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)

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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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demonstrated a diurnal variation in levels of corticosterone in the blood of the rat and cited the importance of such variations in determination of resting steroid levels. Present results confirm the above observation but, in addition, indicate a lack of diurnal rhythm in another material of adrenal origin found in the plasma.

While a few paper chromatographic analyses of rat plasma have indicated the presence of more than a trace of 17-hydroxycorticosterone (Compound F), several workers have reported the presence of a Porter-Silber chromogen in plasma extracts(7,8). Since Silber and Porter reported that corticosterone (Compound B) reacted with their color reagent and had an absorption maximum near 350 mμ(9), a way was open to determine the compound in plasma. If indeed, 2 steroids were present in rat plasma, with different absorption maxima, technics of simultaneous determination could be applied(10,11). While this possibility was being investigated, Rosenman *et al.* reported on such a simultaneous determination of corticoids applied to rat plasma(12).

During this study, evidence was found for the presence of an unknown material in rat plasma, reacting with the Porter-Silber reagent and having an absorption maximum near 410 mμ(13). Additional data indicated the component to be of adrenal origin. Since colorimetric studies on 17-hydroxycorticoids in plasma have involved a chromogenic comparison of plasma extracts with Compound F, in this study the absorption of the unknown material was also compared with that of Compound F. For this reason the unknown substance is termed "F-like material" (to indicate color comparison to the standard) which should not be taken to infer identification as a 17-hydroxycorticoid.

*Methods.* Groups of 5-10 rats of the Purdue strain, 60 to 90 days of age and all of the same sex, were placed in a large wire cage in a light proof room. The room was illuminated with artificial light for 12 hours each day, 8 a.m. to 8 p.m., and was maintained under controlled temperature conditions. The animals were fed the standard laboratory diet and given tap water *ad libitum*. The animals were allowed to acclimate to the new environment for 14 days before plasma samples were obtained for analysis.

Four time intervals were selected as the periods during which blood samples would be obtained: 9-10 a.m., 2-3 p.m., 9-10 p.m., and 2-3 a.m. At the beginning of one of the intervals, the animals were given a single intraperitoneal injection of Nembutal (Abbott) 30 mg/kg body weight, and were removed from the room 20 minutes later when all were anesthetized. Blood was withdrawn by cardiac puncture using a heparinized syringe and plasma obtained by centrifugation. Plasma from 3 to 5 animals was pooled to obtain a 15 ml sample which was stored in the deep freeze for subsequent corticoid determination.

The technic for extraction was that of Peterson *et al.*(14) modified for use with rat plasma(13). In all cases, plasma samples were extracted with dichloromethane and the extract divided into two 10 ml aliquots. One aliquot was treated with the Porter-Silber reagent, phenylhydrazine, in a sulfuric acid-alcohol mixture, and the other aliquot was treated with the alcohol-sulfuric acid mixture as a blank. Absorbancy values for plasma extracts and corticoid solutions of known concentration were obtained at 355 and 410 mμ. In all cases, absorbancy values of the chromogen were corrected for background by subtracting the acid blank absorbance. These corrected absorbancy values were used in simultaneous equations to calculate levels of corticosterone and the unknown substance in rat plasma (F-like material).

When this technic was applied to plasma of completely adrenalectomized rats, absorbancy values of the chromogen obtained at both 355 and 410 mμ, when corrected for background color by subtracting the acid blank, were zero. These data indicated that both corticosterone and the F-like material were absent from the blood of adrenalectomized rats. With rare exception, corrected absorbancy values at both wave lengths in samples from normal rats were greater than zero.

*Results.* Levels of the corticoids in the peripheral blood of the rat were found to vary as a function of time of day (Table I). In both male and female rats, highest level of corticosterone was found at the 9-10 p.m.

TABLE I. Peripheral Plasma Corticoid Levels in Normal Rats at Various Time Intervals.

| Time | No. of groups | μg % Comp'd B | μg % Comp'd F-like |
|---|---|---|---|
| | | Male | |
| 9-10 a.m. | 3 | 11.8 | 11.3 |
| 2- 3 p.m. | 7 | 22.0 | 12.7 |
| 9-10 p.m. | 6 | 31.2 | 9.3 |
| 2- 3 a.m. | 3 | 7.2 | 11.1 |
| | | Female | |
| 9-10 a.m. | 2 | 12.8 | 9.2 |
| 2- 3 p.m. | 5 | 29.8 | 15.0 |
| 9-10 p.m. | 5 | 33.4 | 16.6 |
| 2- 3 a.m. | 2 | 17.7 | 12.6 |

period with a slightly lower value evident during the 2-3 p.m. interval. Minimum concentrations of corticosterone were found in the plasma obtained from animals at 2-3 a.m. and 9-10 a.m. There was little variation in levels of the F-like material in the blood of the female animals obtained at the 2-3 p.m. and 9-10 p.m. intervals.

The presence of a diurnal rhythm in activity of the adrenal gland of the mouse was shown by variation in levels of corticosterone in the blood. The highest concentration was found during the afternoon(3). Similar results were obtained in the study on the rat(6) where maximum concentrations of corticosterone were observed between 4 p.m. and midnight. In the data reported here, the highest levels of corticosterone, in both male and female rats, occurred between 9-10 p.m. with somewhat lower levels during the 2-3 p.m. interval. Though there is some difference in time of illumination in this study and that used by Guillemin *et al.*(6), these data support the evidence that there is a diurnal variation in concentration of corticosterone in the blood of the rat with a maximum activity during early afternoon and evening. Interestingly enough, no diurnal rhythm was found for the F-like material in the plasma of the rat. These results tend to indicate that the F-like component does not respond to the day-night stimulus as seen with corticosterone and supports the concept that corticosterone is the glucocorticoid of physiological significance in the rat.

*Summary.* Concentration of corticosterone in blood of the rat varied as a function of time of day. Highest levels of corticosterone were found between 2 and 3 p.m., and 9 and 10 p.m. There was no diurnal variation in concentration of an unknown component of adrenal origin found in blood. These data supply additional evidence for presence of a diurnal rhythm in secretory activity of the adrenal cortex and support the concept that corticosterone is the glucocorticoid of physiological significance in the rat.

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