Mechanisms of Amethopterin Resistance in Leukemia II. Effect of Cortisone on Sensitive and Resistant Mouse Leukemias. (19070)

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Although cortisone does not cause a prolongation of survival time of mice injected with the Ak4 strain of transplanted leukemia, large single doses will cause a fall in the total leukocyte count in the advanced stages of the disease(1). Since children with acute leukemia may respond well at first but eventually develop resistance either to amethopterin (2-4) or cortisone(5), and since a subline of the Ak4 strain of mouse leukemia has been produced which is resistant to amethopterin (4-amino-N^{10}-methyl PGA), it was thought worthwhile to test the effects of cortisone† on this amethopterin-fast strain (Ak4R)(6) in the hopes that these studies in mice might have some bearing on the problems of clinical resistance to these compounds.

Method. The method used was similar to that previously reported(7). Young Akm mice, 18-22 g in weight, were injected intraperitoneally with 0.1 cc of a saline suspension of spleen from a mouse in the advanced stages of Ak4 or Ak4R leukemia, so diluted as to contain one million cells. Six to 12 days later, when the mice had developed signs of advanced leukemia, and the total leukocyte count had begun to rise, a base line count was taken. Within two hours thereafter a single dose of cortisone was administered subcutaneously. Leukocyte determinations were made at 24, 48, and occasionally 72 hours thereafter.

Results and discussion. From the results with strain Ak4 in Table I it can be seen that a single dose of cortisone at a level of 100-250 mg/kg prevented (temporarily) the precipitous rise in WBC that occurred in the controls, and frequently caused some fall in count. The results in Table II show strain Ak4R to be somewhat more affected by cortisone than its parent amethopterin-sensitive strain. Here single doses of from 25 to 250 mg/kg caused a significant fall in total leukocyte count in 24 hours.

This demonstration of the lack of cross resistance to cortisone in this amethopterin-resistant strain of mouse leukemia is supported by clinical studies(8-10) which show that patients whose disease has developed resistance to aminopterin or amethopterin will still respond to ACTH or cortisone. Clinical studies have also demonstrated that patients whose disease is no longer responding to hormone therapy can often be benefited by treat-

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TABLE I. Effect of Cortisone on Total Leukocyte Count of Mice with Advanced AK4 Leukemia.

Control or treated	Dose, mg/kg	Hr after injection of cortisone: 0 WBC x 10^3 Mean	0 Range	24 WBC x 10^3 Mean	24 Range	48 WBC x 10^3 Mean	48 Range
Control	—	86 D	46-157	146 D	62-233	133 A	59-207
Cort.	250	95 E	34-160	36 E	14-68	66 D	23-126
Control	—	49 F	16-95	130 F	30-231	158 C	81-232
Cort.	250	26 G	14-38	42 G	6-72	66 G	9-107
Control	—	18 D	12-27	57 D	33-93	93 B	44-146
Cort.	250	45 F	22-97	38 F	11-67	49 D	39-65
"	125	21	9-41	45	4-95	65 F	6-134
Control	—	29 G	14-49	106 G	29-277	91 B	36-230
Cort.	250	62 E	19-168	66 E	5-140	62 E	11-92
Control	—	20 A	11-28	117 A	75-158		
Cort.	200	49	19-82	34	5-53		
"	100	58	28-91	41	5-83		
"	50	60	12-144	78	36-136		
"	25	45 G	25-74	57 G	10-97		

Ten mice included in average unless otherwise indicated: A-2, B-4, C-5, D-6, E-7, F-8, G-9.

TABLE II. Effect of Cortisone on Total Leukocyte Count of Mice with Advanced AK4R Leukemia.

