Biochemical Pharmacology, 1963, Vol. 12, pp. 1081–1090. Pergamon Press Ltd., Printed in Great Britain.

STRUCTURE–ACTION RELATIONSHIPS OF CORTICOSTEROID COMPOUNDS AS INHIBITORS OF LEUKEMIC L5187Y CELL REPRODUCTION *IN VIVO* AND *IN VITRO**

J. J. JAFFE,† G. A. FISCHER and A. D. WELCH

Department of Pharmacology, Yale University School of Medicine, New Haven, Conn., U.S.A.

(*Received* 17 *April* 1963; *accepted* 29 *May* 1963)

Abstract—Corticosteroids of the "glucocorticoid-anti-inflammatory" class inhibit the reproduction of leukemic L5178Y cells in mice and in culture. The following modifications of the basic cortisol molecule were found to be associated with enhanced growth-inhibitory activity: (1) an α- or β-methylation at C-16; (2) formation of a 16α,17α-o-isopropylidene derivative; and (3) α-halogenation at C-6 or C-9. Single modifications such as the introduction of the Δ^1-double bond or α-methylation at C-6 did not enhance the growth-inhibitory activity of the basic cortisol molecule. When combined with other substituent groups, however, both the Δ^1-double bond and α-methylation at C-6 revealed their activity-enhancing potential in the L5178Y system. The most potent corticosteroids studied—triamcinolone acetonide and dexamethasone—exerted a marked cytolytic action upon the leukemic cells *in vivo*; however, these steroids also were comparably more toxic in leukemic mice, indicating that the poor therapeutic indices of both these compounds limit the full expression of their capacity to destroy leukemic cells. The similarity of structural requirements for the ability to inhibit cellular reproduction of pure populations of leukemic L5178Y cells *in vitro* and *in vivo* suggests that the corticosteroids act directly upon these cells. Accordingly, it is believed that leukemic L5178Y cell cultures provide a convenient and reliable system for the evaluation of new antileukemic corticosteroids prior to their trial in appropriate animal systems.

SEVERAL glucocorticoid or anti-inflammatory steroids can be employed beneficially in the palliative treatment of certain types of acute leukemia in man. Little is known concerning the mechanism of the action of these steroidal compounds upon susceptible neoplastic cells; indeed, it cannot be stated with certainty that these steroids, while possessing the ability (in rather massive doses) to induce temporary remissions, both clinical and hematologic (including the bone marrow), inherently do not possess a curative potential with regard to the proliferative diseases involving the white blood cells. Although the possibility exists, therefore, that further modifications of the basic chemical structure of these agents could lead to the development of compounds that might cause permanent remissions in these disorders, the available findings do not justify any special optimism in this regard. Nevertheless, as new steroid derivatives are synthesized, it would be helpful to have available experimental neoplasms that are

* These studies were supported by Grant CY-2817 from the National Cancer Institute, and Grants T-17 and T-23 from the American Cancer Society.

† Present address: Department of Pharmacology, University of Vermont, College of Medicine, Burlington, Vt.

sufficiently sensitive to these agents to serve as screening systems and as tools in mechanistic studies. Data presented in this paper will indicate that strains of the transplantable murine lymphoblastic neoplasm, L5178Y, possess properties that should make them especially useful tools in the hands of those interested in the antileukemic action of steroid derivatives.

MATERIALS AND METHODS

Description of the experimental tumor. L5178Y, which is transplantable as either a solid or an ascites tumor in susceptible strains of mice, can produce a highly lethal, acute lymphocytic leukemia. The constituent cells of this neoplasm are descendents of a single cell isolated from a culture of L5178Y cells.[1, 2] This neoplasm grows best in mice of homozygous DBA/2 genotype, but it also grows well in hybrids that possess the DBA/2 genome, such as strains $AKDF_1$ and BDF_1. In addition, a medium has been developed that permits the clonal reproduction in culture of these leukemic cells, whether obtained from the animal or from culture.[3, 4] It has been established that L5178Y cells, after four years of continuous reproduction in culture, are still capable of producing, when injected into mice of the appropriate strain, the originally described form of leukemia without loss of virulence. Similarly, L5178Y cells that have been maintained by many successive transplantations in mice have been quantitatively recovered in culture as spherical, free-living cells with generation times in the range of 8 to 14 hr, corresponding to their rate of reproduction as ascites cells in mice.

