A possible explanation of the results obtained in these experiments may lie in the effect of the sulfur-amino acids on muscular dystrophy. Since arginine was the first limiting amino acid in the basal diet, utilization of the remaining amino acids of the diet for protein synthesis was depressed. More methionine and cystine would then become available to the animal for use in other functions, one of which may be involved in prevention of muscular dystrophy. If this is the case, deficiencies of essential amino acids other than arginine may have the same dystrophy-depressing effects.

These experiments also provide evidence that the sulfur amino acid effect is not a nonspecific effect, observed only because of a deficiency of an essential amino acid. As opposed to the effect of methionine a deficiency of the essential amino acid arginine did not result in muscular dystrophy in chicks fed a Vit. E deficient diet.

Summary. Nutritional muscular dystrophy in the chick did not occur when an arginine deficient diet was fed even though the diet was low in Vit. E and sulfur amino acids. When arginine was included in the basal diet, a high incidence of muscular dystrophy was observed by 5 weeks of age, even when growth was restricted in the chicks receiving adequate arginine by paired feeding with the basal group.

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Growth of Human Cancer Cells (HEP 2) in Newborn Rats.* (25987)

LEON J. KUTNER AND CHESTER M. SOUTHAM
Sloan-Kettering Inst. for Cancer Research, N. Y. City

Transplantation of antigenically foreign tissue is usually followed by a prompt host reaction resulting in rejection of the transplant unless special conditions prevail. Conditions which permit growth of heterologous tissues include special implantation sites such as the brain and the anterior chamber of the eye(1,2), "conditioning" of recipient animal by agents such as cortisone and x-ray which cause a general depression of defense mechanisms(3), and use of an embryonic or newborn recipient such as the chick embryo(4). Each of these methods has been used successfully for the heterologous growth of human cancer cells. More recently it has been reported that the human epidermoid cancer cell line HEp #3 will grow in rats inoculated *in utero* (5) in newborn unconditioned hamsters†, and in newborn unconditioned mice(6). The present report describes growth of another human epidermoid carcinoma cell line (HEp #2) in normal newborn rats.

Methods. The HEp #2 cell line of Toolan (7) was grown in monolayer tissue cultures, harvested with trypsin and washed once with Gey's salt solution. The cells were resuspended in Gey's solution, counted in a hemocytometer, and adjusted to 8 to 10 million cells per ml. Non-inbred albino rats less than 24 hours old were inoculated intravenously in the facial or submental veins with 0.25 ml of this suspension. Inoculation failures were discarded from this study. Rats which died within 2 to 3 days after inoculation were also excluded. Litter mate controls were inoculated with an equal volume of Gey's salt solution.

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† Personal communication.

