

assay in serum, a variance in rate of steroid conjugation may be implicated in the findings with this 6-methyl derivative of prednisolone. A study of free 17-OHCS levels in urine under comparable experimental conditions is being made to pursue this possibility further.

As reported earlier(1,2,9,10), triamcinolone does not give a Porter-Silber reaction. Thus the drop in measurable 17-OHCS signifies suppression of endogenous adrenocortical secretion.

**Summary.** The influence of small oral doses of hydrocortisone analogs on serum and urine 17-hydroxycorticosteroids was studied in 281 human subjects. With the exception of medrol, the drugs produced changes in 17-OHCS which tended to be parallel in serum and urine. The drugs differed greatly in response produced.

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## Influence of Body Weight and Growth Rate on Nitrogen and Electrolyte Excretion in Rats.\* (26414)

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Fasting weight loss in growing rats decreases with age and, therefore, with size(1). Weight loss is probably also a function of the higher growth rates of the younger rats. The resultant of these forces (growth rate and attained body weight) should be reflected in excretion rates of nitrogenous compounds and electrolytes. This possibility was examined with young male rats of different body weights and growth rates as subjects. The influence of differences in food intake was minimized by making the comparison on each day of a 3-day fast.

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**Experimental.** Twelve Sherman strain male rats between 67 and 70 days old (220 g) and 12 between 110 and 113 days old (265 g) constituted the light and heavy groups (L and H), respectively. All were raised in an animal room at 24°C. In the 2-week period before the experiment, Group L rats were gaining at the average rate of 3.0 g/day with food intake averaging 21.2 g/day (69.7 g/kg<sup>3/4</sup> body weight), while Group H rats were gaining at a much slower rate of .36 g/day with a food intake of 19.0 g/day (60.9 g/kg<sup>3/4</sup> body weight). The rats were placed into individual plastic-coated metabolism cages for 6 days (24°C). For the first 3 days food (Purina Dog Chow) and water were furnished *ad libitum* to accustom the animals to the cages. During the last 3

days they received only water. Urine samples (24-hour) were collected into acid on the 3 fasting days. These were pooled at random in each group into 4 samples from the 3 rats each. Clean cages were substituted daily. These samples were analyzed

for 9 constituents by methods previously cited(2).

*Results and Discussion.* Significant overall group differences (including group  $\times$  day interactions) occurred with most variables (Tables 1, 2, 3). As others have observed

TABLE I. Influence of Body Weight on Physiological Changes of Rats during Fasting. General variables.\*

Variable	Wt group	Days (means) fasted			Error terms		Pertinent significant "F" ratios		
		1	2	3	$\sqrt{MSE_1}$	$\sqrt{MSE_2}$	bg	bd	$g \times d$
Body wt (g)	L	198	191	171	14.7	3.997	55.	82.6	N.S.
	H	244	231	220					
Wt loss (g/24 hr)	L	24	7	20	3.56	8.12	N.S.	4.58	"
	H	21	13	11					
Wt loss (% of initial wt)	L	10.6	3.7	10.3	1.435	3.788	29.	N.S.	"
	H	8.0	5.3	4.7					
Water intake (ml/24 hr)	L	21	12	7	14.10	6.488	N.S.	9.84	"
	H	23	18	8					
Urine vol (ml/24 hr)	L	14	5	.5	9.772	6.738	"	7.57	"
	H	14	7	.8					
Ratio: Urine/Water	L	.69	.47	.06	.210	.214	"	12.	"
	H	.50	.32	.09					

\* Means of 2 wt groups (light, L, and heavy, H) for 3 consecutive days of fasting are presented. Only significant "F" ratios are presented (N.S. = not significant; N.A. = not applicable, as where there was a significant interaction).  $\sqrt{MSE_1}$  = mean square between subjects in the same group used to test between group differences;  $\sqrt{MSE_2}$  = mean square pooled subjects  $\times$  days interaction used to test the between day differences and the group  $\times$  day interaction. Individual group means may be compared on the same day by Cochran's approximate t-test(3, p. 272).

