man with yellow fever or influenza virus vaccine prepared in eggs.

Summary. An egg-adapted strain of measles virus has been propagated in cultures of chick embryo cells throughout 24 serial passages. Beginning with the 6th passage an abrupt decrease occurred in the time required to attain maximal concentration of virus in the fluid. The concentration of virus was comparable to that found in cultures of infected primate cells. In the 5th chick cell passage the virus began to produce cytopathic changes closely resembling those occurring in human epithelial cells infected with measles virus. The identity of the agent was confirmed in neutralization tests with measles antisera. In chick cells maintained with a medium of known composition virus production approached that in cultures nourished with serum and tissue extracts. Inoculation of rabbits with the virus propagated in chick cells was followed by development of neutralizing antibodies specific for the agent of measles.

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Increase of Urinary Citrovorum Factor Activity in Patients Receiving Methotrexate (Amethopterin) (23638)

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Regression of metastatic trophoblastic tumors has been observed in women treated with methotrexate, a folic acid antagonist(1). The mechanism of action of the folic acid antagonist has been shown to include an inhibition of the synthesis of citrovorum factor (CF) in liver and prevention of its utilization by tissues in laboratory animals(2,3). It, therefore, was of interest to attempt to correlate the daily excretion of urinary CF activity with tumor response in patients with trophoblastic tumors receiving methotrexate therapy.

Methods. All patients received a constant diet composed of a liquid mixture of milk, cream, eggs and dextrose during the period of study. The mixture contained approximately 1,000 calories, 5 μg of folic acid and 6 μg of CF/liter. The dietary intake of these patients ranged from 1,800 to 2,500 calories/day. Daily 24 hour urines were carefully collected and refrigerated. 25-35 mg of methotrexate was given daily for 5 days either by mouth or intramuscularly. Toxicity, manifested by anorexia, nausea, oral ulcerations and occasional emesis was present but did not interfere with the constant dietary intake. The CF activity was assayed by the microbiological technic of Sauberlich and Baumann (7) using Difco assay medium. Turbidimetric readings were made after 16 to 18 hours incubation at 37°C. Since *Leuconostoc citrovorum* #8081 (*Pediococcus cerevisiae* ATCC 8081) was susceptible to methotrexate inhibition, a methotrexate resistant strain *L. citro-*

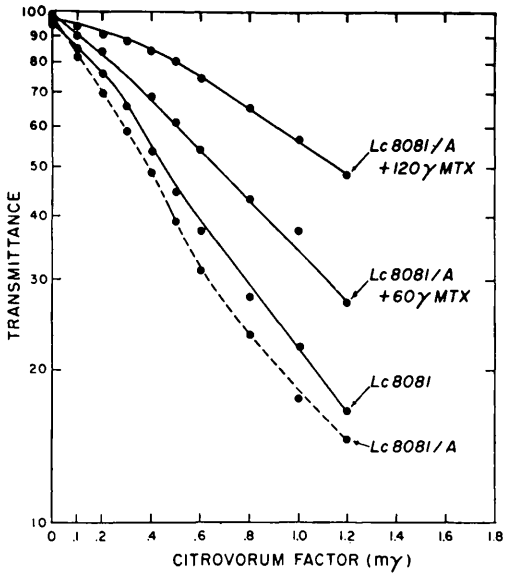

FIG. 1. Growth curves of *Leuconostoc citrovorum* #8081/A.

vorum #8081/A obtained from Hutchinson's laboratory(4) was used as the test organism. All assay tubes had a total of 6 ml containing 15 μg of methotrexate/tube. *Leucovorin* (synthetic DL 5 CHOTHFA) was used as a reference standard. The amount of CF referred to throughout these studies was calculated on the basis of naturally-occurring material this being twice as active as leucovorin(8). In order to obtain maximal CF activity, 2 parts of urine were mixed with 1 part of 5% sodium ascorbate solution at pH 6.0 and autoclaved for 10 minutes at 120°C before assay(5). Whatman #1 paper strips of 1 inch width and 15 inches in length, 0.1 M phosphate buffer at pH 7.6 and descending technic were used for chromatographic separation of CF. The Rf value for CF activity was usually between .70-.75 with this solvent system. The urinary nitrogen was determined by the standard micro-Kjeldahl method.

