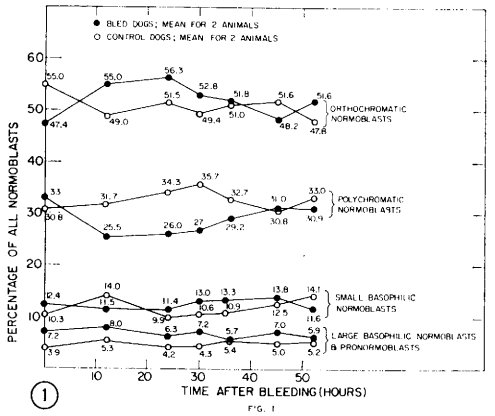
of the marrow did not differ from the controls as measured by percent labeling and grain counts in morphologically differentiated cells.



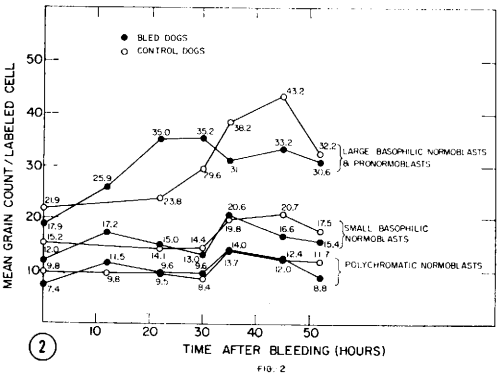


FIG. 1. Percentage of normoblasts in dog bone marrow as a function of time after bleeding.

FIG. 2. Mean grain count of labeled normoblasts in dog bone marrow as a function of time after bleeding. Each point represents mean of 2 animals.

Also, no alterations from control values were found with regard to morphological distribution, relative numbers of the marrow normoblasts, and mitotic index. The data suggest that the acute blood loss did not influence detectably the chosen parameters for the time intervals studied.

1. Wintrobe, M. M., *Clinical Hematology,* Lea & Febiger, 5th Ed., Phila. 1961.

2. Alpen, E. L., Cranmore, D., *Kinetics of Cellular Proliferation*, F. Stohlman, Jr., ed., Grune & Stratton, New York, 1959, p290.

3. Steele, B. F., *J. Exp. Med.,* 1933, v57, 881.

4. Sabin, F. R., *Physiol. Rev.,* 1928, v8, 191.

5. Quastler, H., Sherman, F. G., *Exp. Cell Res.,* 1959, v17, 429.

6. Bond, V. P., Odartchenko, N., Cottier, H., Feinendegen, L. E., Cronkite, E. P., *Erythropoiesis,* L. Jacobson and M. Doyle, Eds., Grune & Stratton, New York, 1962, p173.

7. Cronkite, E. P., Bond, V. P., Fliedner, T. M., Killman, S. A., Rubini, J. R., *Tritium in the Physical and Biological Sciences,* IAEA, Vienna, 1962, vII.

8. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R., Brecher, G., Shork, P. K., *Acta Haemat.,* 1959, v21, 1.

9. Thomas, E. D., Lochte, H. L., *Blood,* 1957, v12, 1086.

10. Rubini, J. R., Cronkite, E. P., Bond, V. P., Keller, S., *J. Nuclear Med.,* 1961, v2, 223.

11. Doniach, I., Pelc, S. R., *Brit. J. Radiol.,* 1950, v23, 184.

12. Alpen, L. E., Cranmore, D., Johnston, M. E., *Erythropoiesis,* Jacobson, L. O., Doyle, M., Eds., Grune & Stratton, New York, 1962, p184.

Received February 13, 1964. P.S.E.B.M., 1964, v116.

Bioassay of Adrenocorticosteroids by the Dog Eosinophil Response. (29291)

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Numerous investigators have elaborated on the bioassay of adrenocorticoids in adrenalectomized mice, based on a decrease in circulating eosinophils(1-3). Tolksdorf(4,5) reported eosinopenic potencies of natural and synthetic corticoids in this species. The adrenalectomized dog also has been used for assessment of eosinopenia. Liddle *et al*(6) measured the decrease in eosinophils 4 hours following intravenous administration of graded doses of steroids. When assessed on an equimolar basis, 9α-fluorohydrocortisone acetate was 20 and 9α-chlorohydrocortisone acetate 8 times more active than the non-

TABLE I. Diurnal Variation of Circulating Eosinophils in Intact, Untreated Dogs.

