TABLE III. Influence of Hog Serum Fractions II, IV, V and IV + V on Growth of Hog Kidney Epithelial Cells in Tissue Culture.

Hog serum fractions*	% of cultures exhibiting various degrees of growth				
	0	1+	2+	3+	4+
II	100	0	0	0	0
IV	11	44	45	0	0
V	43	28	29	0	0
IV + V	0	0	0	0	100
Whole serum	12	13	37	38	0

* In synthetic mixture #199.

vation of hog kidney epithelial cells were determined. The data indicate that cultures grown in a "simple" medium containing Synthetic Mixture #199 (92.5%) and hog serum (7.5%) were consistently more proliferative than cultures cultivated in more complex media containing embryo extract, serum ultrafiltrate and serum of homologous and heterologous origin. 2. None of the media tested adequately supported growth of either splenic or testicular fibroblasts. 3. The influence of various Porcine Serum Fractions (II, IV, V and IV + V) on growth of hog kidney epithelial cells was also investigated. When Fraction IV + V was used, excellent growth was achieved. When Fraction II, IV or V was used independently, growth inferior to that of whole serum resulted.

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Some Metabolic Effects of $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione (Meticorten) Administered Orally to Dogs. (22324)

PRESTON L. PERLMAN, JAMES W. CASSIDY, AND ALIDE TALI.

Biochemistry Department, Schering Corp., Bloomfield, N. J.

The discovery of the glucocorticoid and anti-inflammatory activities of Meticorten ($\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione) in experimental animals(1,2) and of its anti-rheumatic activity in human subjects(3) prompted studies on the metabolic actions of this steroid. Most experimental animal studies with Meticorten have utilized mice or rats. There are, to our knowledge, no published studies of the effects in dogs of large doses of Meticorten on water and nitrogen balance, or of the effects on blood sugar level or on the glucose tolerance curve before and during prolonged oral treatment. The production of a marked polyuria and polydipsia by daily *subcutaneous* treatment of dogs with 10 mg/kg of cortisone has been reported(4). Dogs given the same dose orally did not exhibit these effects. Also, it has been noted(5) that daily intramuscular injections of cortisone (50-300 mg/day) to dogs produced polydipsia and polyuria but there were no changes in fasting blood sugar levels or on the glucose tolerance curves. More recent studies with

dogs(6) have confirmed the observations on the absence of effect of cortisone on blood sugar level and also showed a small but apparently significant elevation of R.Q., a decrease in basal metabolic rate, and an increased diuresis and nitrogen excretion during treatment.

Methods and materials. Four intact male beagles were used in this study. Each animal was conditioned to a separate metabolism cage for the entire period of the experiment and was housed under the same conditions for at least 6 months prior to the study. The feeding regimen, diet composition (high nitrogen), and the methods for evaluating nitrogen-balance status have been described previously(7). Meticorten was administered orally, once a day, in a gelatin capsule. Water intake, urine output and specific gravity were measured each morning, and tests for glycosuria and albuminuria were run on aliquots of each 24-hour urine collection. Fasting blood sugar samples were analyzed by a standard method. Oral glucose tolerance tests were run by administering 3.0 g glucose /kg body weight via stomach tube and analyzing blood samples for glucose at 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 hours. The dosage regimen in this study was one in which the steroid dose was increased at intervals until consistent polyuria and polydipsia were obtained and then a gradual reduction of dose was instituted until these changes had disappeared.

Results. Table I summarizes certain of the data obtained with the 4 dogs but does not indicate the rate at which the various changes occur. Fig. 1 and 2 provide some indication of the responses over a period of time and at increasing or decreasing dosage. These figures, representing data for dog No. 18, are quite similar to results obtained with the 3 other dogs used in this study.

It is evident from Table I that with a large dose of steroid it was possible to produce a marked polydipsia and polyuria in all 4 dogs, an increased urinary nitrogen excretion, a decrease in urine specific gravity, and a complete or almost complete eosinopenia. It is also apparent that these effects continued to manifest themselves even as the dose was reduced from the highest level (250 mg/day) to doses of 50-75 mg (Fig. 1).

TABLE I. Effect of Oral Meticorten Treatment on Blood Sugar, Water and Nitrogen Balance.

