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Adrenocortical Properties of $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione (Meticorten) and $\Delta^{1,4}$-pregnadiene-11β,17α,21-triol-3,20-dione (Meticortelone).* (22429)

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The therapeutic usefulness of cortisone and hydrocortisone prompted a search for agents possessing anti-inflammatory actions similar to these steroids but producing a lower incidence of undesirable side effects. Efforts in these laboratories led to the development of two new steroids, Meticorten ($\Delta^{1,4}$-pregnadiene-17α, 21-diol-3,11,20-trione) and Meticortelone ($\Delta^{1,4}$-pregnadiene-11β,17α,21-triol-3,20-dione), which differ from cortisone and hydrocortisone in possessing an additional double bond between C_1 and C_2 in the A ring(1-3). Their effectiveness in the treatment of rheumatoid arthritis was first demonstrated by Bunim *et al.*(4).

The glucocorticoid potency of Meticorten and Meticortelone was found to be 3 to 4 times greater than that of cortisone and hydrocortisone by 3 different bioassay methods. Further comparison of the adrenocortical activities of the new steroids and their parent substances included 1) the life maintaining action in adrenalectomized rats and 2) the degree of hypercorticism induced in intact rats by prolonged steroid administration as well as the regression of these changes after cessation of treatment. The results of electrolyte excretion studies will be reported separately. Preliminary biological data have been reported previously(5,6). It is the purpose of this paper to present in detail the biological work carried out with Meticorten and Meticortelone.

Methods. 1. *Eosinophil response.* The procedure employed was based on the methods of Speirs and Meyer(7) and Rosemberg and co-workers(8). Steroids were screened in adrenalectomized C_{57} brown, male mice‡ at a dose of 24 μg without pretreatment with epinephrine. Preliminary potency estimates were performed with 3-fold dilutions of Meticorten and Meticortelone (6, 2, and 0.67 μg) and cortisone and hydrocortisone (12, 4, and 1.33 μg). The potency was calculated on

* Meticorten and Meticortelone, Schering Corp. brand of prednisone and prednisolone were used.

† The authors wish to thank Mrs. Shirley W. Bolles, Miss Janet Maley and Mrs. Alide Tali for valuable technical assistance. Mr. Gordon B. Thomas carried out statistical analysis of data, and members of the Chemical Research Division provided the steroids.

‡ Obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me. We wish to thank the members of our Pharmacology Department for performing the adrenalectomies.

