strains. In contrast, however, no leukemias have appeared to date in those offspring born to "infected" mothers but foster-nursed upon C57Bl/Ka mothers. Stable high-leukemic and low-leukemic lines of different C3H strains have been established simply through foster-nursings. Foster-nursing upon low-leukemic mothers combined with thymectomy through successive generations of high-leukemic AKR strain mice did not influence the frequency of or mean age at death from lymphocytic neoplasms. The pattern of transmission of leukemic potentialities in AKR, as well as in other established high-leukemic strains, *viz.*, C58, F, and C3Hf/Fg, is of a chromosomal type in contrast to the extra-chromosomal pattern of transmission of MLV and presumably of Gross Passage A virus. Further data are given concerning the inability of "infected" males to transmit MLV.

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## Cytotoxicity of Steroids to Mammalian Cells in Tissue Culture. (27872)

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In an earlier report(1) we mentioned that growth of Earle's L cells was markedly inhibited when small amounts of progesterone, testosterone, or androstenedione were added to the medium. Further investigation showed that certain steroids were more toxic than others to this cell line. Our observations on the relationship of steroid structure to cytotoxicity to Earle's L cells (NCTC 929), Gey's adrenal cells (3G29), and Ehrlich ascites cells (Squibb #2) growing in suspension culture are summarized here.

*Materials and methods.* The 3 cell lines used were maintained in suspension culture using previously described technics(2,3,4). Waymouth's medium MB 752/1(5) supplemented with calf serum (10%, w/v) and 4000 cps Methocel® (0.03%, w/v) (Dow Chemical Co.) was used as basal medium. The media used for the adrenal cells and the Ehrlich ascites cells also contained Darvan® #2 (0.05%, w/v) (R. T. Vanderbilt Co.). Inoculum for the cytotoxicity tests was grown in shaken rubber-stoppered Erlenmeyer flasks placed on a shaker rotating at 150 rpm (1 inch throw) located in a 37°C

TABLE I. Cytotoxicity of Steroids to Mammalian Cells Growing in Suspension.

| Steroid | Concentration required for 50% inhibition of cell multiplication, μg/ml | | |
|---|---|---|---|
| | Earle's L cells (NCTC 929) | Gey's adrenal cells (3G29) | Ehrlich ascites cells (Squibb #2) |
| Cholesterol | >50 | >50 | >50 |
| Ergosterol | >50 | >50 | >50 |
| Sitosterol | >50 | >50 | >50 |
| Cortisone | 35 | 100 | 40 |
| $\Delta^4$-pregnene-11α,17α,21-triol-3,20-dione | 30 | 35 | 25 |
| 11-ketoprogesterone | 18 | 30 | 20 |
| 11α-hydroxyprogesterone | 12 | 45 | 30 |
| 17α-hydroxyprogesterone | 12 | 45 | 40 |
| $\Delta^4$-pregnene-20β-ol-3-one | 5 | 7 | 7 |
| Progesterone | 7 | 7 | 7 |
| $\Delta^{16}$-progesterone | 9 | 10 | 11 |
| $\Delta^{9,11}$-progesterone | 3 | 10 | 5 |
| $\Delta^4$-pregnene-17α,21-diol-3,20-dione | 9 | 30 | 14 |
| $\Delta^4$-pregnene-11β,17α,21-triol-3,20-dione | 5 | 20 | 9 |
| 16α,17α-oxidoprogesterone | 3 | 30 | 5 |
| 9α,11α-oxido-$\Delta^4$-pregnene-17α,21-diol-3,20-dione | 5 | >50 | |
| 9α-fluoro-11-ketoprogesterone | 7 | 18 | 11 |
| 9α-fluoro-11β-hydroxyprogesterone | .2 | 10 | 3 |
| 9α-fluoro-$\Delta^4$-pregnene-11β,17α,21-triol-3,20-dione | .1-.3 | 45 | .7 |
| 6α-fluoro-$\Delta^4$-pregnene-17α,21-diol-3,20-dione | .6 | 15 | |
| Triamcinolone | 1 | 18 | 1 |
| Aldosterone | .6 | | |

incubator. The cytotoxicity tests were carried out in ¾ × 6 inch screw capped glass tubes containing 15 ml of medium, or ½ × 6 inch screw capped plastic test tubes (Falcon Plastic Co.) containing 10 ml of medium. The inoculated tubes were placed in a drum rotating at 60 rpm(2). Cell densities of samples taken at 0 time and after 3 days' incubation were determined with a Coulter Electronic Counter®. Dose response curves were prepared for each steroid and the amount required for 50% inhibition of cell multiplication was calculated graphically (2).

