to free lungworm-bound influenza virus. Aqueous extracts of *Ascaris* also stimulate increased leucinamidase to provoke the virus (5). This eliminates tissue damage by migrating larvae as necessary to provoke influenza infection.

No tests have been made on serums from pigs infected experimentally with pathogenic bacteria or leptospira. These obviously must be made.

Enzyme activity is a sensitive health indicator. In our laboratory, 2-3-day-old pigs, infected with *Ascaris* larvae, show a rise followed by a partial decline in leucinamidase activity when the larvae pass through the intestine, when they pass through the liver (most marked), and again when they pass through the lungs. Established base lines of serum enzyme activity might be useful indicators of health in other species. Amino acid amidase activity might indicate latent infections or parasitism which would eliminate potential hosts from experiments that would be voided by a complex etiology.

Summary. A moderate increase in serum leucinamidase activity occurred in colostrum-deprived pathogen-free pigs experimentally infected with swine influenza virus. Activity also increased during migration of *Ascaris suum* larvae through the liver and again when the larvae reached the lungs. Greatest leucinamidase activity was stimulated when influenza virus was provoked from its latent lungworm state by migrating *A. suum* larvae.

1. Sabina, L. R., Young, G. A., *Virology,* 1959, v9, 480.

2. Sabina, L. R., Ph.D. Thesis, Univ. of Nebraska, Aug., 1960.

3. Young, G. A., Hinz, R. W., Underdahl, N. R., *Am. J. Vet. Res.,* 1955, v16, 123.

4. Shope, R. E., *J. Exp. Med.,* 1941, v74, 41.

5. Sen, H. G., Kelley, G. W., Underdahl, N. R., Young, G. A., *ibid.,* 1961, v113, 517.

6. Underdahl, N. R., Kelley, G. W., *J. Am. Vet. Med. Assn.,* 1957, v130, 173.

7. Underdahl, N. R., *ibid.,* 1958, v133, 380.

8. Sabina, L. R., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 824.

Received January 12, 1961. P.S.E.B.M., 1961, v106.

Serum and Urine 17-Hydroxycorticosteroid Levels Following Small Oral Doses of Synthetic Analogs of Hydrocortisone.* (26413)

JOHN R. PRIGMORE AND IRA L. SHANNON
School of Aviation Medicine, USAF, Brooks AFB, Texas

Earlier reports from these laboratories enumerated changes in body fluid 17-hydroxycorticosteroid (17-OHCS) levels following oral administration of synthetic analogs of hydrocortisone(1,2). Parotid fluid steroid findings have reliably reflected serum and urine results. However, the *magnitude* of steroid response differed with each drug, even though all doses were 50 mg. The present study was designed to investigate this variability in steroid response by employing smaller doses of hydrocortisone analogs.

Materials and methods. Volunteer subjects were 281 young adult male airmen, all judged physically qualified for military service by recent medical examination. Diet, environmental exposure, times of arising and retiring, and other conditions were virtually identical in all instances. Each day, 16 subjects were randomly divided into experimental and control groups, and these groups further divided into 4-hour and 7-hour groups. At 0800, blood was collected by venipuncture and the serum retained. A force-voided urine specimen was discarded. A second blood sample was collected at 1200 or 1500 hours, *i.e.*, either 4 or 7 hours after the initial sampling. Urine was collected over the respective 4 or 7-hour periods. The drugs

* The opinions expressed herein are those of the authors and are not to be construed as representing USAF policy.

TABLE I. Free 17-Hydroxycorticosteroid Levels in Serum Following Oral Administration of Synthetic Analogs of Hydrocortisone.

	0800-1200 hr					0800-1500 hr				
		Free 17-OHCS, γ %					Free 17-OHCS, γ %			
Drug	No.	Time	Mean	S.D.	P	No.	Time	Mean	S.D.	P
Control	57	0800	13.78	4.86	—	58	0800	12.02	4.37	—
		1200	12.50	5.63			1500	10.44	4.63	
Prednisolone, 10 mg	12	0800	11.32	2.82	<.01	16	0800	14.31	2.88	N.S.
		1200	18.62	4.12			1500	12.16	3.64	
Decadron, 2.5 mg	20	0800	11.83	4.08	<.01	18	0800	12.37	3.97	<.01
		1200	6.67	3.02			1500	4.07	2.24	
Triamcinolone, 10 mg	12	0800	11.44	4.00	<.01	16	0800	15.01	6.04	<.01
		1200	3.72	2.64			1500	3.01	2.19	
Medrol, 10 mg	19	0800	12.17	3.08	N.S.	19	0800	13.84	4.23	<.01
		1200	10.02	4.40			1500	6.15	2.70	
2-CH_3-F-F, 1.5 mg	19	0800	13.98	4.61	<.01	15	0800	12.24	3.80	<.01
		1200	6.39	2.71			1500	3.31	2.04	