Methods used to study the effect of steroid derivatives on the growth of L5178Y cells in vivo. $AKDF_1$ mice of either sex, weighing 20 to 25 g, in groups of 5 were inoculated intraperitoneally with approximately 2×10^6 viable L5178Y cells (determined by hemocytometer count). Treatment was begun on the fourth day, when the growth of the ascitic form of the tumor was fairly well advanced (Table 1). Steroidal compounds

TABLE 1. THE EFFECT OF VARIOUS DOSES OF CORTISONE ACETATE UPON THE GROWTH OF LEUKEMIA L5178Y CELLS (ASCITIC FORM) IN $AKDF_1$ MICE

Exper.	No. of mice per group	No. of tumor cells ($\times 10^6$) injected i.p.	Day of sacrifice and cell counting	Total ascites tumor-cell counts ($\times 10^6$) Average (range) Controls	Cortisone acetate (50 mg/kg)*			
1	5	2·8	4	128 (102–195)				
	5	2·8	5	458 (385–502)	216 (98–360)			
	5	2·8	6	734 (515–900)	286 (130–362)			
	5	2·8	7	750 (605–958)	250 (152–357)			
2	5	2·9	4	133 (72–214)				
	5	2·9	5	520 (455–582)	264 (180–380)			
	5	2·9	6	754 (628–850)	296 (247–400)			
	5	2·9	7	847 (765–980)	293 (235–355)			
				Controls	Cortisone acetate (mg/kg)*			
					6·2	12·5	25	50
3	5	2·6	4	251 (205–325)				
	5	2·6	7	786 (630–980)	644 (495–908)	541 (280–708)	360 (230–465)	196 (162–235)

* Cortisone acetate was injected subcutaneously as a suspension once daily on days 4, 5, and 6. The tumor-bearing mice were $AKDF_1$ females, 6 to 8 weeks old, weighing 18 to 22 g.

were suspended with the aid of carboxymethylcellulose (0·25%), in such manner that a given dose was contained in 0·5 ml; mice were given daily subcutaneous injections for periods of not more than three consecutive days, receiving either steroidal suspensions or vehicle only. The mice were maintained on Purina lab chow and water *ad libitum*.

In the first series of experiments, groups of control animals were sacrificed on the fourth, fifth, sixth, and seventh days, while groups of steroid-treated mice were examined on the fifth, sixth, and seventh days. In later experiments, control groups were sacrificed on day 5 and day 7; the steroid-treated mice were examined only on day 7 (24 hr after the last of three consecutive daily injections of a given steroid). The technique employed for determining the total number of tumor cells in the ascitic population was that described by Potter and Law,[5] whereby the average total ascites tumour cell population for each group was calculated after thrice rinsing by immersion the peritoneal cavities and viscera of each member of the group and counting the number of tumor cells contained in the combined washings.

All steroids were administered initially in doses of 50 mg/kg daily, and their effect on the increase in number of L5178Y cells was compared with that achieved by cortisone acetate, the effect of which at this dose was unequivocal and highly reproducible (Table 1). If a compound proved to be more potent (or more toxic) than cortisone acetate in the above-mentioned dose, assays were made with smaller daily doses, with a view to obtaining a growth-inhibitory effect comparable to that produced by the cortisone acetate standard.