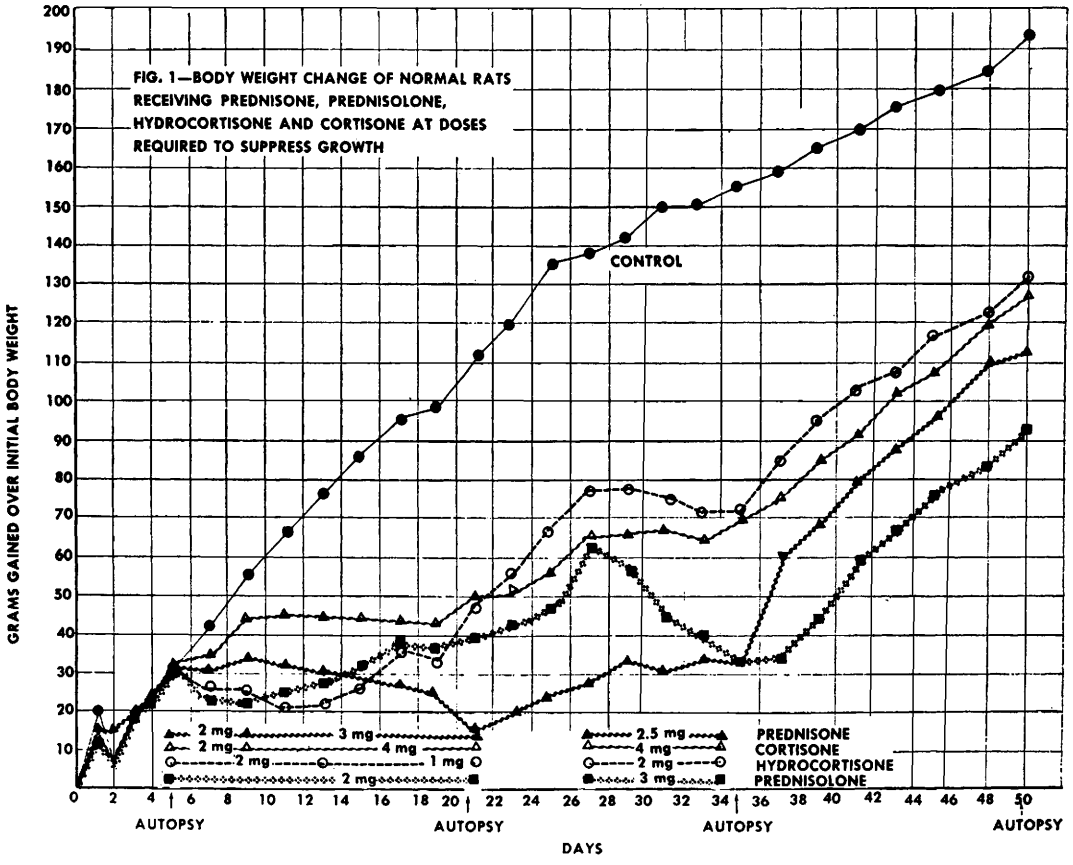

FIG. 1.

logarithmically transformed counts as described by Finney(9). 2. *Liver glycogen deposition.* Liver glycogen deposition was assayed in adrenalectomized male rats§ by the methods of Olson *et al.*(10) and Pabst *et al.*(11). Glycogen was estimated by the anthrone procedure of Kahan(12). 3. *Thymus involution.* The assay was patterned after that described by Stephenson(13) using intact, immature, female, litter-mate rats which had been rested for 1 week after shipment from the breeder. The steroids were dissolved in cottonseed oil and injected subcutaneously 3 times daily for 2 consecutive days.‡ On the morning of the third day, the animals were sacrificed and their thymi weighed to the nearest milligram. 4. *Life maintenance and growth.* Adrenalectomized, male rats weighing 50-70 g received 14 daily subcutaneous injections of Meticorten, Meticortelone, cortisone, hydrocortisone and desoxycorticosterone acetate (DCA) suspended in 1 part of ethanol and 9 parts of 50% aqueous propylene glycol. The animals were given tap water and Purina Lab Blox *ad libitum* and were weighed at 3-day intervals. The number of rats dying during each 24-hour period was tabulated until all completely operated animals had succumbed. Animals surviving for more than 3 weeks following the last injection and showing growth rates similar to those of the normal controls were eliminated from the experiment because of probable functional adrenal tissue. 5. *Effects of chronic administration of steroids on growth, organ weights and peripheral blood elements.* The degree of hypercorticism induced by pro-

§ All rats, both intact and adrenalectomized, were obtained from Charles River Breeding Laboratories, Brookline, Mass.

‡ Personal communication from Dr. Stephenson indicating this change in his published procedure.

longed steroid treatment and the reversibility of the resulting changes were studied in intact, growing rats. Suppression of somatic growth was selected as an easily defined physiological response and served as the basis for comparing the effects of the 4 steroids, Meticorten, Meticortelone, cortisone and hydrocortisone. Twenty-two groups of intact, male rats, weighing approximately 125 g, were housed 5 animals to a cage. They were maintained on stock Purina Chow diet and tap water and were weighed daily for the first 11 days and every other day thereafter. Each animal in a given cage received 1 of 5 treatments (Meticorten, Meticortelone, cortisone, hydrocortisone and diluent). After a 5-day observation period, the animals were injected subcutaneously daily for 19 days with saline suspensions of the appropriate steroid, rested for 3 days, and injected again daily for 8 days. The initial dose of 2 mg was adjusted periodically to insure the desired plateaued body weight. The injection schedule is indicated in Fig. 1. A 2-week recovery period was observed after the last injection. At various intervals, groups of animals were withdrawn from the experiment for autopsy. Tail blood was obtained before autopsy for the hematological examination, and an additional blood count was performed during the recovery period.