Group	Dog No.	Sex	Exp No.	Eosinophils/mm³ blood 0 hr	2 hr	5 hr	7 hr	9 hr
I	163	♂	1	1026	1037 (101)*	866 (85)	1019 (99)	
			2	1350	1379 (102)	1218 (90)	1355 (101)	
	164	♂	1	725	694 (96)	664 (92)	634 (87)	
			2	669	707 (106)	565 (84)	667 (99)	
	165	♂	1	566	458 (81)	400 (71)	411 (73)	
			2	426	426 (100)	488 (114)	474 (111)	
					92.7 ±11.8†	82.7 ±12.4	86.3 ±15.3	
					102.6 ± 3.5	96.0 ±17.7	103.3 ± 7.0	
II	168	♂	3	920	1016 (110)	802 (87)	842 (92)	
			4	832	840 (101)	932 (112)	907 (109)	
	169	♂	3	654	656 (100)	798 (122)	854 (130)	
			4	518	452 (87)	542 (105)	563 (109)	
	170	♂	3	928	973 (105)	901 (97)	400‡	
			4	661	838 (126)	776 (117)	955 (144)	
					105.0 ± 5.9	102.0 ±20.6	111.0 ±33.6	
					104.6 ±23.0	111.3 ± 7.1	120.6 ±20.6	
III	207	♀	5	640		566 (89)		610 (95)
	208	♀	5	387		421 (109)		483 (125)
	209	♀	5	386		323 (84)		421 (109)
						94 ±14.8		110 ±17.7
IV	211	♂	5	654		787 (120)		699 (107)
	213	♀	5	720		666 (93)		730 (101)
	214	♀	5	981		1062 (108)		966 (98)
						107 ±16.0		102 ±5.3

* Numbers in parentheses represent % of 0 hr. † Mean % of 0 hr ± S.D. ‡ Clot.

halogenated parent steroids. One mg orally of 16α-methyl-9α-fluoroprednisolone produced maximal eosinopenia in 6 hours; a comparable response was observed with 5 mg prednisolone(4).

The present communication concerns an assay procedure based upon the eosinopenic response of intact dogs to orally and intravenously administered adrenocorticosteroids, and compares potency estimates with those obtained in man.

Materials and methods. Twelve intact beagle dogs, 12-15 months old and weighing 6.7 to 10 kg, were divided equally into 4 groups. Animals of Groups I and II were litter mates (6 males) as were those in Groups III and IV (5 female and 1 male). They were fasted the day prior to and during the test period; water was allowed *ad libitum*. All dogs were rested 1-2 weeks between tests. A minimum of 3 dogs was used for each dose of steroid.

For oral administration, sufficient distilled water was added to weighed amounts of steroid so the dose (μg/kg) was contained in 0.1 ml. Uniform suspensions were obtained by the use of glass homogenizers. The total dose was contained in a No. 00 gelatin capsule. The intravenous dose (μg/kg) was dissolved in 0.1 ml distilled water. Total volume (0.67-1.0 ml) was injected in about 10 seconds *via* the radial vein.

Eosinophil measurements. Blood samples were obtained from the jugular vein at —0.5, 0, 2, 4 (or 5), 7, 9 (or 12) and 24 hours following steroid administration. Number of eosinophils was determined by diluting 0.5 ml blood with 4.5 ml Randolph's solution and counting in a Fuchs-Rosenthal chamber(7). Eosinophils in the total ruled area from both sides of the chamber were counted, averaged, corrected for dilution and expressed as eosinophils per cubic mm blood.

Statistics. Dose-response lines were obtained by the method of least squares. Slopes of dose-response regression lines were combined using the reciprocals of slope variance (8). Assay precision (λ) was calculated by

TABLE II. Eosinopenic Effects of Orally Administered Adrenocorticosteroids.