Results. The growth curves of *L. citrovorum* #8081/A with and without methotrexate are shown in Fig. 1. It is noted that this variant maintains its dependence on CF logarithmically for growth stimulation. Methotrexate alone, 60 μg, and 120 μg/tube did not support growth of *L. citrovorum* #8081/A in the absence of CF. Instead, there was some inhibition of growth even in the presence of CF as was originally observed by Hutchinson. However, it is an appropriate organism for assaying CF in the presence of methotrexate provided the methotrexate concentration does not go beyond 40 μg per assay tube. The metabolic studies on patient R.S., a 34-year-old man with metastatic embryonal carcinoma and choriocarcinoma to lungs and abdomen is illustrated in Fig. 2. It is noted that the urinary CF activity was fairly constant on the days when the patient received no methotrexate. When methotrexate was given, urinary CF activity rose to 3-5 times that of the control values. These findings were confirmed by chromatographic separation and assaying with *L. citrovorum* #8081. The rise of CF activity occurred within the first 24-hour period and subsided in similar fashion after the drug was discontinued. Change of urinary nitrogen excretion was not marked, although there was a suggestion of an increase of nitrogen excretion from 0.5 to 1 g on the days when the patient received methotrexate. In spite of the marked increase of urinary CF activity there was no significant favorable effect on this patient's disease.

The studies on patient A. A., a 28-year-old woman with extensive choriocarcinoma of uterus and with metastases to the lungs is il-

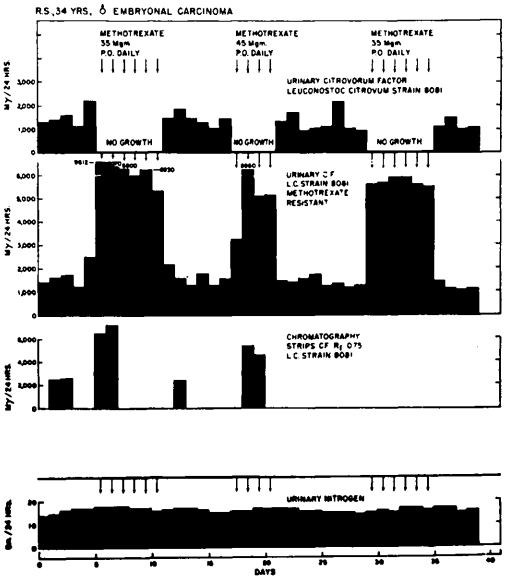

FIG. 2. Patient R. S. urinary excretion of CF and nitrogen.

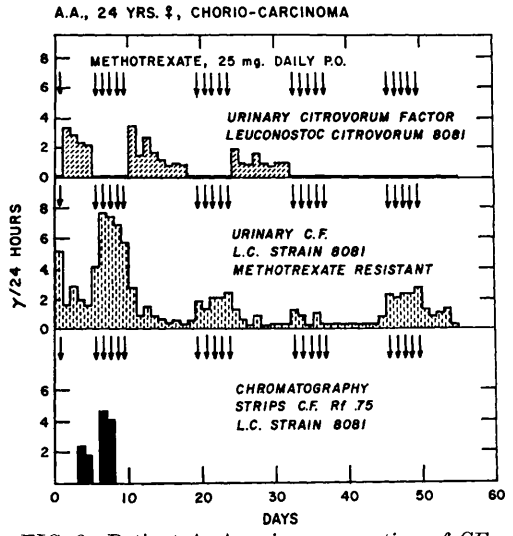

FIG. 3. Patient A. A. urinary excretion of CF.

lustrated in Fig. 3. A similar increase of urinary CF activity was again obtained on the days when methotrexate was given. However, the increase seemed to diminish toward the end of each course and the rise on subsequent courses was less prominent than the preceding ones. Tumor regression was obtained in her case. The studies on patient G.S., a 30-year-old man with metastatic choriocarcinoma to lungs and abdomen are illustrated in Fig. 4. The data, which are similar to those obtained in patient R.S., show an increase in urinary CF activity upon methotrexate administration.

Discussion. In 56 observations on 3 patients, a rise in excretion of urinary CF activity occurred when the patients received methotrexate. Using *L. citrovorum* #8081, this increase was masked, but could be measured after the separation of the material with CF activity from the other materials in the urine. This increase in urinary CF activity could be measured using the *L. citrovorum* #8081/A test organism without a preliminary separation of this material (Fig. 2 and 3). Thus, *L. citrovorum* #8081/A is a more valid microorganism for the measurement of CF activity of materials containing methotrexate.