*Results. Cytotoxicity of steroids.* Some of the data collected in experiments studying the cytotoxicity of a number of the more common steroids are summarized in Table I. All 3 cell lines were relatively insensitive to the sterols cholesterol, ergosterol, and sitosterol. The Earle's L cells were quite sensitive to $\Delta^4$-pregnene-3,20-dione (progesterone), and its derivatives, and especially sensitive to the fluorinated steroids 9α-fluoro-11β-hydroxyprogesterone (9α-fluoro-$\Delta^4$-pregnene-11β-ol-3,20-dione), 9α-fluoro-hydrocortisone (9α - fluoro - $\Delta^4$ - pregnene - 11β,17α,21-triol-3,20-dione), triamcinolone (9α-fluoro-$\Delta^{1,4}$ - pregnadiene - 11β,16α,17α,21 - tetrol-3, 20-dione), and to aldosterone (18-oxo-$\Delta^4$-pregnene-11β,21-diol-(11 → 18 hemi-acetal)-3,20-dione). Introduction of the hydroxyl groups or a keto group into the progesterone nucleus did not markedly affect the toxicity of the steroids to this cell line. On the other hand, the 11-keto derivative of Reichstein's compound S($\Delta^4$-pregnene-17α, 21-diol-3,20-dione) was less toxic than the 11β-ol, or the 9β,11β-oxido compound, and the 11α-ol was relatively non-toxic. The toxicity of the fluorinated progesterones was not noticeably reduced by introduction of other functional groups into the nucleus.

The sensitivity of Gey's adrenal cells (3G29) to the progesterone derivatives resembled that seen with the Earle's L cells. Gey's adrenal cells were markedly less sensitive to the fluorinated steroids. The Ehrlich ascites cells (Squibb #2) were approximately as sensitive to progesterone and its derivatives as Earle's L cells and less sensitive to the fluorinated steroids than the Earle's L cells.

These experiments show that cell lines dif-

fer in their response to cytotoxic steroids. Preliminary studies on the origin of this difference in response have shown that potassium leakage from the Earle's L cells is greater in the presence of the fluorinated steroids than that observed with Gey's adrenal cells. Since Gey's adrenal cells and the Ehrlich ascites cells are tumorgenic after passage in tissue culture, this difference in response to cytotoxic steroids may be of interest in evaluation of tissue culture response as a method of selection of anti-tumor agents.

*Metabolism of steroids by Gey's adrenal cells and Ehrlich ascites cells.* Earlier studies(1) showed that Earle's L cells (NCTC 929) converted progesterone to a mixture of $\Delta^4$-pregnene-20$\alpha$-ol-3-one and $\Delta^4$-pregnene-20$\beta$-ol-3-one. Similar studies using Gey's adrenal cells (3G29) showed that progesterone was converted to the following compounds: $\Delta^4$-pregnene-20$\alpha$-ol-3-one; $\Delta^4$-pregnene-20$\beta$-ol-3-one; $\Delta^4$-pregnene-6$\alpha$-ol-3,20-dione; and $\Delta^4$-pregnene-6$\beta$-ol-3,20-dione. These cells converted 11$\alpha$-hydroxyprogesterone to: $\Delta^4$-pregnene-11$\alpha$,20$\alpha$-diol-3-one; $\Delta^4$-pregnene-11$\alpha$,20$\beta$ - diol - 3 - one; $\Delta^4$ - pregnene - 6$\alpha$, 11$\alpha$ - diol - 3,20 - dione; and $\Delta^4$ - pregnene-6$\beta$,11$\alpha$-diol-3,20-dione. The Ehrlich ascites cells (Squibb #2) converted the progesterone to $\Delta^4$-pregnene-20$\alpha$-ol-3-one and $\Delta^4$-pregnene-20$\beta$-ol-3-one, transformations also noted with Ehrlich ascites cells taken from mice implanted with this tumor. All of the metabolic products formed from the steroids were identified by filter paper chromatographic behavior and spot tests(6).

*Discussion.* The data collected in these experiments show that different cell lines vary considerably in their sensitivity to the cytotoxic action of steroids. The adrenal cell line (3G29) apparently is much more tolerant of fluorinated steroids than the other 2 cell lines tested. It also metabolizes the steroid more extensively, inserting an hydroxyl group in position 6 in addition to reducing the 20-ketone function. These two differences, the tolerance of fluorinated steroids and hydroxylation at position 6, may be useful markers in distinguishing this cell line from others derived from various tissues.

*Summary.* The cytotoxicity of a number of steroids to Earle's L cells (NCTC 929), Gey's adrenal cells (3G29), and Ehrlich ascites cells (Squibb #2) was determined using cells grown in suspension culture in serum-containing media. The L cells were much more sensitive to fluorinated steroids than they were to the non-fluorinated parent compounds; progesterone derivatives were more toxic than corticosteroids. The adrenal cells were quite insensitive to the fluorinated steroids, while the Ehrlich ascites cells were less sensitive than the L cells but more sensitive than the adrenal cells. The adrenal cells were found to convert progesterone to: $\Delta^4$-pregnene-20$\alpha$-ol-3-one; $\Delta^4$-pregnene-20$\beta$-ol-3-one; $\Delta^4$-pregnene-6$\alpha$-ol-3,20-dione; and $\Delta^4$-pregnene-6$\beta$-ol-3,20-dione, as determined by filter paper chromatographic methods and spot tests. A similar series of compounds was formed from 11$\alpha$-hydroxyprogesterone by this cell line.

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