Results. Preliminary assays by the eosinophil method showed Meticorten to be 3.0 times as potent as cortisone (95% fiducial limits 1.6-5.4). Meticortelone exhibited 3.3 times the activity of hydrocortisone (95% fiducial limits 1.6-6.6). This potency ratio was confirmed by the liver glycogen deposition method (Table I) and by the thymus involution method (Table II). The results of the cross-potency assays were in satisfactory agreement with the values expected from the potency ratios of Meticorten and Meticortelone and their respective standards. In general, the results of the 3 bioassay methods were remarkedly concordant in spite of the relatively large inherent error of the individual assays.

In the life maintenance test (Table III), too, Meticorten and Meticortelone were approximately 4 times as potent as cortisone and hydrocortisone and seemed about as active as DCA. Meticorten and Meticortelone were, however, less effective than DCA in enhancing the body weights of immature, adrenalec-

TABLE I. Summary of Liver Glycogen Deposition Assays of Meticorten and Meticortelone in Adrenalectomized Male Rats.

Reference standard 100%	Substance assayed	Potency (%)	95% fiducial limits	Index of precision (λ)
Cortisone	Meticorten	326	190- 570	.44
		226	102- 457	.42
		260	125- 500	.48
		359	193- 665	.46
	W't'd mean*	286	214- 384	.45
	Meticortelone	1240	810-2120	.32
		829	461-1721	.46
	W't'd mean	1010	702-1456	.40
	Hydrocortisone	179	103- 310	.44
		248	164- 376	.31
	W't'd mean	220	158- 306	.38
Hydrocortisone	Meticortelone	422	225-1670	.30
		386	235- 658	.41
	W't'd mean	395	260- 599	.36
	Meticorten	220	144- 335	.30
		83	32- 165	.48
	W't'd mean	141	52- 380	.39
Meticorten	Meticortelone	231	128- 484	

* Weighted mean.

TABLE II. Summary of Thymus Involution Assays of Meticorten and Meticortelone in Immature Female Rats.

Reference standard 100%	Substance assayed	Potency (%)	95% fiducial limits	Index of precision (λ)
Cortisone	Meticorten	325	217- 577	.258
	Hydrocortisone	314	167- 629	.526
		255	179- 363	.298
	W't'd mean*	269	197- 367	
	Meticortelone	1410	940-2100	.319
		611	391- 956	.344
		900	600-1420	.376
	W't'd mean	923	570-1490	
Hydrocortisone	Meticortelone	164	46- 398	.750
		339	64-2330	1.012
		440	234- 859	.538
	W't'd mean	318	196- 515	
Meticorten	Meticortelone	174	110- 280	.288
		248	167- 359	.347
	W't'd mean	216	160- 292	

* Weighted mean.

TABLE III. Survival of Adrenalectomized, Immature Male Rats Treated for 14 Days with Meticorten, Meticortelone, Cortisone, Hydrocortisone and Desoxycorticosterone Acetate.

Daily dose, μg	Exp. I Treatment*	Exp. I No. of rats	Exp. I Mean survival in days	Exp. II Treatment*	Exp. II No. of rats	Exp. II Mean survival in days
180	DCA	13	21.5 ± 1.20†	DCA	14	21.4 ± .79†
90		18	13.7 ± 1.02		14	13.9 ± 1.19
45		17	9.4 ± .81		13	8.0 ± .76
480	F	11	19.5 ± .58	E	7	15.3 ± 1.26
240		11	14.9 ± .70		13	12.2 ± 1.09
120		10	13.0 ± 1.12		13	9.8 ± 1.09
120	Δ^1F	7	15.6 ± 1.29	Δ^1E	12	18.3 ± .68
60		9	14.1 ± 1.94		12	12.0 ± 1.14
30		10	10.3 ± 1.24		12	8.4 ± .57
15		9	8.0 ± .97			
—	C	5	8.2 ± 1.59	C	13	5.8 ± .44

* Treatment groups are indicated by the following letters: DCA = desoxycorticosterone acetate, F = hydrocortisone, Δ^1F = Meticortelone, E = cortisone, Δ^1E = Meticorten, C = controls.