The increase in urinary CF activity occurred both in the presence and the absence of a response to therapy. Thus, there was not a correlation of response and increase in urinary CF activity. This would also suggest that the urinary CF was not coming from the resolution of tumor tissue. It was thought, probably, not to be coming from the breakdown of normal tissue either, as the result of drug toxicity, since the catabolic effect of the drug was minimal as indicated by the essentially constant daily nitrogen excretion.

Pharmaceutical preparations of methotrexate contain 1 to 2 or more per cent contaminant of folic acid which might contribute to an increased urinary CF activity. If this were occurring one might expect to see the same amount of increased urinary CF activity after the same dose each time it was administered. Instead, the same amount was not excreted by each subject receiving the same amount and less was excreted after successive doses.

Nichol(2) has concluded from observations in animal and *Streptococcus faecalis* that the folic acid antagonists have at least 2 actions, one on the conversion of folic acid to CF and one on the utilization of CF after it is formed. If utilization of CF is blocked, then one might expect a part of this preformed, unusable CF to be excreted in the urine. Nichol(2) when measuring urinary CF after aminopterin administration to rats has observed such an increased excretion in some of the rats, and has interpreted this as possible displacement of CF. Swenseid(6) has examined the urine

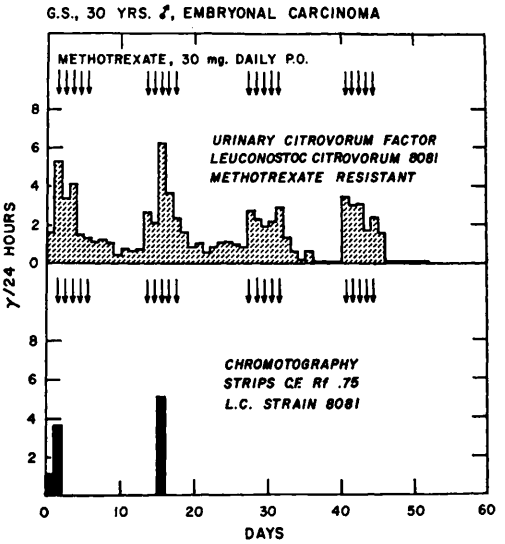

FIG. 4. Patient G. S. urinary excretion of CF.

of leukemic patients upon giving test doses of folic acid 6-10 days after aminopterin administration and has observed an increase in the excretion of a material that would support the growth of *Streptococcus faecalis*. She has interpreted her data to suggest a displacement by the antagonist. Thus, in agreement with Swenseid and Nichol, the present direct finding of a 3-5 fold increase in urinary CF activity in man after methotrexate administration probably reflects a displacement of CF by the drug.

Summary. *L. citrovorum* #8081/A of Hutchinson's laboratory has been shown to be an appropriate microorganism for measurement of urinary CF activity under the experimental conditions described. A 3-5 fold increase of urinary CF activity was repeatedly observed in subjects receiving large doses of methotrexate (Amethopterin). The possible sources of this increased urinary CF activity were discussed.

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Effects of DON (6-Diazo-5-Oxo-L-Norleucine) and Azaserine on the Sand-Dollar Embryo.* (23639)

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The antimetabolites, DON (6-diazo-5-oxo-L-norleucine) and azaserine (0-diazo-acetyl-L-serine), are highly toxic, and often produce developmental abnormalities in chick(1,2), rat(3-5) and dog(6) embryos. It was of interest to examine the effects of these agents on echinoderm embryos, since there are many studies on the biochemical events associated with their development(7-9). Sand-dollar embryos (*Echinarchius parma*) are available in quantity during July and August in Salisbury Cove, Me., and their development, while slower, parallels that of the more extensively studied sea-urchin embryo.

Materials and methods. Eggs and sperm were obtained from sand-dollars by the KCl-injection method(10). The eggs were fertilized in a finger-bowl by adding 5 drops of dilute sperm. In most experiments 200 to 500 fertilized eggs were then transferred to compartments of plastic ice-cube trays, each compartment containing 10 cc of filtered sea-water. The trays were floated on circulating fresh sea-water, so that the embryos were kept at the temperature of the sea, 13-15°C. The embryos were examined under a dissecting microscope at regular intervals up to 72 hours after fertilization. At 36 hours, 5 cc of fresh sea-water was added to each compartment to replenish the oxygen supply. The chemicals used were dissolved in filtered sea-water; appropriate dilutions were made in sea-water from stock solutions which were prepared

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