	Dog No. 17	18	31	32
Before treatment (control)*				
Wt, kg	11.6	10.3	7.6	8.0
N, g N/kg/day	E¶	−.07	−.04	E
Water intake, cc†	200	540	520	800
Urine, cc†	200	260	325	420
Specific gravity	1035	1040	1032	1030
Blood sugar, mg %	55	59	62	67
During treatment‡				
Wt, kg	10.8	9.5	6.5	7.2
N, g N/kg/day	−.35	−.27	−.38	−.45
Water intake, cc	1400	1500	1400	1500
Urine, cc	1400	1610	1250	1620
Specific gravity	1022	1010	1012	1008
Blood sugar, mg %	61.2	59	65	53
Max Meticorten dose, mg/day	250	250	250	250
% fall in eosinophils§	100	100	100	100
After treatment‖				
Wt, kg	11.2	9.5	7.0	7.4
N, g N/kg/day	+.04	+.02	+.08	E
Water intake, cc	100	500	410	250
Urine, cc	100	380	315	150
Specific gravity	1035	1035	1040	1025
Blood sugar, mg %	59	71	55	59

* Twelve-day control period.
† Maximum 24-hr value.
‡ Avg values at maximum dose. Period at this dose 12 days for dogs 18, 31 and 32, and 21 days for dog 17.
§ Compared to "Before treatment (control)."
‖ Post-treatment period 17 days for dogs 18 and 31, and 3 days for dogs 17 and 32.
¶ E = equil.

At no time during these experiments did any animal exhibit hypo- or hyperglycemia, glycosuria or albuminuria. Glucose tolerance curves obtained before and during treatment at the maximum dose level (250 mg/day) were normal in every instance.

We have also confirmed (unpublished observations) the published observations on the effects of cortisone on water metabolism. In our experience, the minimal doses which produce significant eosinopenia do not necessarily produce negative nitrogen balance or polydipsia and polyuria. Similarly, larger doses which produce eosinopenia and negative nitrogen balance do not cause polydipsia or polyuria when the dose is not high (100-150 mg/day). However, with still larger cortisone doses (200-300 mg/day) all these effects usually can be produced, although we

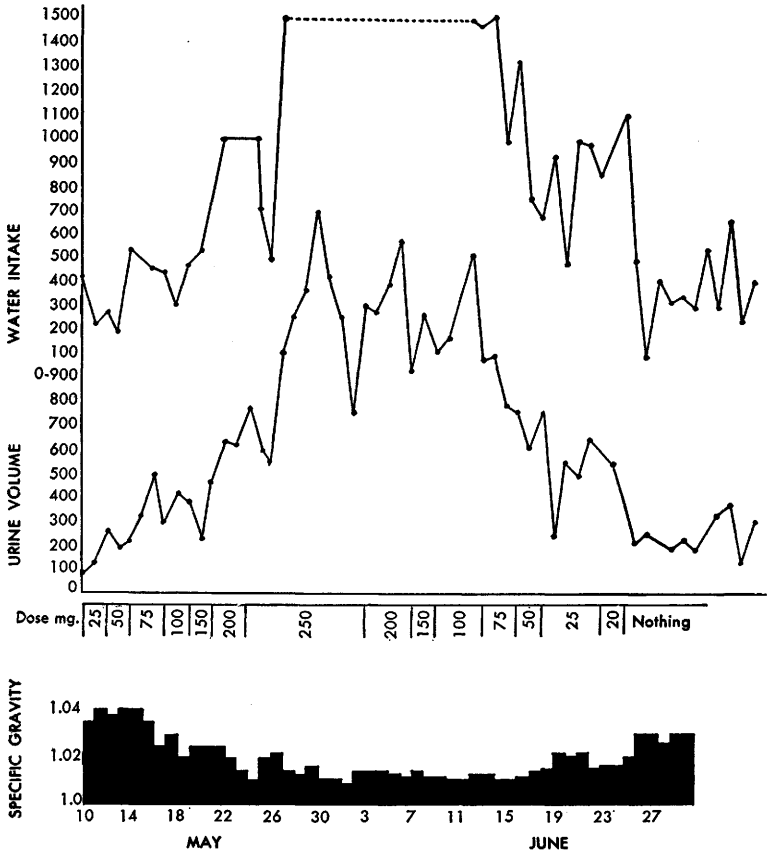

FIG. 1. Effect of Meticorten on water balance and urinary specific gravity in normal dog 18.