† Stand. error of mean.

tomized rats. Twelve days after initiation of treatment, animals receiving 90 μg of DCA daily had gained 20 g whereas a daily dose of 120 μg of the 11-oxygenated steroids produced only the following weight increases: cortisone 7 g; hydrocortisone 12 g; Meticorten 17 g; and Meticortelone 12 g.

Changes in body weights observed during and after prolonged steroid treatment are plotted in Fig. 1. The daily dose required to suppress growth in young male rats may be estimated to be 4 mg for cortisone, 2.5 mg for Meticorten and 2 mg for hydrocortisone and Meticortelone. However, the effects of all 4 steroids on body weight were transient. This is clearly evident from the "escape" which occurred during the 3-day rest period and from the prompt resumption of growth at the termination of treatment.

The mean absolute and relative organ weights obtained at different intervals during the experiment are presented in Tables IV and V. All 4 steroids produced striking decreases in the absolute and relative weights of the thymus confirming the thymic involution observed in the short-term experiments described above. Meticorten and Meticortelone also suppressed the absolute and relative weights of the spleen, whereas in the cortisone and hydrocortisone groups, the spleen weights remained proportionate to the body weights. The absolute weights of the adrenals were more than 50% below the control values at the end of the injection period, in contrast to the slight reduction of the relative adrenal weights. All 3 organs resumed growth after cessation of treatment and showed signs of over-compensation 2 weeks after the last injection. The absolute weights of the liver, kidney, testes, seminal vesicles, thyroid and pituitary were only moderately decreased in the treated animals (Table IV), while the relative weights of these tissues actually exceeded those of the controls, with the exception of the seminal vesicles (Table V). In general, Meticorten and Meticortelone exerted a greater effect on organ weights than cortisone and hydrocortisone. Two weeks after cessation of treatment the organ weights had returned to essentially normal levels. Histological examination of the tissues revealed no pathological changes beyond the expected involution of lymphoid tissue and atrophy of the fascicular and reticular zones of the adrenal cortex.

The mean hematological data are presented in Table VI. The most striking change noted in the peripheral blood elements was the rapid and profound decrease in the percent of lymphocytes with the concomitant increase in polymorphonuclear leucocytes in the animals treated with Meticorten and Meticortelone, and to a lesser degree, in the cortisone and hydrocortisone groups. The total number of

TABLE IV. Absolute Organ Weights for Groups of Male Rats Treated with Meticorten, Meticortelone, Cortisone and Hydrocortisone.