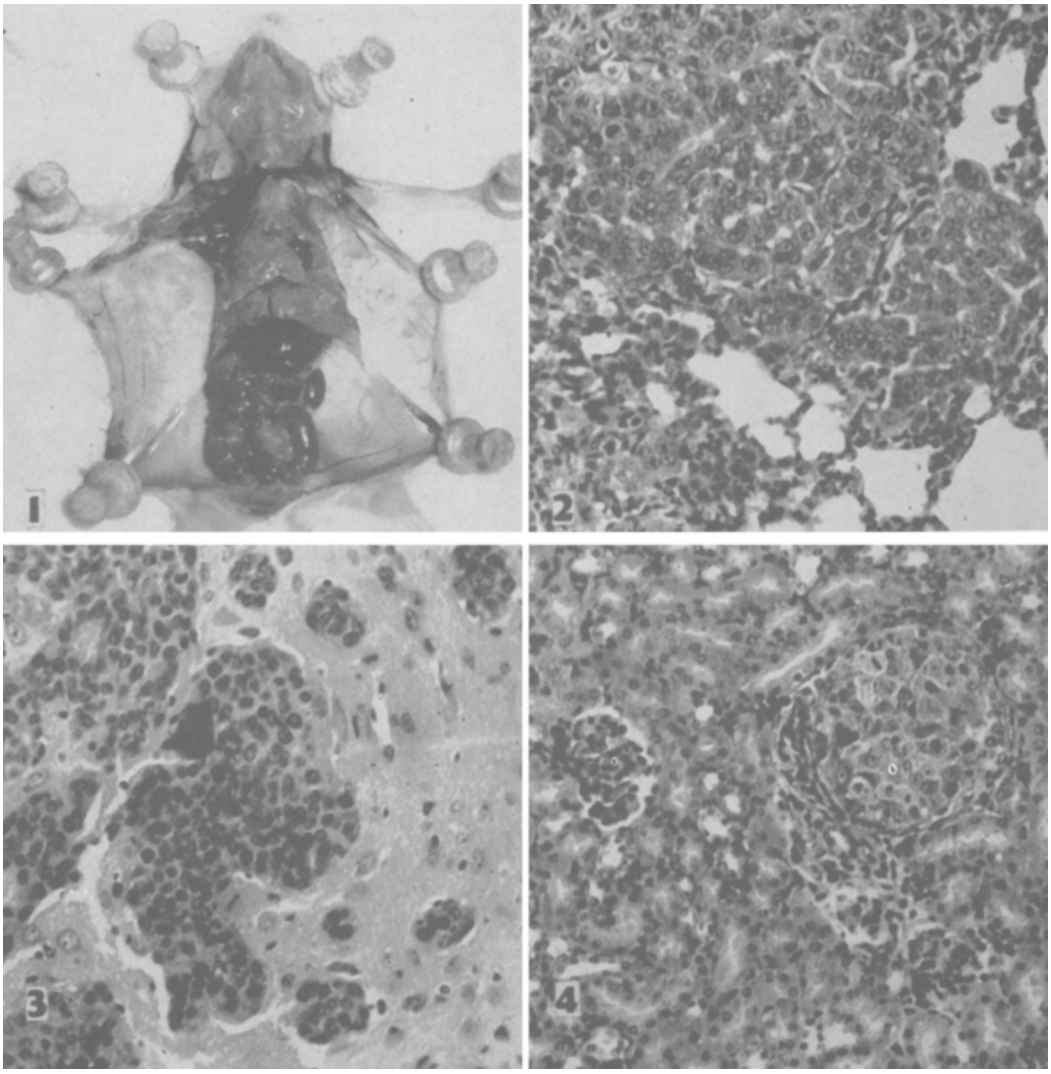

FIG. 1. Massive tumor in lung of rat 26 days old inoculated with HEp 2 cells intrav. when approximately 4 hr old.

FIG. 2. Histologic section of lung of rat age 26 days inoculated with HEp 2 cells intrav. when approximately 10 hr old. Lung parenchyma collapsed and displaced by epidermoid cancer cells.

FIG. 3. Multiple foci of tumor cells in brain stem of rat 27 days old inoculated with HEp 2 cells intrav. when 6 hr old.

FIG. 4. Histologic section of kidney of rat 26 days old inoculated with HEp 2 when approximately 10 hr old. Bowman's capsule is filled with tumor cells which displace glomerular tuft.

Results. As early as 15th to 19th day cell inoculated rats showed signs of illness. By 4 to 5 weeks their weight was 30% to 50% less than that of litter-mate controls. Terminally, they had ruffled fur, severe cachexia, thoracic enlargement, rapid and labored breathing, cyanosis, and sometimes signs of central nervous system disorder. Death usually occurred in the 4th or 5th week. At the end of 5 weeks 92% (77/84) had died of respiratory failure or had been sacrificed when moribund. Mortality in litter-mate controls was only 3% (1/35).

All animals were autopsied except a few which were cannibalized or severely autolyzed. On gross examination the lungs of all

cell inoculated rats were enlarged, firm, white, and did not collapse when the thorax was opened (Fig. 1). Other organs rarely had gross abnormalities.

Microscopic examination consistently revealed masses of the HEp #2 cancer cells almost completely obliterating normal lung tissues (Fig. 2). Cancer cells were also found in brain (Fig. 3), and kidney (Fig. 4). The frequency with which lesions occurred in organs other than lungs is not known because these lesions were not visible grossly and only lung was sectioned routinely.

Subcutaneous and intraperitoneal inoculations of newborn rats and intravenous, intraperitoneal or subcutaneous inoculations of newborn mice with 100,000 to 1,000,000 cells gave no evidence of transplant growth or stunting of the recipients.

These studies are being continued to determine effect of age of rats and size of inoculum on growth of the implant, and to study other human cell lines. These incomplete studies suggest that progressive growth of HEp #2 will occur only with an inoculum in the magnitude of a million or more cells and in rats less than 3 days of age. The cell lines HEp #1, J-111 and Detroit 6 grow in a similar manner.

Discussion. The factors permitting growth of these heterotransplants are unknown and are being investigated further. The simplest explanation is that the human cells were introduced at an age when rejection mechanisms were incompetent, and grew at the expense of lung tissue to a size where death was caused by anoxia before rejection could be initiated. Alternative possibilities are that early introduction of these heterologous cells caused specific immune tolerance or immunologic "paralysis" due to antigen excess.

The human identity of this HEp #2 cell line was established by antigenic studies with the tanned erythrocyte agglutination technic during these experiments.

Summary. Tissue cultured cells of human cancer cell line HEp #2 inoculated intravenously into newborn rats at a dose of 1 million or more cells resulted in growth of the heterologous tumor cells in lungs and sometimes in other organs and produced death by respiratory impairment in 4 to 5 weeks.

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Diurnal Rhythm in Plasma Corticosterone and Lack of Diurnal Rhythm in Plasma Compound F-like Material in the Rat.* (25988)

J. L. MCCARTHY,† R. C. CORLEY AND M. X. ZARROW
Dept. Biological Sciences and Chemistry, Purdue University, Lafayette, Ind.

A diurnal variation in activity of the adrenal cortex has been shown to occur in the mouse, dog and human being(1-5) on the basis of changes in circulating levels of eosinophils and in concentrations of the adrenocorticoids in blood and urine. In conjunction with a study on corticoids of rats, it was deemed advisable to examine the possibility of such a diurnal variation in plasma adrenocorticoid concentrations. Recently, Guillemin *et al.*(6)

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† Present address: Dept. Biology, Southern Methodist Univ., Dallas, Texas.