Control or treated	Dose, mg/kg	Hr after injection of cortisone: 0 WBC x 10^3 Mean	0 Range	24 WBC x 10^3 Mean	24 Range	48 WBC x 10^3 Mean	48 Range
Control	—	154 F	119-219	177 F*	107-275*	176 A	148-203
Cort.	250	163	112-205	48 *	14-92*	15 F	7-24
Control	—	76	25-150	79	29-180	71 C	29-154
Cort.	250	73	22-113	29	17-36	35	14-58
"	125	67	20-135	17	6-24	28 G	9-66
"	62.5	59	12-130	25	7-44	21 G	6-51
"	31.0	63	31-116	26	14-42	37 F	21-85
Control	—	76	31-106	76	33-110		
Cort.	200	88	17-129	19	8-31		
"	100	62	36-89	17	8-35		
"	50	77	28-120	20	7-37		
"	25	95	66-138	21	11-28		
Control	—	70	32-119	98	40-141	98 F	58-155
Cort.	125	60	22-97	21	2-38	26 F	8-52
"	62.5	64	17-110	30	11-45	41 G	18-97
Control	—	69 F	26-155	92	29-143	98 D	62-137
Cort.	100	95 G	30-116	47 G	5-66	72 E	29-132
"	50	81	25-113	52	8-74	95 G	69-137
"	25	100 G	42-144	64 G	10-115	85 D	12-133
Control	—	35	18-74	73	50-107	110	41-202
Cort.	31	45	23-85	26	15-34	67 G	25-122
"	15.6	32	17-73	46	15-64	91 G	25-168
"	7.8	30	12-66	62	33-85	94 G	65-158

* Counts taken at 19 hr. Ten mice included in avg unless otherwise indicated: A-2, B-4, C-5, D-6, E-7, F-8, G-9.

ment with the folic acid antagonists(10).

Summary. (1) The administration of large single doses of cortisone to mice with advanced transplanted leukemia of strains Ak4 (amethopterin sensitive) and Ak4R (amethopterin resistant) caused a fall in the elevated leukocyte count. (2) This leukotoxic effect was considerably more marked and occurred

at lower cortisone dosage with the Ak4R than with the parent Ak4 strain, and demonstrates the lack of cross resistance to cortisone in this amethopterin-fast strain (Ak4R) of mouse leukemia.

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Observations on Antiviral Activity of Viscosin.*† (19071)

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A culture of *Pseudomonas viscosa* was isolated by Kochi in the laboratories of the Yokohama Medical College during a systematic search for microorganisms producing antibiotic substances(1). This culture was found to produce an antibiotic which was active *in vitro* against various pathogenic and saprophytic mycobacteria but which was inactive against representative species of other groups of bacteria. This antibiotic, provisionally designated "P-preparation," was obtained in crystalline form and was found to be heat-stable and soluble in ethanol, methanol, ether, acetone, and alkaline phosphate buffer. Taro Miuri(2) reported that the crystalline antibiotic was found to be an acidic polypeptide having a melting point of 264-268°C (Corr.). In preliminary tests this substance was found to exert a demonstrable therapeutic effect on experimental tuberculosis in guinea pigs(1,3) which was minimal when compared to that of streptomycin(3).

A quantity of the crystalline substance was prepared in this laboratory, and the antibiotic was named viscosin(4). In addition to its antibiotic activity against mycobacteria, viscosin was reported to exert a protective effect in embryonated eggs infected with infectious bronchitis virus of chickens and to exert a slight but detectable suppressive effect on the progress of infection in mice infected with influenza A virus(4). A report of the studies on the antiviral properties of viscosin is presented here.

Methods. The following viruses were used in this study: Vaccinia virus, New York City Board of Health strain; Newcastle disease virus, California strain; influenza A virus, PR-8 strain; influenza B virus, Lee strain; infectious bronchitis virus of chickens, original strain isolated by Beaudette and Hudson; and *Miyagawanella felis,* original strain of feline pneumonitis virus isolated by Baker. Inoculum pools of the various viruses containing 30 to 50% yolk or inactivated normal horse serum were prepared, distributed into suitable ampules, and stored at —70°C. In each experiment the infectivity titers of the various viral inocula were determined in the usual manner by inoculation of serial decimal dilutions of the respective pools into groups of 6-10 eggs each. The contact test, a combined *in vitro* and *in vivo* test was performed as follows: Equal volumes of solutions of various concentrations of the antibiotic and appropriate dilutions of virus were mixed *in vitro* and incubated at room temperature for 2 hours. One ml amounts of the various antibiotic-virus mixtures were then inoculated

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