Treatment*	Body wt (g)	Spleen (g)	Liver (g)	Kidney (g)	Adrenals (mg)	Testes (g)	Thymus (g)	Seminal vesicles (g)	Pituitary (mg)	Thyroids (mg)
C‡	163 ± 4.37†	.702 ± .139	7.27 ± .221	1.50 ± .005	27.7 ± 1.17	1.99 ± .073	.50 ± .25	.194 ± .018		
△¹E§	149 ±13.7	.482 ± .087	7.95 ± .468	1.71 ± .079	14.4 ± .758	2.43 ± .073	.043 ± .0053	.464 ± .100	9.50 ± .634	
E	193 ± 6.41	.878 ± .064	8.52 ± .461	1.82 ± .040	25.8 ± 4.86	2.44 ± .157	.134 ± .014	.730 ± .056	10.60 ± .52	
F	180 ± 7.08	.716 ± .132	7.66 ± .374	1.80 ± .066	22.6 ± 1.99	2.61 ± .096	.174 ± .014	.436 ± .065	9.62 ± .63	
△¹F	161 ± 7.92	.520 ± .118	7.94 ± .564	1.68 ± .081	20.6 ± 3.29	2.43 ± .114	.114 ± .012	.424 ± .060	8.08 ± .77	
C	237 ± 6.58	1.09 ± .233	9.26 ± .287	1.96 ± .088	33.9 ± 1.94	2.74 ± .104	.520 ± .028	.670 ± .064	11.54 ± 1.68	
△¹E‖	152 ± 8.76	.56 ± .101	8.19 ± .387	1.78 ± .071	14.4 ± 2.49	2.50 ± .039	.078 ± .022	.516 ± .093	9.7 ± 1.80	15.5 ± 2.08
E	210 ±21.29	.80 ± .110	10.10 ± .911	2.14 ± .143	16.9 ± 1.63	2.47 ± .139	.186 ± .001	.658 ± .129	8.0 ± 1.68	15.7 ± 1.76
F	199 ± 3.45	.78 ± .144	9.49 ± .445	1.88 ± .062	15.6 ± 1.50	2.74 ± .092	.117 ± .018	.520 ± .034	6.7 ± .75	16.1 ± 2.67
△¹F	159 ±11.19	.35 ± .050	8.79 ± .797	1.92 ± .146	17.5 ± 1.91	2.60 ± .071	.036 ± .006	.348 ± .034	7.1 ± .45	12.1 ± 1.29
C	287 ± 6.69	1.22 ± .339	11.28 ± .438	2.71 ± .767	33.3 ± 2.71	3.00 ± .047	.286 ± .068	.822 ± .130	9.9 ± .22	19.9 ± 1.06
△¹E¶	240 ± 9.48	1.36 ± .138	10.59 ± .792	2.46 ± .115	30.9 ± 2.19	2.77 ± .060	.504 ± .022	.844 ± .051	10.7 ± .69	19.0 ± 2.70
E	247 ±15.76	1.48 ± .104	10.78 ± .865	2.29 ± .283	39.9 ± 4.44	2.86 ± .233	.542 ± .051	.934 ± .089	10.4 ± .90	17.46 ± 5.57
F	238 ±16.35	1.56 ± .157	9.81 ± .610	2.36 ± .054	30.8 ± 2.12	2.89 ± .177	.514 ± .060	.726 ± .102	9.7 ± .99	20.66 ± 1.55
△¹F	236 ± 8.63	1.82 ± .204	10.90 ± .656	2.42 ± .137	32.3 ± 3.76	2.92 ± .137	.434 ± .048	1.00 ± .066	9.24 ± .54	16.8 ± 1.80
C	312 ±12.14	1.52 ± .158	12.41 ± .557	2.80 ± .143	43.6 ± 3.01	3.13 ± .169	.586 ± .062	1.02 ± .054	12.24 ± .63	21.44 ± 1.19

* Treatment groups are indicated by following letters: C = controls, △¹E = Meticorten, E = cortisone, F = hydrocortisone, △¹F = Meticortelone. † Means and stand. dev. of mean for 10 rats/group on day 5 and for 5 rats/group for subsequent autopsies. ‡ Autopsied at start of inj. § Autopsied on day 21 at end of inj. ‖ Autopsied on day 35 at end of inj. ¶ Autopsied on day 50 at end of 2 wk recovery period.

TABLE V. Organ Weights per 100 g Body Weight for Groups of Male Rats Treated with Meticorten, Meticortelone, Cortisone and Hydrocortisone.