Methods used to study the effect of steroid derivatives on the growth of L5178Y cells in vitro. The medium and techniques used in growth experiments of leukemic L5178Y cells[3, 4] were modified to study the growth-inhibitory properties of certain steroid derivatives. Appropriate aliquots (0·1 to 0·3 ml) of the steroids, dissolved in acetone, were added to sterile culture tubes, 16 × 125 mm (in duplicate), to provide an approximate 3-fold dilution series (for example: 100, 30, 10, 3, and 1 mμg of triamcinolone acetonide per tube); the acetone was evaporated by incubation at 45° for 18 hr. The inoculum was collected by the centrifugation of cell populations for 5 min at about 800 × *g*; this was done during the exponential phase of reproduction of the cells (range of generation time: 8 to 14 hr) in medium that contained 10% horse serum as the undefined component. The supernatant fraction was discarded, and the pellet of cells was resuspended in 5 ml of a medium containing only 2% dialyzed horse serum as an undefined component. The serum was prepared by dialysis at 0 to 4° for 18 hr against 50 volumes of a solution (0·9%) of sodium chloride and was sterilized by filtration before use. After the cells had been washed twice in 5-ml portions of the modified growth medium, they were diluted in the same medium in order to provide a cell concentration of 1 × 10^5/ml; 5-ml aliquots were delivered to the culture tubes containing the steroids (see above) and to drug-free control tubes. After incubation at 37° for 52 to 70 hr, the cells were counted in hemocytometer chambers, and the amount of steroid that would have permitted only one-half the number of generations achieved by the drug-free controls was determined graphically (GD_{50}, Table 3). The drug-free controls underwent only 2·5 to 3·5 successive divisions, a rate of reproduction lower than the established maximum. This decrease in the rate of reproduction was attributable to the growth-limiting levels of serum present and to the initial lag phase of 6 to 20 hr before the onset of logarithmic growth.

RESULTS

Structural requirements for growth-inhibitory activity in vivo

The data in Table 1 indicate the degree of reliability of the system using L5178Y ascites tumor cells and the sensitivity of these cells to cortisone acetate, when this steroid was injected subcutaneously into tumor-bearing mice in doses of 50 mg/kg for three consecutive days. The total ascites tumor cell counts obtained in the controls during days 4 through 7 of tumor growth show that between the fourth and fifth days the tumor cell population was still increasing exponentially (with a characteristic doubling time of about 12 hr), while from day 5 onward the rate of increase in cell number diminished (asymptotic phase). It is evident that treatment with cortisone acetate suppressed the growth of the tumor cell population, and that this growth-suppressive activity was dose dependent (Table 1).

The ability of a variety of steroid derivatives to inhibit the reproduction of L5178Y cells *in vivo* has been assessed (Table 2). The data suggest that the following chemical features, all present in the same molecule, may be essential for significant antileukemic activity to be exhibited in this system: (1) Δ^4-cyclopentanoperhydrophenanthrene nucleus; (2) 2-carbon side chain at C-17 (consisting of a carbonyl group at C-20 and a hydroxyl group at C-21); (3) α-hydroxyl group at C-17; (4) carbonyl group at C-3; (5) oxygen function at C-11 (for activity to be expressed, oxygen must eventually be in the form of β-hydroxyl).

TABLE 2. THE EFFECT OF VARIOUS STEROID DERIVATIVES UPON THE GROWTH OF L5178Y ASCITES TUMOR CELLS IN $AKDF_1$ MICE*

Chemical name	Other name	Dose (mg/kg)	Total ascites tumor cell counts ($\times 10^6$) Treated Average (range)	Controls Average (range)
1. 17α-Hydroxy-21-acetoxy-4-pregnene-3,11,20-trione	Cortisone acetate	50	196 (162–235)	786 (630–980)
		25	360 (230–465)	786 (630–980)
		12·5	541 (280–708)	786 (630–980)
2. 11β,17α,21-Trihydroxy-4-pregnene-3,20-dione	Cortisol (hydrocortisone)	50	248 (207–282)	792 (560–852)
		25	666 (570–848)	896 (802–1045)
3. 11α, 17α,21-Trihydroxy-4-pregnene-3,20-dione	Epicortisol	50	693 (440–825)	800 (605–980)
4. 9α-Bromo-4-pregnene-3, 11, 20-trione	9α-Bromo-11-ketoprogesterone	50	772 (602–870)	800 (605–980)
5. 11β,21-Dihydroxy-4-pregnene-3,20-dione	Corticosterone (compound B)	50	738 (658–868)	700 (532–852)
6. 17α-21-Dihydroxy-4-pregnene-3,20-dione	17α-Hydroxy-11-desoxycorticosterone (compound S)	50	688 (620–845)	700 (532–852)
7. 4-Pregnene-3,20-dione	Progesterone	50	723 (615–882)	754 (678–910)

