gave remarkably constant results. Occasionally a result would vary as much as 0.05 mg/100 ml.

Lactic dehydrogenase was purchased from C. F. Boehringer and Soehne and uricase was purchased from Sigma Chemical Company.

Results. As seen in Fig. 1 the infusion of lactate resulted in a 70–80% diminution, compared to preinfusion values, in the renal clearance of uric acid. The nadir of uric acid clearance was reached between 60 and 120 min in these studies.

In each experiment the serum uric acid is higher 30 min after beginning the infusion of lactate. In subjects N.K., T.K., and R. B. the serum uric acid level reaches its zenith at 120 min. In subject D.F. the serum uric acid levels at 60 and 120 min are unchanged in relation to the 30-min value. In all cases the serum uric acid levels at 180 min are below the 120-min values; this finding correlates well with the increased renal uric acid clearance observed at approximately 3 hours in each case. In control studies without any infusion, serial determinations of renal uric acid clearance at the same time of day that the lactate infusions were carried out failed to show a diminution in uric acid clearance below the preinfusion values herein reported. Thus, a normal periodic shift in uric acid clearance cannot be invoked as an explanation for our results.

Discussion. In each subject the infusion of lactate resulted in a rise in blood L-(+)-lactate. The elevated blood lactate, in turn, is associated with a marked diminution in the renal clearance of uric acid. A decrease in renal uric acid clearance is closely related in time to a rise of 8-18% in serum uric acid levels.

Studies which showed that pyruvate (1,9) and 0.4 *M* acetate (5) enhance the renal uric acid excretion, as well as studies with lesser concentrations of acetate and with citrate and bicarbonate (5) which showed only slight effect, if any, on renal uric acid excretion, indicate that the effect of lactate on renal clearance is not mediated through a metabolic product of lactate. Further, since pyruvate and acetate administration do not evoke a