under study were administered orally immediately after the initial blood sampling. The compounds† and relatively comparable dosages employed were as follows: prednisolone (delta-1 hydrocortisone) 10 mg; medrol (delta-1, 6-methyl hydrocortisone) 10 mg; triamcinolone (delta-1, 9-alpha-fluoro, 16-hydroxy hydrocortisone) 10 mg; decadron (delta-1, 9-alpha-fluoro, 16-methyl hydrocortisone) 2.5 mg; and 2-methyl, 9-alpha-fluoro-hydrocortisone (2-CH_3-F-F) 1.5 mg.

Concentration of free 17-OHCS in serum was determined by the method of Peterson *et al.*(3), utilizing a methylene chloride extraction procedure. Total 17-OHCS levels in urine were determined by a modification combining the butanol extraction methods of Reddy(4) and Clayson(5). Both blood and urine technics are based upon the Porter-Silber(6) color reaction between 17, 21-dihydroxy-20-ketosteroids and phenylhydrazine in sulfuric acid.

Results and discussion. The effect of the drugs on the free 17-OHCS concentration in serum is shown in Table I. Serum steroid values at 0800 hours were relatively constant throughout the experiment, as were control values at 1200 and 1500 hours. Decadron, triamcinolone, and 2-CH_3-F-F dosage prompted highly significant decreases at 1200 and 1500 hours in the concentration of the free steroids in serum. Prednisolone produced a significant steroid increase at 1200 and a return to normal level at 1500 hours. Administration of medrol had little effect at 1200; however, a significant decrease in steroid concentration was observed at 1500. There was considerable variation between drug groups in magnitude of response, ranging from a decrease of 12.00 γ % at 1500 for triamcinolone to an increase of 7.30 γ % at 1200 for prednisolone.

Data on urinary excretion of total 17-OHCS are presented in Table II. Prednisolone and medrol administration prompted marked increases in urine steroid levels during the 4-hour and 7-hour collection periods. Following decadron and triamcinolone dosage 17-OHCS values were not significantly different from control values. A highly significant decrease in urinary steroid excretion rate was observed following dosage with 2-CH_3-F-F.

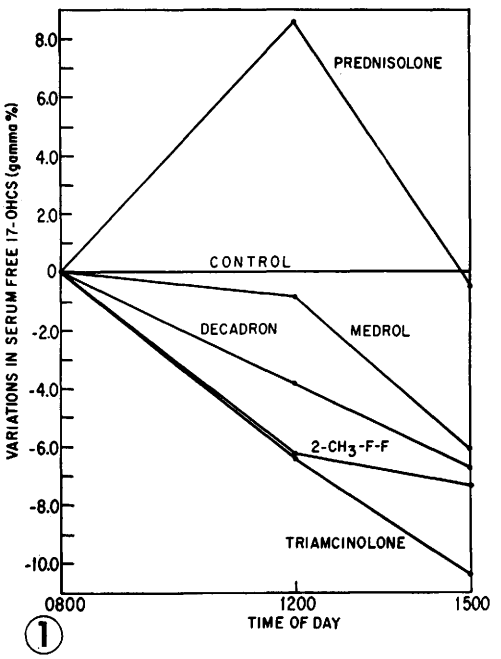
Relative differences in 17-OHCS levels in serum and urine are seen in Fig. 1 and 2, respectively. Changes in the 2 fluids tended to be parallel for each except medrol. A significant decrease in serum steroid concentration followed medrol dosage, while a marked increase in urinary 17-OHCS was found.

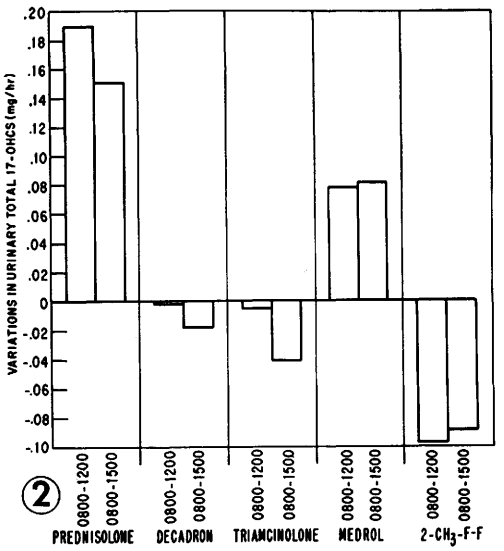
The divergent responses produced by the drugs under study suggest that the absorption and/or metabolic degradation of the

† Drugs furnished by Lederle Laboratories, Merck, Sharp and Dohme Co., and Upjohn Co.