* Groups of 5 $AKDF_1$ mice of either sex, weighing 20 to 25 g, were inoculated intraperitoneally with approximately 2×10^6 viable L5178Y cells, suspended in 0·1 ml Ringer–Locke's solution. Treatment with steroids was started on day 4, when the average total ascitic tumor cell counts were 150 to 200 $\times 10^6$. Steroids were injected subcutaneously as suspensions in 0·25% carboxymethylcellulose once daily on days 4, 5, 6. In each instance a given dose was contained in 0·5 ml. Controls received vehicle only. The average total ascites tumor cell counts in steroid-treated and control groups were compared on day 7.

TABLE 2.—*cont.*

Chemical name	Other name	Dose (mg/kg)	Total ascites tumor cell counts ($\times 10^6$) Treated Average (range)	Controls Average (range)
8. 11β-Hydroxy-4-pregnene-3,20-dione	11β-Hydroxy progesterone	50	748 (710–804)	754 (678–910)
9. 3α,17α-Dihydroxy-21-acetoxy-5-pregnene-11,20-dione	Allodihydrocortisone acetate	50	744 (580–992)	754 (678–910)
10. 6α-Methyl-11β,17α-dihydroxy-4-pregnene-3,20-dione	21-Desoxy-6α-methylcortisol	50	631 (454–778)	754 (678–910)
11. 6α-Methyl-11β,17α,21-trihydroxy-4-pregnene-3,20-dione	6-Methylcortisol	50	144 (55–227)	786 (692–902)
		25	362 (250–455)	786 (692–902)
		12·5	603 (520–778)	786 (692–902)
12. 21-Hydroxy-4-pregnene-3,20-dione	11-Desoxycorticosterone	50	738 (622–918)	730 (550–890)
13. 9α-Fluoro-11β,17α,21-trihydroxy-4-pregnene-3,20-dione	9α-Fluorocortisol	50	110 (55–170) (2/5 dead)	854 (760–952)
		25	188 (72–472)	774 (680–942)
		12·5	392 (222–522)	672 (560–852)
		6	593 (487–662)	791 (630–910)
14. 9α,21-Difluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione	9α,21-Difluorodesoxycortisol	50	880 (770–975)	854 (760–952)
15. 9α-Fluoro-11β,16α,17α,21-tetrahydroxy-4-pregnene-3,20-dione	16α-Hydroxy-9α-fluorocortisol	50	153 (95–210)	672 (560–852)
		25	410 (288–583)	672 (560–852)
16. 9α-Fluoro-11β,21-dihydroxy-16α,17α-*o*-isopropylidene-4-pregnene-3,20-dione	9α-Fluorocortisol-16α,17α-acetonide	50	10 (4–12)	672 (560–852)
		25	19 (10–30)	704 (639–845)
		10	296 (130–380)	704 (639–845)
		5	579 (492–655)	704 (639–845)
		1	566 (507–675)	704 (639–845)
		0·5	576 (557–595)	704 (639–845)
17. 9α-Fluoro-11β-hydroxy-17α,21-oxido-4-pregnene-3,20-dione	9α-Fluorocortisol-17α,21-oxide	25	765 (595–982)	791 (630–910)
18. 6α-Fluoro-11β,17α-dihydroxy-21-acetoxy-4-pregnene-3,20-dione	6α-Fluorocortisol acetate	50	86 (44–156)	754 (678–910)
		25	203 (127–460)	754 (678–910)
		12·5	350 (230–518)	754 (678–910)
		6	580 (463–690)	754 (678–910)
19. 9α-Chloro-11β-17α-dihydroxy-21-acetoxy-4-pregnene-3,20-dione	9α-Chlorocortisol acetate	50	53 (28–108)	612 (565–675)
		25	130 (72–175)	612 (565–675)
		12·5	280 (212–325)	612 (565–675)
		6	512 (357–580)	612 (565–675)
20. 11β,17α,21-Trihydroxy-1,4-pregnadiene-3,20-dione	Prednisolone	50	232 (137–395)	848 (750–998)
		25	315 (243–422)	848 (750–998)
		12·5	432 (372–508)	848 (750–998)
		6	761 (634–840)	848 (750–998)
21. 6α-Methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione	6α-Methylprednisolone	25	377 (212–557)	785 (692–902)
22. 6α-Methyl-9α-fluoro-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione	9α-Fluoro-6α-methyl prednisolone	25	294 (237–347)	824 (755–1120)