Treatment*	Spleen (g)	Liver (g)	Kidney (g)	Adrenals (mg)	Testes (g)	Thymus (g)	Seminal vesicles (g)	Pituitary (mg)	Thyroids (mg)
C‡	.433 ± .064†	4.47 ± .128	.918± .018	16.9 ± .432	1.26 ± .053	.306 ± .015	.119 ± .008		
△¹E§	.324 ± .047	5.49 ± .471	1.17 ± .073	10.1 ± 1.22	1.72 ± .210	.029 ± .007	.308 ± .063	6.0 ± .61	
E	.523 ± .046	4.40 ± .127	.94 ± .027	10.6 ± 2.85	1.26 ± .068	.069 ± .008	.312 ± .039	5.0 ± .37	
F	.409 ± .103	4.26 ± .113	1.00 ± .040	10.5 ± 1.34	1.47 ± .083	.098 ± .010	.245 ± .039	5.0 ± .31	
△¹F	.326 ± .074	4.99 ± .412	1.06 ± .066	11.5 ± 1.56	1.53 ± .118	.068 ± .007	.258 ± .028	5.0 ± .44	
C	.452 ± .091	3.93 ± .094	.83 ± .027	14.3 ± .735	1.17 ± .056	.222 ± .013	.238 ± .018	5.0 ± .61	
△¹E‖	.39 ± .051	5.44 ± .210	1.18 ± .025	9.6 ± 1.61	1.67 ± .079	.053 ± .014	.35 ± .075	6.2 ± .85	10.4 ± 1.01
E	.38 ± .063	4.80 ± .099	1.03 ± .030	8.2 ± .78	1.20 ± .059	.083 ± .022	.29 ± .046	3.7 ± .62	7.5 ± .66
F	.39 ± .078	4.76 ± .262	.94 ± .039	7.8 ± .75	1.37 ± .042	.057 ± .009	.26 ± .019	3.3 ± .35	8.0 ± 1.19
△¹F	.22 ± .036	5.50 ± .138	1.25 ± .181	11.1 ± 1.02	1.67 ± .079	.022 ± .0027	.22 ± .041	4.6 ± .041	7.9 ± 1.13
C	.42 ± .116	3.95 ± .221	.95 ± .092	11.7 ± 1.04	1.05 ± .031	.118 ± .015	.28 ± .042	3.5 ± .026	6.9 ± .34
△¹E¶	.57 ± .063	4.44 ± .356	1.03 ± .057	12.6 ± .519	1.16 ± .046	.21 ± .014	.35 ± .022	4.5 ± .299	7.9 ± .87
E	.62 ± .086	4.08 ± .190	.92 ± .095	16.2 ± 1.49	1.16 ± .062	.22 ± .019	.37 ± .021	4.2 ± .255	6.6 ± 1.84
F	.67 ± .084	4.10 ± .134	1.01 ± .052	13.0 ± .658	1.21 ± .009	.22 ± .033	.31 ± .050	4.0 ± .340	8.7 ± .50
△¹F	.77 ± .076	4.62 ± .178	1.02 ± .036	13.7 ± 1.34	1.25 ± .081	.18 ± .021	.43 ± .025	3.8 ± .177	7.2 ± .65
C	.49 ± .057	3.97 ± .077	.90 ± .042	14.7 ± .754	1.04 ± .056	.19 ± .019	.34 ± .017	4.1 ± .404	7.1 ± .38

* Treatment groups are indicated by following letters: C = controls, △¹E = Meticorten, E = cortisone, F = hydrocortisone, △¹F = Meticortelone. † Means and stand. dev. of mean for 10 rats/group on day 5 and for 5 rats/group for subsequent autopsies. ‡ Autopsied at start of inj. § Autopsied on day 21 at end of inj. ‖ Autopsied on day 35 at end of inj. ¶ Autopsied on day 50 at end of 2 wk recovery period.

TABLE VI. Changes in Peripheral Blood Elements of Male Rats Treated with Meticorten, Meticortelone, Cortisone and Hydrocortisone.