TABLE II. Influence of Body Weight on 24-Hour Electrolyte Excretion by Rats during Fasting (meq).\*

Variable	Wt group	Days (means) fasted			Error terms		Pertinent significant "F" ratios		
		1	2	3	$\sqrt{MSE_1}$	$\sqrt{MSE_2}$	bg	bd	$g \times d$
Sodium	L	1.02	.41	.24	.0539	.0601	N.A.	N.A.	27.
	H	.65†	.38	.29					
Potassium	L	1.31	.70	.78	.1276	.0802	N.S.	127.	N.S.
	H	1.25	.68	.79					
Ratio: Na/K	L	.77	.59	.31	.0596	.07006	N.A.	N.A.	10.2
	H	.52†	.55	.37					
Magnesium	L	.21	.34	.24	.0386	.0293	"	"	7.44
	H	.14†	.30†	.23					
Calcium	L	.091	.070	.083	.00775	.00424	"	"	8.89
	H	.086	.082†	.086					
Ratio: Mg/Ca	L	2.3	5.0	2.9	.4808	.4130	"	"	4.92
	H	1.7†	3.6†	2.7					
Phosphate (as P)	L	1.18	1.20	1.16	.0566	.0245	14.	N.S.	N.S.
	H	.94†	.90†	.88†					
Ratio: Ca/P	L	.078	.060	.073	.0172	.00866	12.	"	"
	H	.092†	.093†	.098†					

\* See footnote of Table I.

† Significant group difference (Cochran's approximate t-test(3, p. 272).

TABLE III. Influence of Body Weight on 24-Hour Excretion of Nitrogenous Compounds by Rats during Fasting (mg).\*

Variable	Wt group	Days (means) fasted			Error terms		Pertinent significant "F" ratios		
		1	2	3	$\sqrt{MSE_1}$	$\sqrt{MSE_2}$	bg	bd	$g \times d$
Urea	L	517	408	317	47.2	24.7	N.A.	N.A.	5.78
	H	546	353†	310					
Uric acid	L	2.4	2.6	2.3	.369	.158	N.S.	14.	N.S.
	H	2.1	2.4	2.0					
Creatinine	L	6.4	6.6	6.5	.541	.275	N.A.	N.A.	11.
	H	6.8	8.3†	7.7†					
Ratio: Uric acid/ Creatinine	L	.38	.36	.31	.0452	.0226	21.	18.	N.S.
	H	.31†	.28†	.26†					
Histidine	L	3.4	2.4	1.6	1.72	1.46	N.S.	37.	"
	H	2.8	2.0	1.7					

\* † See footnotes of Table II.

(1), the lighter (and younger) rats lost more weight during the fast than the heavier ones, and they excreted slightly less total calcium (4) over the 3 days. Since they excreted more phosphate, their Ca/P ratios also were lower. The lighter rats excreted more magnesium, and the slight retention of calcium (mentioned above) becomes quite apparent in the elevated Mg/Ca ratios. The heavier rats retained more sodium on day 1 of the fast; this was also reflected in the Na/K ratio.

Since the younger rats were eating more, higher total loss of urea was to be expected. Likewise, their smaller muscle mass as well

as their higher growth rate could explain their lower creatinine excretion. Also, since mean uric acid excretion was slightly elevated each day in these lighter rats, their uric acid/creatinine ratios were uniformly higher.

These differential day-to-day changes are illustrated in Fig. 1, where various ratios of heavier to lighter rats are presented. On succeeding fast days ratios for sodium and magnesium increased in near-linear manner from their initial low values. Calcium also was low initially, but increased temporarily on Day-2 to return nearly to unity by Day-3. The creatinine ratio started high and increased further on Day-2. Although it decreased slightly on Day-3, it remained higher than the initial value. Urea on the other hand with the same initial ratio as creatinine fell on Day-2 and returned to unity on Day-3.