TABLE 2.—*cont.*

Chemical name	Other name	Dose (mg/kg)	Total ascites tumor cell counts ($\times 10^6$) Treated Average (range)	Controls Average (range)
23. 11β,17α-Dihydroxy-21-acetylthio-1,4-pregnadiene-3,20-dione	21-Mercaptoprednisolone acetate	50	623 (472–717)	698 (640–755)
24. 11β,17α-Dihydroxy-21-phenylthio-1,4-pregnadiene-3,20-dione	21-Phenylthioprednisolone	50	629 (545–690)	698 (640–755)
25. 6α-Methyl-9α-fluoro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione	6α-Methyl-9α-fluoro-21-desoxyprednisolone	50	545 (490–765)	824 (755–1120)
26. 9α,11β-Dichloro-17α-hydroxy-21-acetoxy-1,4-pregnadiene-3,20-dione	9α-11β-Dichloroprednisolone acetate	50	692 (495–812)	688 (582–710)
27. 11β-Hydroxy-17α,21-oxido-1,4-pregnadiene-3,20-dione	Prednisolone-17,21-oxide	50	635 (552–690)	746 (525–930)
28. 9α-Fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione	Triamcinolone	50	117 (48–185)	876 (847–912)
		25	380 (250–427)	876 (847–912)
		12·5	602 (530–729)	876 (847–912)
		6	767 (700–923)	876 (847–912)
29. 9α-Fluoro-11β,21-dihydroxy-16α,17α-*o*-isopropylidene-1,4-pregnadiene-3,20-dione	Triamcinolone acetonide	50	8 (3–12)	712 (542–882)
		25	8 (5–18)	703 (620–845)
		10	100 (20–195)	703 (620–845)
		2	580 (390–730)	703 (620–845)
30. 9α-Fluoro-16α-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione	Dexamethasone	25	12 (3–20)	672 (510–960)
		12·5	13 (6–19)	672 (510–960)
		6	125 (50–175)	698 (640–755)
		3	254 (162–375)	698 (640–755)
		1	572 (403–687)	698 (640–755)
31. 9α-Fluoro-16β-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione	9α-Fluoro-16β-methylprednisolone	25	24 (16–30)	698 (640–755)
32. 9α-Fluoro-16α-methyl-11β,17α-dihydroxy-21-oxido-1,4-pregnadiene-3,20-dione	17,21-Oxidodexamethasone	50	14 (2–20)	896 (802–1045)
33. 9α-Fluoro-17α-methyl-11β,17β-dihydroxy-4-androstene-3-one	Fluoxymesterone	50	783 (687–990)	736 (610–827)
34. 17α-Methyl-17β-hydroxy-4-androstene-3-one	Methyltestosterone	50	663 (522–822)	700 (532–852)
35. 17β-Hydroxy-5α-androstane-3-one	Dihydrotestosterone	50	752 (615–900)	682 (508–795)
36. 17β-Hydroxy-4-androstene-3-one	Testosterone	50	801 (722–1012)	700 (532–852)
37. 1,4-Androstadiene-13,17-lactone-3,17-dione	Δ′-Testololactone	50	755 (685–855)	712 (542–882)
38. 17α-Ethynyl-17α-hydroxy-4-androstene-3-one	Ethisterone	50	703 (607–817)	736 (610–827)
39. 17α-Ethynyl-17β-hydroxy-19-nor-4-androstene-3-one	Norethindrone	50	878 (600–790)	736 (610–827)
40. 17α-Ethynyl-17β-hydroxy-5α,10α-estrane-3-one	Dihydronorethynodrel	50	674 (600–790)	736 (610–827)

In addition, certain other modifications of the above basic structure were associated with changes in relative antileukemic potency.

1. Unsaturation at carbons 1 and 2 moderately increased activity (e.g. cortisol vs. prednisolone; 6α-methylcortisol vs. 6α-methylprednisolone).