Compound	Dose, μg/kg	Avg eosinophils/mm³ blood							
		0 hr*	2 hr	4 hr	5 hr	7 hr	9 hr	12 hr	24 hr
Hydrocortisone	1000 †	947	750 (79 ± 5)‡	379 (44 ± 7)	315 (29 ± 6)	446 (46 ± 7)	677 (79 ± 8)	759 (89 ± 9)	843 (98 ± 7)
	500	569	509 (90 ± 10)		269 (47 ± 10)	355 (65 ± 12)	539 (100 ± 21)	644 (115 ± 10)	745 (142 ± 45)
	250	836	804 (97 ± 13)	724 (87 ± 13)		763 (91 ± 11)			870 (105 ± 7)
Triamcinolone	250 †	849	749 (91 ± 5)	214 (21 ± 7)	165 (26 ± 3)	26 (4 ±.29)	4 (.3 ±.12)	0 (0)	360 (33 ± 7)
	60	1026	1075 (106 ± 14)	465 (44 ± 4)		80 (8 ± 3)	16 (2 ±.9)	14 (1 ±.29)	806 (75 ± 16)
	15	741	727 (99 ± 7)	417 (56 ± 1)		144 (19 ± 1)		295 (39 ± 7)	720 (96 ± 15)
	3.75	1418	1211 (87 ± 7)	1096 (76 ± 9)		829 (54 ± 10)		986 (68 ± 9)	918 (69 ± 14)
	.94	781	741 (94 ± 5)		647 (83 ± 6)	625 (79 ± 5)		654 (86 ± 10)	750 (96 ± 5)
Triamcinolone acetonide	60	723	661 (91 ± 6)		335 (46 ± 3)	184 (26 ± 4)	221 (31 ± 7)	393 (55 ± 8)	514 (76 ± 19)
	3.75	644	633 (100 ± 5)		609 (97 ± 12)	635 (101 ± 12)			
Dexamethasone	25	841	697 (85 ± 25)		240 (28 ± 13)	114 (14 ± 8)	122 (14 ± 6)	283 (33 ± 17)	820 (98 ± 21)
	3.75	432	378 (87 ± 3)		317 (71 ± 11)	317 (73 ± 10)	438 (104 ± 8)	427 (100 ± 5)	443 (104 ± 8)
Prednisolone	250	749	642 (86 ± 6)		105 (14 ± 1)	20 (3 ± 1)	13 (2 ±.69)	197 (25 ± 10)	891 (114 ± 10)
	100	687	651 (95 ± 2)		334 (48 ± 8)	370 (53 ± 10)	556 (81 ± 13)	773 (112 ± 14)	691 (99 ± 19)
	10	675	674 (98 ± 4)		664 (94 ± 12)	663 (94 ± 12)	697 (101 ± 6)	797 (115 ± 8)	690 (100 ± 7)
6α-Methylprednisolone	250	952	721 (78 ± 9)		73 (8 ± 1)	20 (2 ±.34)	27 (3 ±.34)	235 (23 ± 7)	1024 (113 ± 13)
	10	650	571 (87 ± 9)		499 (76 ± 10)	469 (72 ± 13)	603 (93 ± 5)	680 (105 ± 5)	498 (76 ± 7)
16α-Hydroxyhydrocortisone	500	714	606 (85 ± 4)		363 (51 ± 8)	451 (65 ± 5)		657 (99 ± 20)	759 (110 ± 9)
	100	727	651 (90 ± 7)		623 (87 ± 8)	716 (100 ± 10)	827 (115 ± 10)	830 (116 ± 14)	759 (108 ± 20)

* Avg of −0.5 and 0 hr counts. † 6 dogs. For all other doses, 3 dogs. ‡ Numbers in parentheses represent avg % change from 0 hr ± S.D.