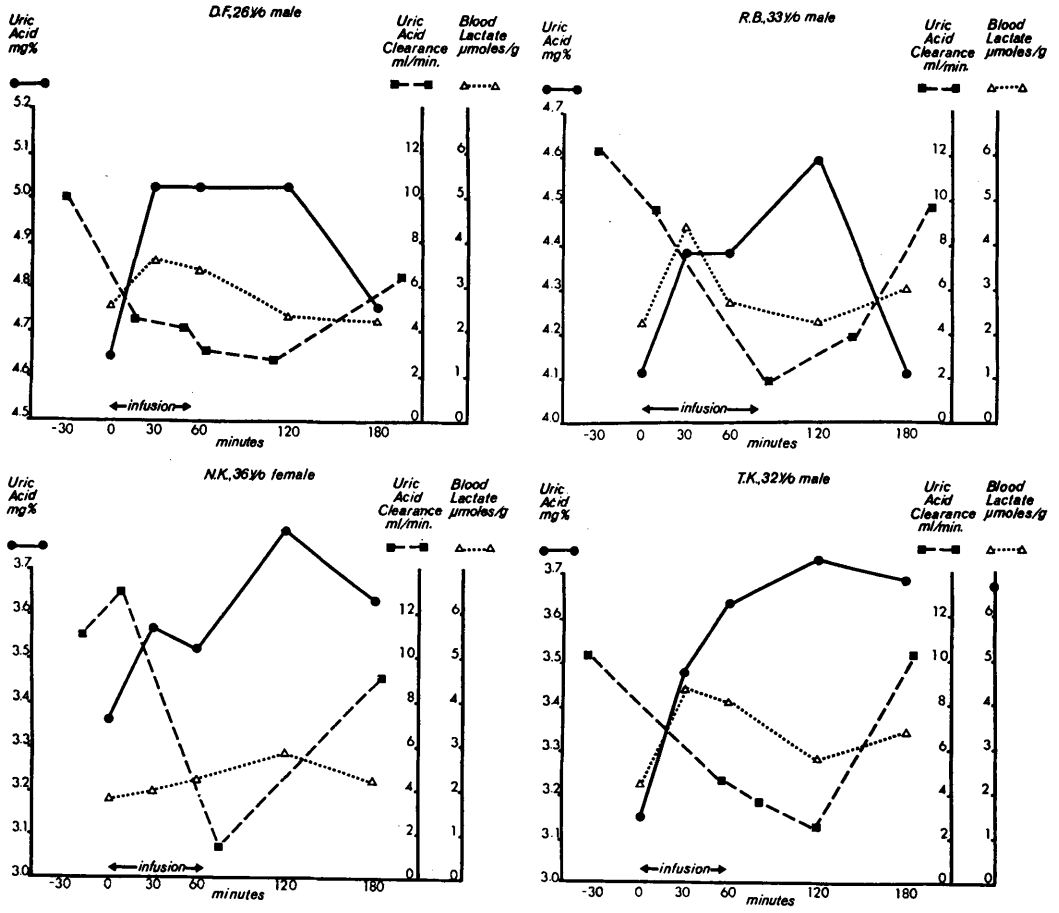

FIG. 1. The effect of intravenous administration of 1000 ml of 1/6 *M* racemic sodium lactate on blood lactate, serum uric acid, and renal uric acid clearance. Lactate was infused intravenously at approximately 15 ml per min. Blood samples were obtained by a free flow technique without a tourniquet at 0, 30, 60, 120, and 180 min. Urine for clearance studies was obtained as often as possible during each experiment. The collection of samples and the enzymatic determination of lactate and uric acid are described under "Methods and Materials."

decrease in renal uric acid excretion, the possibility that acetoacetate and/or β-hydroxybutyrate (10,11), produced from the infused lactate, mediate the renal effect would seem quite remote. Therefore, it appears that the renal excretion of uric acid is diminished because of a direct effect of lactate. The rise in serum uric acid which parallels the decreased renal clearance seems to be adequately explained by the latter.

Summary. Four normal volunteers were given 1000 ml 1/6 *M* racemic sodium lactate intravenously over a 60-min period. In each subject there was a marked diminution in the renal clearance of uric acid and a concomitant rise in the serum uric acid.

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Received Nov. 1, 1967. P.S.E.B.M., 1968, Vol. 127.

Influence of Mammary Tumor Virus Infection on the Acceptance or Rejection of Transplanted ML+ Leukemias* (32611)

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The ML (Mammary Leukemia) antigenic system was first recognized during a study of cytotoxic antibodies produced by mice immunized against histoincompatible leukemias of DBA/2 mice (1,2). These antisera, after removal of isoantibodies by absorption *in vivo* in normal DBA/2 mice, were still cytotoxic for several DBA/2 leukemias. Absorption analysis of the sera *in vitro* indicated that the antigen identified in DBA/2 leukemias was present also in lactating mammary tissues and mammary tumors of mice infected with the mammary tumor virus (MTV), but was not demonstrable in any other tissues, normal or malignant, from either MTV+ or MTV— mice. On the basis of these findings it was concluded that this cellular antigen was derived from MTV. The designation ML (Mammary Leukemia) was chosen to indicate its presence in both mammary tumors and certain DBA/2 leukemias (designated ML+ leukemias).

Recently we have demonstrated by immunodiffusion a soluble antigen, MTV-s1, which like ML antigen is present both in mammary tissues infected with MTV and in certain leukemias of DBA/2 mice (3). This antigen is a subunit of MTV and is released by treatment of the intact virion with ether. It is not known whether these two antigens, ML cellular antigen and MTV-s1 soluble antigen, have the same specificity or different specificities; their occurrence is invariably linked, however, because they both are associated with infection by MTV.

This report is concerned with the question whether ML antigen in ML+ leukemias is capable of eliciting resistance to the growth of transplanted ML+ leukemias or of transplanted mammary tumors. For this purpose ML+ leukemias were inoculated into the following hosts, all genotypically histocompatible: (a) mice infected with MTV, (b) mice of strains freed of MTV by foster-nursing, and (c) reciprocal F_1 hybrids from various crosses between MTV+ and MTV— mice. Experiments in which resistance to mammary tumor transplants was successfully transferred by peritoneal cells from donors immunized with an ML+ leukemia confirmed that the specificity of this resistance was determined by MTV.

Materials and Methods. Mice. These were obtained from our own colonies. To simplify the description of mouse strains the abbreviations used are shown in Table I.

Preparation and inoculation of cell suspensions. Leukemia cells. Cell suspensions were prepared from leukemic spleens by mincing with curved scissors in Earle's Balanced Salt Solution (EBSS). The released cells were washed once and resuspended in fresh EBSS.

Mammary tumor cells. Suspensions of mammary tumor cells were prepared by slow circulation of tissue fragments in EBSS containing 250 mg trypsin 2× crystallized), 25 mg collagenase, and 2 mg deoxyribonuclease

* This work was supported by grant CA 08748 from the National Cancer Institute, NIH, USPHS, and by a grant from the John A. Hartford Foundation, Inc.