2. α-Methylation at C-6 moderately increased activity (e.g. cortisol vs. 6α-methylcortisol). It is of interest that the presence in the same molecule of both unsaturation at carbons 1 and 2 and the α-methyl at C-6 apparently did not confer upon the compound an antileukemic potency greater than that found with steroids that incorporated these structural modifications individually (e.g. 6α-methylcortisol vs. prednisolone vs. 6α-methylprednisolone).

3. α-Halogenation at either C-6 or C-9 increased the relative antileukemic potency of a steroid (e.g. cortisol vs. 9α-fluorocortisol, 9α-chlorocortisol acetate, and 6α-fluorocortisol acetate).

4. Methylation (either α- or β-) at C-16 markedly increased activity, apparently conferring upon the compound the ability to exert a cytolytic as well as a cytostatic action upon the ascitic leukemic cell population (e.g. 9α-fluoro-6α-methylprednisolone vs. dexamethasone and 9α-fluoro-16β-methylprednisolone).

5. α-Acetonidation—i.e. the formation of an α,α-isopropylidene derivative at C-16 and C-17—increased the antileukemic potency of steroids to a degree comparable to that of compounds with methyl-substitution at C-16 (e.g. 9α-fluorocortisol vs. 9α-fluoro-16α,17α-*o*-isopropylidene cortisol; triamcinolone vs. triamcinolone acetonide).

6. Substitutions at C-21, with resultant loss of a functional hydroxyl group, usually caused loss of antileukemic potency (e.g. 9α-fluorocortisol vs. 9α,21-difluorodesoxycortisol and 9α-fluorocortisol-17α,21-oxide.) An interesting exception was dexamethasone-17α,21-oxide, which proved almost as potent as dexamethasone itself.

Structural requirements for growth-inhibitory activity in vitro

The steroidal structural requirements for ability to inhibit the growth of L5178Y cells in culture are remarkably similar to those found *in vivo* (Table 3). This finding indicates that the antileukemic activity observed in most instances represents a direct cytotoxic effect of the steroid upon the leukemic cells, without the intervention of the host to convert the steroid to a cytolytic derivative. An evident exception to this generalization is the absolute requirement *in vitro*, but not *in vivo*, for the 11β-hydroxyl group (e.g. cortisone acetate vs. cortisol), most likely a reflection of the inability of these leukemic cells to perform the stereospecific reduction which presumably occurs in the liver cells of the mouse.

It is evident that those steroids, the GD_{50} of which *in vitro* is lower than 0·04 μg/ml, will demonstrate antileukemic activity in mice and, furthermore, that cytotoxicity *in vitro* is correlated with potency *in vivo*. It is also evident that those derivatives that had to be present in concentrations higher than 0·04 μg/ml to attain a GD_{50} were not active in the circumstances of the assay *in vivo*. These findings suggest that the culture system described above may be profitably exploited as a convenient and reliable tool in the search for more effective steroids to be used in the management of leukemia.

Effect of triamcinolone acetonide upon the survival time of mice bearing the advanced ascitic form of leukemia L5178Y

Having determined that certain adrenocorticoid homologs, such as triamcinolone acetonide and dexamethasone, can strikingly reduce the size of the ascitic population of L5178Y cells, it was desirable to learn whether these steroids can prolong the lives

TABLE 3. INHIBITION BY ANTI-INFLAMMATORY STEROIDS OF THE REPRODUCTION OF L5178Y CELLS IN CULTURE*

	Common name	GD_{50} Average (μg/ml)	Range	Effective dose in animals (mg/kg)
1.	Cortisone acetate	7	1–10	25
2.	9α-Bromo-11-ketoprogesterone	>0·1		>50
3.	Corticosterone	>0·1		>50
4.	11-Desoxycorticosterone	0·1	0·07–0·2	>50
5.	11α-Hydroxyprogesterone	0·4		
6.	11β-Hydroxyprogesterone	0·06	0·05–0·08	>50
7.	Testosterone	>0·1		>50
8.	Δ'-Testololactone	>0·1		>50
9.	Cortisol	0·02	0·01–0·04	50
10.	Prednisolone	0·03	0·01–0·06	12·5
11.	Triamcinolone	0·03	0·02–0·06	25
12.	9α-Fluorocortisol	0·008	0·006–0·01	12·5
13.	Triamcinolone acetonide	0·003	0·002–0·004	10