TABLE III. Eosinopenic Effects of Intravenously Administered Adrenocorticosteroids.*

Compound	Dose, μg/kg†	Avg eosinophils/mm³ blood						
		0 hr‡	2 hr	5 hr	7 hr	9 hr	12 hr	24 hr
Hydrocortisone	1000	1052	852 (81 ± 3)§	358 (36 ± 11)	524 (52 ± 16)	807 (78 ± 10)	941 (89 ± 4)	1003 (94 ± 9)
	500	543	480 (93 ± 14)	332 (65 ± 14)	446 (87 ± 13)	597 (119 ± 26)	787 (158 ± 39)	688 (139 ± 34)
	50	455	478 (111 ± 12)	559 (135 ± 26)	603 (148 ± 36)	650 (158 ± 35)	714 (180 ± 52)	346 (82 ± 21)
Triamcinolone	15	852	697 (81 ± 7)	123 (14 ± 4)	49 (6 ± 2)	83 (10 ± 2)	282 (35 ± 5)	619 (74 ± 3)
	3.75	805	805 (101 ± 3)	663 (84 ± 1)	601 (76 ± 7)	655 (85 ± 20)	677 (89 ± 26)	765 (101 ± 28)
	.94	953	952 (101 ± 4)	982 (103 ± 3)	889 (94 ± 6)	938 (99 ± 6)	899 (95 ± 12)	763 (81 ± 11)
Triamcinolone acetonide	60	1021	821 (79 ± 9)	202 (19 ± 9)	64 (19 ± 7)	67 (6 ± 3)	252 (24 ± 2)	858 (82 ± 21)
	3.75	624	492 (79 ± 12)	127 (20 ± 7)	73 (11 ± 6)	99 (16 ± 7)	317 (52 ± 10)	696 (115 ± 23)
	.94	756	709 (93 ± 2)	665 (87 ± 8)	613 (79 ± 10)	677 (89 ± 2)	771 (101 ± 9)	885 (116 ± 10)
Dexamethasone	25	785	677 (88 ± 7)	188 (26 ± 10)	130 (19 ± 9)	228 (33 ± 14)	390 (53 ± 12)	692 (87 ± 3)
	12.5‖	803	606 (75 ± 3)	186 (23 ± 3)	131 (34 ± 2)	124 (19 ± 1)	349 (44 ± 5)	641 (80 ± 4)
	6.25	922	827 (90 ± 2)	630 (68 ± 6)	670 (73 ± 8)	—	732 (79 ± 3)	738 (80 ± 1)
	3.75	950	943 (98 ± 5)	883 (92 ± 7)	905 (95 ± 2)	921 (97 ± 3)	868 (91 ± 4)	713 (75 ± 2)

* Administered as the C-21-disodium phosphate esters.
† Calculated on basis of free alcohol.
‡ Avg of −0.5 and 0 hr counts.
§ Numbers in parentheses represent avg % change from 0 hour ± S.D.
‖ 6 dogs. For all other doses, 3 dogs.

dividing within assay standard deviation (s) by the slope (b) of the line(9).

Results. Diurnal variation of eosinophil counts in intact, untreated dogs. The variation in 0 hour eosinophil count from animal to animal was large: for female dogs values ranged from 386-981 and for males from 426-1350 cells/mm³ blood (Table I). Statistical analyses of data from male dogs revealed that variations in counts from dog to dog were appreciably greater than from hour to hour ($P<0.01$). Also, there were no indications of a systematic increase or decrease in circulating eosinophils with repeated bleedings. It was concluded that treatment response would best be expressed as per cent of the pretreatment eosinophil count. Moreover, the precision of the procedure would be enhanced if eosinophils were assessed at −0.5 and 0 hours, averaged, and the mean taken as the pretreatment value. These techniques were employed throughout the steroid studies.

Eosinopenia following administration of adrenocorticoids. The effects of single oral or intravenous doses of steroids on number of circulating eosinophils are shown in Tables II and III, respectively. Not only the degree, but the duration of eosinopenia appeared to be a function of steroid dosage. Significant depression of eosinophils was not evident until 2 hours postinjection. With hydrocortisone and its 21-phosphate ester, maximal eosinophil depression occurred at 5 hours, whereas with the synthetic steroids maximal eosinopenia was usually evident at 7 hours.