Treatment*	Hematocrit, %	Total WBC/mm³	Lymphocytes, %	Polymorphonuclear leukocytes
C‡	47.5 ± .864†	20,850 ± 2,083	78.9 ± 1.63	18.6 ± 1.576
Δ^1E§	54.4 ± 1.641	16,520 ± 2,478	46.8 ± 5.19	52.5 ± 4.74
E	59 ± 3.74	15,975 ± 1,669	73 ± 1.64	25 ± 1.80
F	60 ± 4.93	16,750 ± 1,286	66 ± 8.26	32.4 ± 7.94
Δ^1F	51.2 ± 1.11	23,140 ± 6,358	40.4 ± 7.7	58.4 ± 8.14
C	61.5 ± 4.53	19,620 ± 493	79.6 ± 2.34	14.6 ± 1.51
Δ^1E‖	49.6 ± 1.00	21,250 ± 1,448	41 ± 11.94	58.8 ± 10.77
E	50 ††	14,484 ± 1,178	65 ± 7.00	34 ± 7.00
F	50 ††	19,100 ± 4,843	58.4 ± 8.22	50.8 ± 6.98
Δ^1F	51 ††	16,290 ± 2,803	10 ± 1.60	90 ± 1.60
C	49 ††	18,400 ± 1,385	81.2 ± 2.32	16.4 ± 2.35
Δ^1E¶	45.4 ± 1.08	20,870 ± 4,445	73.2 ± 4.77	25.8 ± 4.86
E	47.6 ± 1.46	20,110 ± 2,669	80.4 ± 2.81	17.6 ± 1.95
F	49.6 ± .61	14,190 ± 3,390	58.4 ± 10.25	39.6 ± 10.36
Δ^1F	45.4 ± .73	13,420 ± 1,336	60.6 ± 6.60	38.4 ± 7.11
C	49.6 ± .54	20,370 ± 1,949	78.6 ± 4.72	20.4 ± 4.53
Δ^1E**	46.6 ± 1.91	15,340 ± 2,636	79.4 ± 4.12	19.2 ± 3.99
E	53.6 ± 1.89	17,390 ± 1,765	80.4 ± 5.35	17.6 ± 4.93
F	52.4 ± 1.25	15,980 ± 1,502	79.6 ± 4.28	20.4 ± 4.41
Δ^1F	53.0 ± 1.72	16,500 ± 1,909	63.8 ± 4.54	33.8 ± 4.22
C	49.4 ± .36	20,080 ± 6,444	79.2 ± 3.90	18.6 ± 3.39

* Treatment groups are indicated by following letters: C = controls, Δ^1E = Meticorten, E = cortisone, F = hydrocortisone, Δ^1F = Meticortelone.

† Means and stand. dev. of mean for 10 rats/group on day 5 and for 5 rats/group for subsequent bleedings.

‡ Bled at start of injections. § Bled on day 21. ‖ Bled on day 35 at end of inj. ¶ Bled on day 42 one wk after last inj. ** Bled on day 50 2 wk after last inj. †† Means for less than 5 rats.

leukocytes was variable, and the hematocrit did not show any outstanding or consistent changes. It is of interest that the inverted lymphocyte/polymorphonuclear leukocyte ratio began to return towards normal values 1 week after cessation of treatment. Two weeks after the last injection, all but the Meticorten-treated animals had normal blood counts.

Discussion. The introduction of a second double bond in the A ring of cortisone and hydrocortisone produced a 3- to 4-fold increase in the glucocorticoid activity of Meticorten and Meticortelone. Since completion of our experiments, similar findings have been reported for Meticortelone by Stafford *et al.* (14) and for Meticorten by Ferrari *et al.*(15). Increases in glucocorticoid activity also were observed in this laboratory for Δ^1-corticosterone and Δ^1-desoxycorticosterone. Compared to cortisone (100%) by the liver glycogen assay method, the following activity ratios were found: Δ^1-corticosterone, 294% (95% fiducial limits 197-440); corticosterone, 119% (80-177); Δ^1-desoxycorticosterone, 7.8% (4.9-12.4); desoxycorticosterone, 1.8% (1.1-2.7). Potentiation of the biological activity of compounds possessing the $\Delta^{1,4}$-configuration had been noted previously by Huggins *et al.*(16) in the androstadiene series. They found $\Delta^{1,4}$-androstadiene-3, 17-dione more effective in promoting uterine growth in hypophysectomized rats than either the Δ^1 or Δ^4 analogues.

Unsaturation in both the A and B rings of cortisone apparently does not enhance glucocorticoid activity. Thus the Δ^6 analogue of cortisone acetate was found in this laboratory to possess only 53% (fiducial limits 39-78) of the activity of cortisone acetate in the liver glycogen assay. This observation concurs with the studies of this compound by Higgins *et al.*(17).

Glucocorticoid activity greatly exceeding that of the natural hormones and of Meticorten and Meticortelone has been demonstrated for 9α-halogenated steroids(18), and for steroids methylated at C_2 or possessing both

modifications(19). However, both types of chemical alterations, alone or in combination, resulted in greatly enhanced mineralocorticoid potency(19,20) whereas Δ^1-dehydrogenation did not affect electrolyte activity. In unpublished experiments in this laboratory, it was found that Meticorten and Meticortelone failed to produce sodium retention in adrenalectomized rats. Similar findings have been reported recently for both steroids(14,21) as well as for Δ^1-desoxycorticosterone(22). Likewise, the introduction of an additional double bond between C_1 and C_2 in 9α-fluorohydrocortisone greatly enhanced glucocorticoid but not mineralocorticoid activity(14, 23).