* After the reproduction from an inoculum of 1×10^5 cells/ml for a period of 52 to 70 hr in medium containing 2% dialyzed horse serum and different levels of the steroids, the amount of steriod was determined that would have resulted in one-half the generations achieved in drug-free controls (GD_{50}). The effective dose in animals was calculated as that most nearly approximating one-half the total cell number in the treated animal, when compared with the untreated animals (Table 2).

of mice bearing this invariably fatal, but steroid-sensitive neoplasm. For these studies triamcinolone acetonide was chosen as the representative compound, and initially a daily subcutaneous dose of 15 mg/kg was selected, since this approximates the lowest dosage, when given on two consecutive days, that exerts a reproducible cytolytic action upon leukemic cells in tumor-bearing mice. With this steroid, treatment was deferred until the fourth day after intraperitoneal inoculation of approximately 2×10^6 L5178Y cells into mice, at which time experience had indicated that the animals had an advanced form of this experimental neoplastic disease. The data in Table 4 show that, at best, three or four consecutive daily injections of triamcinolone acetonide, each of 15 mg/kg, prolonged survival time by an inconsequential 10 to 14%; no increase in survival time was observed when the regimen of dosages was more prolonged. In a later study, attempts were made to minimize host toxicity and to increase the efficacy of the steroid by manipulating the schedules of dosage; however, the data in Table 5 indicate that, to all intents and purposes, these attempts were futile. At least one possible reason for the restricted therapeutic efficacy might be deduced from the data presented in Table 6, which indicate that in the dosages that had to be given, triamcinolone acetonide eventually is lethal for mice, a finding that has already been reported by others.[6]

TABLE 4. EFFECT OF DAILY SUBCUTANEOUS INJECTIONS OF TRIAMCINOLONE ACETONIDE UPON THE SURVIVAL TIME OF MICE BEARING ADVANCED ASCITIC FORM OF LEUKEMIA L5178Y*

Days of treatment (15 mg/kg/day)	Cumulative dose (mg/kg)	Survival time in days		Increase in survival time (%)
		Average	Range	
0	0	11·2	10–13	
1	15	11·7	10–15	4
2	30	11·8	11–13	5
3	45	12·3	12–13	10
4	60	12·8	9–14	14
5	75	11·2	8–15	0
6	90	11·2	9–13	0

* Groups of 6 $AKDF_1$ female mice, weighing 20 to 25 g, were inoculated intraperitoneally with $1{\cdot}8 \times 10^6$ viable L5178Y cells. Treatment was started 4 days after inoculation of the tumor cells, when the total number of leukemic cells in the peritoneal cavity averaged 135×10^6.

TABLE 5. EFFECT OF INTERRUPTED DOSE SCHEDULES OF SUBCUTANEOUSLY ADMINISTERED TRIAMCINOLONE ACETONIDE UPON SURVIVAL TIME OF MICE BEARING ADVANCED ASCITIC FORM OF LEUKEMIA L5178Y*

Dose (mg/kg)	Days between treatments	No. of injections	Cumulative dose (mg/kg)	Survival time in days		Increase in survival time (%)
				Average	Range	
0			0	10·7	10–11	
15	2	5	75	11·7	11–13	9
15	3	4	60	12·0	11–13	12
15	4	3	45	11·5	10–14	7
30	2	6	180	12·8	12–15	20
30	3	4	120	12·7	11–16	19
30	4	3	90	12·3	11–15	15

* Groups of 6 $AKDF_1$ male mice, weighing 20 to 22 g, were inoculated intraperitoneally with $2{\cdot}0 \times 10^6$ viable L5178Y cells. Treatment was started 4 days after inoculation of tumor cells, when the total number of leukemic cells in the peritoneal cavity average 190×10^6.