The 2 to 12 hour eosinopenic effects of the compounds, expressed as a weighted average per cent(10) was used for the dose-response

TABLE IV. Comparative Eosinopenic Potencies in the Dog and Man.

	Dog		Man
	I.V.*	Oral	Oral†
Hydrocortisone	1.0	1.0	1.0
Triamcinolone	107 (77– 150)	292 (160 –540)	5
Triamcinolone acetonide	700 (380–1300)	55 (23 –128)	3
Dexamethasone	140 (84– 230)	158 (36 –690)	28
Prednisolone		20 (11 – 38)	4
Methylprednisolone		46 (25 – 87)	5
16α-Hydroxyhydrocortisone		1.0 (.2– 4.5)	

* Administered as the C-21-disodium phosphate esters.
† Single administration(15).

calculations. Precision (λ) of the bioassay was 0.33 when steroid was administered orally, and 0.13 following intravenous injection. Combined slopes for compounds were —0.37 and —0.76, respectively.

Comparative eosinopenic potencies of several adrenocorticosteroids are shown in Table IV. Orally, triamcinolone was appreciably more effective than the other steroids; however, because 95% confidence limits overlapped with those of dexamethasone, a significant difference between the activities of these steroids was not established. Moreover, it was obvious that quantitatively, eosinopenic potency in the intact dog had little relationship to that recorded in man. When steroids were administered intravenously as the disodium phosphate esters, triamcinolone acetonide was significantly more efficacious than either triamcinolone or dexamethasone. It was noteworthy that triamcinolone acetonide given intravenously as the phosphate ester was significantly more effective than when given orally. This was not true of either triamcinolone or dexamethasone esters.

Discussion. Though the data of West and collaborators(11,12), Liddle(13), McMahon and Gordon(14), and Ringler *et al*(15) exemplify the excellent agreement between clinical eosinopenic and anti-inflammatory activities of orally administered adrenocorticoids, application of the eosinopenic assay methodology to intact dogs failed to result in comparable steroid potencies. Canine relative eosinopenic potencies were significantly greater than those observed in man (Table IV). Moreover, the potency ratios based on eosinopenia in the dog exceed those reported for rat thymus involution(16).

On the premise that eosinopenic potencies reflect glucocorticoid activity in the dog, choice of steroid dosage for dog studies, based on relative clinical efficacy, may be somewhat tenuous. Fielder *et al*(17) administered prednisolone (2.5 and 5.0 mg/kg), methylprednisolone (2.5 and 5.0 mg/kg) and triamcinolone (0.5, 2.5 and 5.0 mg/kg) orally to dogs daily for 6 weeks. Two of four dogs receiving 5.0 mg/kg triamcinolone and 3 of 4 on 2.5 mg/kg died during the study. Significant loss of body weight, diuresis and decreases in hemoglobin and packed cell volumes were noted in other animals receiving triamcinolone. These effects were minimal in dogs given the other steroids; moreover, no mortality occurred. Faludi *et al*(18) administered triamcinolone (20 mg); dexamethasone (4 mg); methylprednisolone (20 mg); prednisolone (25 mg); and hydrocortisone (100 mg) intramuscularly to dogs daily for 5 weeks in an attempt to induce myopathy. It was concluded that "weight loss and signs of muscle wasting could be observed in all groups except the control group; they were most pronounced in the triamcinolone treated dog."

Both these data and the eosinopenic activity support the concept that the biological responsiveness of intact dogs to triamcinolone exceeds that which would be expected from the relative clinical activity. However, it also must be cautioned that eosinophils in the dog, unlike the human, may respond to adrenocorticosteroids in such manner as not to quantitatively reflect other metabolic actions.

Summary. Several glucocorticoids were administered orally or intravenously to fasted, intact dogs and their effects on circulating

eosinophils determined. The calculated eosinopenic potencies were not quantitatively related to those reported in man. The possible implication of these data with respect to dosage selection of steroids for studies in dogs is briefly discussed.