Meticorten and Meticortelone were more active than their parent substances in maintaining the life of young, adrenalectomized rats (Table III). This observation is at variance with that of Swingle¶ who found that Meticorten and Meticortelone had only one-fourth the activity of cortisone and hydrocortisone in maintaining adrenalectomized dogs. Similar species differences between rats and dogs have been reported in life maintenance studies with 9α-halogenated corticoids(20, 24). It will be noted that the daily amounts of steroids required to maintain our rats were considerably higher than the doses of cortisone used by Leatham and Wolf(25). This difference may be attributed to the high protein diet employed by these investigators which is known to have a beneficial effect on the survival of adrenalectomized rats.

The manifestations of hypercorticism induced by prolonged steroid administrations to intact rats were more marked in the Meticorten and Meticortelone groups than in the animals treated with cortisone and hydrocortisone. The suppression of growth and the changes in organ weight during treatment and their subsequent return to normal values were in general agreement with the findings obtained with cortisone acetate(26-28) and with Meticorten(15). The fact that in the present study the relative adrenal weights were not suppressed to the same extent as reported in the literature may be attributed to differences in dose, length of treatment, initial body weight of the animals and to the pair-feeding of the controls carried out by most investigators.

Higgins and co-workers(17) noted a fall in the total leukocyte count of 10,300 cells per cu mm during cortisone treatment, the control count being in the neighborhood of 16,000. As shown in Table VI, the total white cell count of our controls ranged from 18,400 to 20,840; and the lowest count observed in the treated rats was 13,420 in the Meticortelone group 1 week after the last injection. No explanation for this discrepancy can be offered.

Summary. (1) Meticorten and Meticortelone, 2 new steroids, were studied in animals to assess their adrenocortical activities. (2) Glucocorticoid properties were assayed by the eosinophil response in adrenalectomized mice, by the liver glycogen deposition in adrenalectomized rats, and by the involution of the thymus in immature rats. (3) By all 3 methods, the glucocorticoid activity of Meticorten and Meticortelone was found to be 3 to 4 times greater than that of cortisone and hydrocortisone. (4) Meticorten and Meticortelone were more active than cortisone and hydrocortisone in maintaining the life of immature, male, adrenalectomized rats. (5) Prolonged administration of Meticorten, Meticortelone, cortisone and hydrocortisone to young, male rats produced the following biological changes: a) suppression of somatic growth, b) decrease in thymus, spleen and adrenal weights on an absolute basis as well as per 100 g body weight, c) increase in the relative weights of liver, kidney, testes, thyroid and pituitary and inconsistent changes in the weight of the seminal vesicles, d) inversion of the lymphocyte/polymorphonuclear leukocyte ratio in the circulating blood without consistent changes in total white cell count or hematocrit. (6) Meticorten and Meticortelone were more potent than cortisone and hydrocortisone in affecting organ weights and peripheral blood elements. (7) The changes induced by prolonged administration of the 4 steroids were readily reversible after cessa-

¶ We wish to thank Dr. Swingle for making the results of his study available.

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Received April 24, 1956. P.S.E.B.M., 1956, v92.

Distribution of Fetal Hemoglobin in Layers by Centrifugation. (22430)

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Hemoglobin of the fetal blood is of 2 varieties, that can be differentiated by the alkali denaturation technic(1). At birth, 50 to 90% of hemoglobin is of the alkali resistant fetal type, while the remainder is of the normal adult type(2). It is not known, however, whether fetal and adult hemoglobins are distributed evenly inside the fetal red blood cells. In order to study this problem washed erythrocytes of the cord blood were centrifuged and fetal hemoglobin concentrations of the upper and lowest layers were compared.

Material and method. Two samples of blood, 20 cc each, were taken from the cord of 29 normal newborns and added to 0.05 cc of sodium heparin (Abbott, in which 10 mg