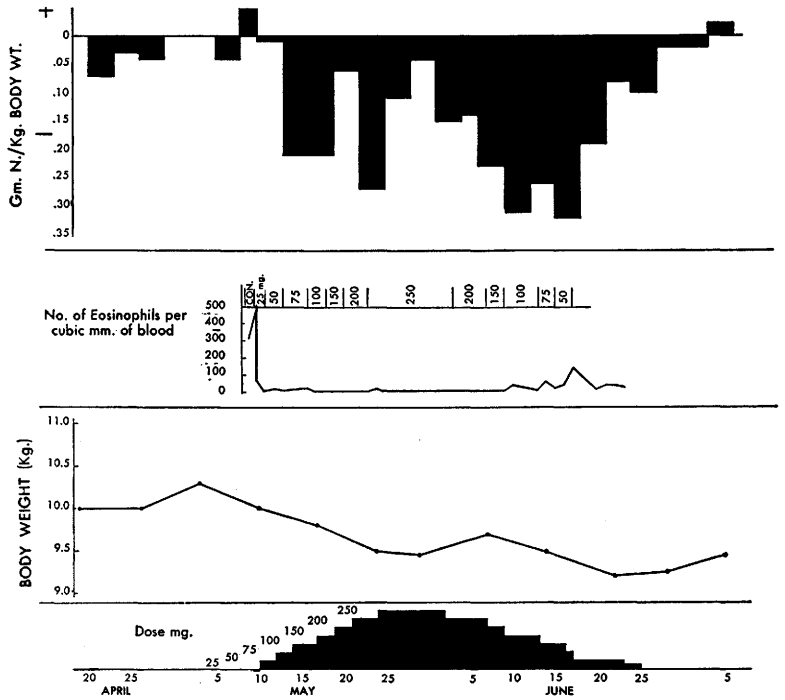

FIG. 2. Effect of Meticorten on nitrogen balance, body wt and eosinophil level in normal dog 18.

have had experience with one male dog which, even at doses producing polydipsia and polyuria as well as negative nitrogen balance, exhibited no change in circulating eosinophil level. Also the sequence in which the various changes (eosinopenia, negative nitrogen balance, polydipsia and polyuria) usually occur would suggest a differential sensitivity of each of these responses to dose.

Summary. 1. In dogs large, prolonged, oral doses of Meticorten induced polydipsia, polyuria, and negative nitrogen balance. 2. Blood glucose levels and glucose tolerance curves were unaffected by the treatment, and no evidence of albuminuria and glycosuria was obtained.

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Protective Action of Glutamine, Cysteine and Other Substances Against Experimental Lathyrism in the Rat. (22325)

WALDEMAR DASLER. (Introduced by Richard G. Roberts.)

Department of Biochemistry, Chicago Medical School, Chicago 12, Ill.

Although the toxic action of sweet peas, *Lathyrus odoratus*, is known to be due to β-aminopropionitrile (occurring as its γ-glutamyl derivative in the sweet pea)(1,2,3), the specific metabolic disturbance caused in the rat by the ingestion of this substance is still undetermined. It is to be expected that discovery of a specific compound having a protective action against sweet pea toxin will aid in revealing the metabolic site at which the toxin acts. The finding of a protective agent may also be of considerable medical importance because of the possible relationship between odoratism (sweet pea lathyrism) and a number of human diseases of unknown etiology(4,5).

Methods. Male, albino, weanling rats, 24-28 days of age and weighing 48-64 g were used in all experiments. All rats were Sprague-Dawley (Holtzman) strain. Because all young rats receiving substantial amounts of sweet pea meal in their diets rapidly develop lathyrism without exception, screening tests for protective agents can be conducted with a relatively small number of rats. For screening purposes, therefore, supplements were usually fed to 3, but sometimes 2, rats at each level. *Diets.* In a few of the earlier experiments the basal diet contained 50% sweet pea meal* and 25% corn starch and was made up as previously described(6). In most of the screening tests, however, the basal diet contained 30% sweet pea meal and 45% corn starch. Supplements were incorporated by replacing an equivalent amount of starch. All diets contained 10% casein (not vitamin-free) except as noted below. In a few instances supplements were added to Rockland Rat Diet containing 0.1% of synthetic β-aminopropionitrile hydrochloride.† Since high levels of casein exhibit some protective action(8,9, 10) the following device was used with supplements which appeared to have anti-lathyrismic properties. Five per cent of supplement was incorporated into a diet containing 30%

* Sweet peas were generously supplied by Mr. Raymond H. Coulter of Ferry-Morse Seed Co.

† β-aminopropionitrile was synthesized by the method of Buc(7), then converted to hydrochloride and crystallized from 95% alcohol.