TABLE 6. REPEATED-DOSE TOXICITY OF TRIAMCINOLONE ACETONIDE IN $AKDF_1$ FEMALE MICE*

Mouse no.	Day 0 weight (g)	Day 7 weight (g)	Day 7 change in weight (%)	Day 10 weight (g)	Day 10 change in weight (%)	Time of death (day)
1	21·0	18·5	−12	—	—	10
2	23·0	21·0	−9	19·0	−17	12
3	21·0	19·0	−9	18·0	−14	12
4	20·5	18·0	−12	18·0	−12	11
5	22·0	19·0	−14	18·0	−18	12
6	20·5	15·5	−24	—	—	9
7	22·0	17·5	−20	—	—	10
8	23·0	19·5	−15	18·0	−22	12
9	21·0	18·0	−14	16·5	−21	11
10	22·0	18·0	−18	17·0	−23	12
Avg.	21·6	18·4	−15	17·8	−18	11

* Triamcinolone acetonide, suspended in 0·25% carboxymethylcellulose and given in a constant volume of 0·5 ml, was injected subcutaneously once daily, 15 mg/kg, for 10 consecutive days.

DISCUSSION

Those corticoids that were effective in suppressing the growth of populations of leukemia L5178Y cells *in vivo* and *in vitro* were glucocorticoids or anti-inflammatory steroids. This finding is not surprising since the growth-inhibitory activity of this class of steroids against other cellular populations of mesenchymal origin is well established.[7–14]

The structural modifications of the basic cortisol molecule invariably associated with enhanced growth-inhibitory activity against L5178Y cells were (1) an α- or β-methylation at C-16; (2) formation of a 16α,17α-*o*-isopropylidene derivative; and (3) an α-halogenation at C-6 or C-9. Other single modifications, such as the Δ^1-double bond and α-methylation at C-6, did not enhance the antileukemic potency of the basic cortisol molecule. When combined with other substituent groups, however, both the Δ^1-double bond and α-methylation at C-6 revealed their activity-enhancing potential in the L5178Y system.

The finding in these studies that one of the more potent steroidal compounds had comparably higher host toxicity is disappointing. Nevertheless, the demonstration that certain modifications of the basic cortisol molecule imparted to it the capacity to exert a cytolytic action upon the leukemic cells may offer promise for future investigations.

The L5178Y cell culture system, as a source of steroid-sensitive leukemic cells, might prove useful for both mechanistic studies[15] and for the development of more effective antileukemic agents and should aid in the selection of new derivatives for study in appropriate animal systems.

Acknowledgements—We are especially indebted to Miss Sheila McDonald and to Mrs. Barbara Moracaldi for their skillful assistance.

REFERENCES

1. G. A. FISCHER and A. D. WELCH, *Science* **126**, 1018 (1957).
2. G. A. FISCHER, *Ann. N.Y. Acad. Sci.* **76**, 673 (1958).
3. E. E. HALEY, G. A. FISCHER and A. D. WELCH, *Cancer Res.* **21**, 532 (1961).
4. G. A. FISCHER and A. C. SARTORELLI. To be published.
5. M. POTTER and L. W. LAW, *J. nat. Cancer. Inst.* **18**, 413 (1957).
6. J. F. NOBLE, M. F. MAZZEI and L. A. KANEGIS, *Pharmacologist* **3**, 57 (1961).
7. V. A. DRILL and B. RIEGEL, *Recent Progr. Hormone Res.* **14**, 29 (1958).
8. J. FRIED, *Cancer* **10**, 752 (1957).
9. J. FRIED and A. BORMAN, *Vitam. and Horm.* **16**, 303 (1958).
10. J. M. LAMPKIN and M. POTTER, *J. nat. Cancer Inst.* **20**, 1091 (1958).
11. J. M. LAMPKIN-HIBBARD, *J. nat. Cancer Inst.* **20**, 1353 (1960).
12. I. RINGLER, S. MAUER and E. HEYDER, *Proc. Soc. exp. Biol. (N.Y.)* **107**, 451 (1961).
13. N. R. STEPHENSON, *J. Pharm. Pharmacol.* **12**, 411 (1960).
14. S. TOLKSDORF, *Ann. N.Y. Acad. Sci.* **82**, 839 (1959).
15. S. S. CARDOSO, *Biochem. Pharmacol.* **11**, 1 (1962).