1. Speirs, R. S., Meyer, R. K., *Endocrinology,* 1951, v48, 316.

2. Bibile, S. W., *J. Endocrinol.,* 1953, v9, 357.

3. Rosemberg, E., Cornfield, J., Bates, R. W., Anderson, E., *Endocrinology,* 1954, v54, 363.

4. Tolksdorf, S., *Ann. N. Y. Acad. Sci.,* 1959, v82, 829.

5. ———, *Inflammation and Diseases of Connective Tissue,* W. B. Saunders Co., Philadelphia and London, 1961.

6. Liddle, G. W., Pechet, M. M., Bartter, F. C., *Science,* 1954, v120, 496.

7. Randolf, T. G., *J. Allergy,* 1944, v15, 89.

8. Finney, D. J., *Statistical Method in Biological Assay,* Charles Griffen & Co., Ltd., London, 1952.

9. Bliss, C. I., *The Statistics of Bioassay,* Academic Press, Inc., New York, 1952.

10. Whittaker, E., Robinson, G., *The Calculus of Observations,* Blackie & Son, Ltd., London, 1946.

11. West, K. M., *Metabolism,* 1958, v7, 411.

12. West, K. M., Johnson, P. C., Kyriakopoulos, A. A., Bahr, W. J., Bloedon, C. E., *Arthritis Rheumat.,* 1960, v3, 129.

13. Liddle, G. W., *Metabolism,* 1958, v7, 405.

14. McMahon, F. G., Gordon, E. S., *Wisconsin Med. J.,* 1961, v60, 291.

15. Ringler, I., West, K. M., Dulin, W., Boland, E. W., *Metabolism,* 1964, v13, 37.

16. Maurer, S. I., Heyder, E., Ringler, I., PROC. SOC. EXP. BIOL. AND MED., 1962, v3, 345.

17. Fielder, F. G., Hoff, E. J., Thomas, G. B., Tolksdorf, S., Perlman, P. L., Cronin, M. T. I., *Tox. and Appl. Pharm.,* 1959, v1, 305.

18. Faludi, G., Mills, L. C., Chayes, W. Z., *Inflammation and Diseases of Connective Tissue.* W. B. Saunders Co., Philadelphia and London, 1961.

Received March 24, 1964. P.S.E.B.M., 1964, v116.

Inheritance of Resistance to Influenza Virus in Mice.* (29292)

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Natural resistance to certain bacterial infections in mice has usually been found to involve many factors and to have a complex mode of inheritance(1). In contrast, there are two viral infections the resistances to which depend on simple genetic mechanisms. Thus, a single dominant gene (or block of closely linked genes) is responsible for the resistance of PRI or BRVR mice to group B arboviruses(2,3). Similarly, resistance of C3H mice to mouse hepatitis virus is associated with one or two recessive genes(4).

The observation that inbred mice of the A2G strain were highly resistant to the lethal action of certain myxoviruses(5,6) prompted research into the genetic mechanism underlying this phenomenon. Crosses were arranged between resistant A2G and fully susceptible A or C3H mice. The results reported below suggest that a single dominant autosomal gene governs resistance to neurotropic influenza virus.

Materials and methods. Mice: A2G mice were obtained by inbreeding from a litter of A2G/EGA mice introduced in 1962 (see 6). A/Jax and C3H/HeJax mice were purchased from Jackson Memorial Laboratory, Bar Harbor, Maine; these mice will be designated A and C3H, respectively. ICR mice (noninbred) were purchased from Dublin Laboratories, Dublin, Va.

Virus: The Stuart-Harris strain of neurotropic influenza A virus, NWS, was used(7). A volume of 0.1×10^{-4} ml of mouse brain passage material was inoculated into the allantoic cavity of 10-day embryonated eggs. After 48 hours of incubation at 35°C a single allantoic fluid with an egg infectivity titer of $10^{7.5}$ 50% egg infective doses per ml was selected. Small aliquots of this virus stock were kept in sealed ampoules at —70°C and thawed only once for use. Virus dilutions

* This work was supported in part by grant from Nat. Inst. Health, USPHS.