TABLE II. Total 17-Hydroxycorticosteroid Levels in Urine Following Oral Administration of Synthetic Analogs of Hydrocortisone.

	0800-1200 hr Total 17-OHCS, mg/hr				0800-1500 hr Total 17-OHCS, mg/hr			
	No.	Mean	S.D.	P	No.	Mean	S.D.	P
Control	57	.40	.17	—	58	.33	.11	—
Prednisolone, 10 mg	12	.59	.19	<.01	16	.48	.14	<.01
Decadron, 2.5 mg	20	.40	.16	N.S.	18	.32	.14	N.S.
Triamcinolone, 10 mg	12	.40	.15	N.S.	16	.29	.10	N.S.
Medrol, 10 mg	19	.48	.10	<.05	19	.41	.14	<.05
2-CH_3-F-F, 1.5 mg	19	.30	.13	<.05	15	.24	.09	<.01





various compounds proceed at different rates or by entirely different pathways. Prednisolone prompted markedly increased steroid levels in both serum and urine, while the methylated derivatives (medrol, decadron, and 2-methyl, 9-alpha-fluoro-hydrocortisone) led to obvious adrenocortical suppression with significantly lower 17-OHCS concentrations in serum. The last two drugs also produced lower steroid levels in urine.

Ely and co-workers(7) studied the effect of orally administered prednisolone on plasma free 17-OHCS, and concluded that the metabolism of prednisolone proceeded at a rate slower than that of hydrocortisone. In "normal", hospitalized patients, Kupperman *et al.*(8) found an increased 17-OHCS excretion in urine following prednisone therapy. They concluded that this increase actually due to the presence of the administered steroid, more than offset the associated endogenous 17-OHCS suppression. These workers also found a decreased steroid excretion in patients dosed with medrol and triamcinolone caused, they felt, by the drugs not being metabolized into 17-hydroxysteroids.

In contrast to certain of the urinary findings of Kupperman *et al.*(8), who gave a 32 mg daily drug dose for a 5-day period, medrol was found in our studies to promote an increased steroid excretion while causing a decrease in serum levels. Since the urinary steroid procedure employed measures *total* 17-OHCS, as opposed to the *free* 17-OHCS

FIG. 1. Relative changes in serum free 17-OHCS levels following oral administration of synthetic analogs of hydrocortisone.

FIG. 2. Relative changes in urinary total 17-OHCS levels following oral administration of synthetic analogs of hydrocortisone.

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.

The authors express appreciation for technical assistance to R. P. Feller, E. Espinosa, J. F. McAnear, E. D. Dinger, D. Weise, and R. R. Hogue.

1. Shannon, Ira L., Prigmore, John R., *Oral Surg., Oral Med., and Oral Path.*, 1960, v13, 878.

2. ———, USAF School of Aviat. Med., 1959 Report 59-79.

3. Peterson, R. E., Karrer, A., Guerra, S. L., *Anal. Chem.*, 1957, v29, 144.

4. Reddy, W. J., *Metabolism*, 1954, v3, 489.

5. Clayson, K. L., *Am. J. Med. Technol.*, 1956, v22, 1.

6. Porter, C. C., Silber, R. H., *J. Biol. Chem.*, 1950, v185, 201.

7. Ely, Robert S., Done, A. K., Kelley, V. C., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 503.

8. Kupperman, H. S., Peh-Ping, H., Epstein, J. A., *Metabolism*, 1958, v7, 463.

9. Hatfield, C. B., Schuster, S., *J. Clin. Path.*, 1959, v12, 140.

10. Walser, A., Schlunke, H. P., *Experientia*, 1959, v15, 71.

Received December 5, 1960. P.S.E.B.M., 1961, v106

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

ROY B. MEFFERD, JR. AND HENRY B. HALE

Houston Veterans Administration Hospital and Baylor University College of Medicine, and Physiology-Biophysics Branch, USAF School of Aviation Medicine, Brooks Air Force Base, Texas

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.

* This research was supported by USAF contract. The experiment proper was conducted while the senior author was at Clayton Foundation Biochemical Inst., Univ. of Texas, Austin. Issued as School of Aviation Medicine Research Rep. 61-10, 1961.

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^{¾} 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^{¾} 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