It may appear that excretion rates should be equalized by expressing the results on a body weight basis. Expressed on this basis, the group differences become quite large with variables such as magnesium, where the lighter group excreted more than the heavier rats, but they are minimized with variables such as phosphate where the converse is the case. Correlations of body weight with urinary variables are essentially unknown. In an earlier study with adult rats(5) correlations with body weight on the whole were quite low and many were not even significant (e.g., sodium,

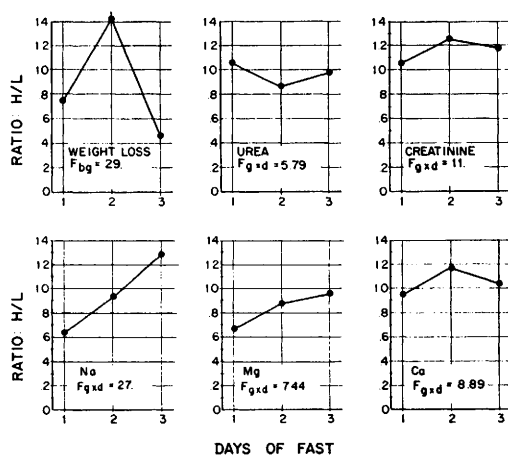


FIG. 1. Differential changes in fasting excretion rates of young male rats as a function of body wt and growth rate. Each value expressed as ratio of heavier to lighter rats; pertinent F-ratios (analysis of variance) are presented:  $F_{bg}$  = between the 2 groups, and  $F_{g \times d}$  = interaction.

calcium, phosphate, uric acid and creatinine). Furthermore, body weight decreases on succeeding days of the fast and possibly the intercorrelations also change. It does not seem justifiable, therefore, to express excretion rates of fasting rats on a body weight basis until much more information is available.

**Conclusion.** Young male rats that differed in body weight by only about 20% had significant ( $p \leq .05$ ) metabolic differences during a 3-day fast. These included calcium, phosphate, urea, creatinine, the Na/K, Mg/Ca, Ca/PO<sub>4</sub> and uric acid/creatinine ratios. There was an almost 10-fold difference in their respective growth rates, however, which undoubtedly contributed to the differences.

These group differences tended to disappear in almost all variables as the fast progressed. The various ratios proved to be quite sensitive to the group differences.

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### A Hemolytic Phenomenon in Ewes Requiring *Leptospira pomona* Antigen and Antibody.\* (26415)

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The properties of *Leptospira pomona* responsible for the hemoglobinemia and hemoglobinuria observed in field and experimental infections of cattle and sheep have not been defined(1,2,3). *In vitro* studies have revealed the presence of hemolytic factors in the culture supernatant fluid(4,5,6). Little hemolytic activity was observed in preparations of washed disrupted cells(4,6). The hemolytic factor was thermolabile (inactivated at 56°C in 10 minutes) inhibited by specific antiserum, independent of complement and most active against erythrocytes of ruminants. Intravenous administration of the material produced marked and fatal hemolysis and hemoglobinuria in nonimmune lambs(7,8). Specific *L. pomona* antibodies

inhibited hemolysis and protected lambs(7). The similarity of the effect of the hemolytic factor in lambs to the syndrome observed following active *L. pomona* infections has been reported(8).

During experimental infections in cattle and sheep, *L. pomona* has been isolated from the blood stream after the appearance of detectable specific antibodies. Ferguson *et al.* (9) suggested that a hemolytic toxin was released from the leptospirae by the effect of a lytic antibody. Morse *et al.*(3) suggested a similar phenomenon as the cause of hemolytic anemia in experimental *L. pomona* infections of sheep.

Hemolysis and hemoglobinuria in cattle and sheep have occurred after the appearance of specific antibodies. Inhibition by specific leptospiral antibodies of the hemolysis from culture supernatant fluids suggests that another factor is responsible for hemolysis in actual infections. This preliminary investigation was undertaken to delineate the relation

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