precision of 0.239. The minimum standard error of the potency estimates from the 48-hour assay was 17%. Potency estimates from the single injection assay were also compared with those from a multiple injection 48-hour assay. The order of thymolytic activity agreed favorably regardless of the assay employed. Quantitative values, however, did not agree. Greatest discrepancies between potency ratios were noted for steroids possessing the 16α,17α-acetonide function. Nevertheless, quantitative differences observed for relative potencies of steroids from thymolytic assays appear to be far less than those recorded using other parameters of glucocorticoid activity.

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Development of a Hydrocortisone-Resistant Sub-Line of Mouse Lymphoma *in vitro*.* (27788)

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Anti-inflammatory steroids are known to inhibit the growth of some experimental transplanted mouse tumors, and there have been reports of the *in vivo* appearance of variants of these tumors which are steroid-resistant (1). We have recently described the properties of a mouse lymphoma cell line (ML-388), growing *in vitro,* which is extraordinarily sensitive to anti-inflammatory steroids (2). The present report describes the isolation, after several weeks cultivation in sublethal concentrations of hydrocortisone, of a sub-line of ML-388 which is no longer sensitive to the steroid. The resistance extends to all other steroids tested, and has remained characteristic of this sub-line even after cultivation for several months in the absence of any added steroid.

Methods and results. ML-388 cells were grown as adherent cultures in 8-oz prescription bottles using methods previously described(2). At 5-day intervals, the spent culture medium was poured off, and the cells suspended in 10 ml of fresh, sterile culture medium. The number of cells present in an aliquot of this suspension was determined, utilizing an electronic cell counter, and fresh bottles were then inoculated with 3×10^5 cells. In this manner, multiplication factors

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could be determined for 5-day intervals in control cultures, and in cultures to which hydrocortisone had been added to the medium at 1×10^{-6} M. The results (Table I) indicate that after about 4 weeks of very poor growth, some cells resume a rapid rate of growth (although not as rapid as control cultures in the absence of steroid), and these cells are able thereafter to grow in the presence of concentrations of steroids which inhibit the cell line of origin.

Cross-resistance extends to other active steroids. The growth of normal and resistant cells at several concentrations of active steroids is shown in Table II. The phenomenon of steroid-resistance, once obtained, seems to persist even after long culture of cells in the absence of added steroid, and seems to be a "one-step" jump to a high level of resistance, rather than a stepwise pattern of resistance, dependent on the concentration of steroid used to select the cell strain. Thus, cells which had been selected out at a concentration of 10^{-6} M hydrocortisone were also resistant to higher concentrations of hydrocortisone and even to high concentrations (10^{-5} M) of 6α-9α-difluoro-16α-hydroxyprednisolone-16,17-acetonide (Synalar®), a steroid about 100 times as potent as hydrocortisone as a growth-inhibitor of ML-388(2).

TABLE I. Development of a Hydrocortisone-Resistant Sub-Line of ML-388 *in vitro*.

Growth period	Multiplication factor: Control cells, no drug	Multiplication factor: 1×10^{-6} M hydrocortisone
1	19.8 ± .6	1.0 ± .2
2	29.9 ± 6.1	2.0 ± .1
3	20.7 ± 1.9	1.0 ± .1
4	22.6 ± 1.6	5.8 ± .4
5	15.5 ± 1.4	15.8 ± 1.9
6	15.2 ± 1.5	8.3 ± 1.1
7	19.0 ± 5.5	13.7 ± 2.3
8	27.6 ± 6.0	13.3 ± 5.0
9	26.6 ± 7.5	14.6 ± 1.0
10	24.3 ± .9	16.3 ± 2.4
11	21.1 ± 2.6	12.8 ± .4

An initial cell inoculum of 3×10^5 cells was allowed to grow for 5 days in normal medium, or a medium containing hydrocortisone at 1×10^{-6} M. The cell count was determined, and 3×10^5 cells (if available) were reinoculated to continue growing. Results are presented as the multiplication factor by which the initial cell count had changed over the 5-day period, and each value is the mean and stand. error of 3 separate bottles.

TABLE II. Cross-Resistance to Various Steroids of the Hydrocortisone-Resistant Sub-Line of ML-388.

Compound		Sensitive cell strain	Resistant cell line #1*	Resistant cell line #2†
Hydrocortisone,	10^{-7} M	84 ± 5	100 ± 9	105 ± 7
	10^{-6} M	39 ± 1	104 ± 7	105 ± 3
	10^{-5} M	25 ± 2	83 ± 6	92 ±10
Corticosterone,	10^{-7} M	61 ± 4	106 ± 6	83 ± 5
	10^{-6} M	37 ± 4	116 ± 5	87 ± 3
	10^{-5} M	27 ± 3	117 ± 5	61 ± 1
Dexamethasone,	10^{-7} M	23 ± 7	96 ±18	92 ± 7
	10^{-6} M	15 ± 3	105 ±11	76 ±17
	10^{-5} M	21 ± 1	65 ±10	72 ±10
Synalar,®	10^{-7} M	13 ± 1	81 ± 2	91 ±16
	10^{-6} M	15 ± 1	133 ±15	111 ± 9
	10^{-5} M	7 ± 1	79 ± 7	82 ± 6

* This sub-line had been maintained for several months in the presence of 1×10^{-6} M hydrocortisone before testing.

† This sub-line had been maintained for 6 mo in the absence of any added steroid before testing.

Cells were inoculated into 8-oz prescription bottles and the following day various steroids were added at the specified concentrations. Initial cell inoculum in all instances was about 1×10^5 cells. Cell count was determined at the end of 5 days. Results are presented as percent of number of cells observed in control bottles, which received vehicle instead of steroid. Each value is the mean and stand. error of a group of 3 bottles. In the control bottles, the sensitive cell strain multiplied 26.5-fold over this period while the resistant cell lines multiplied 16.2-fold (#1) and 11.3-fold (#2).

Summary. A steroid-resistant variant of ML-388 cells has been isolated by culture of the parent cell strain in the presence of 10^{-6} M hydrocortisone for several weeks. The resistant sub-line grows at a somewhat slower rate than its parent cell line, and maintains the characteristic resistance to steroids even after long cultivation in the absence of added steroids. The variant cell line is not only resistant to high concentrations of hydrocortisone; there is cross-resistance to all other potent anti-inflammatory steroids tested.

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