normal serum protein patterns. Four days following intravenous Vit. A acetate administration, Vit. A deficiency syndromes in heifer "A" disappeared and albumin content of sera was elevated (Fig. 1, No. 3). Electrophoretic separations of sera 6 days later (Fig. 1, No. 4) showed elevation in serum and plasma albumin that approximated normality. These results further exemplify that metabolic carotene in the bovine possesses little value in alleviating syndromes predisposed by Vit. A deficiency.

Summary. Bovine serum protein was fractionated with a continuous flow electrophoresis apparatus. Vit. A and carotene were principally associated with albumin. When one co-twin of identical twin heifers was subjected to Vit. A deficiency, serum albumin was progressively reduced. Following an intravenous injection of carotene, the deficient heifer low in serum albumin retained approximately one-half the plasma-carotene concentration as her normal co-twin. Serum albumin returned to normal in the deficient co-twin within 10 days following intravenous Vit. A administration.

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Effect of Corticotrophin on Guinea Pig Plasma Corticosteroids.* (24632)

HERMAN COHEN AND WILLIAM KLEINBERG
Princeton Labs., Inc., Princeton, N. J.

In view of the similarity between the major blood adrenocortical steroids of man and guinea pig we became interested in using the guinea pig for preclinical assessment of corticotrophins and repository media. Liddle, *et al.*(1) have used the 24-hour urinary steroid output of this species as a method for evaluating effectiveness of corticotrophin. We decided to explore the plasma steroid concentration as possibly offering a more rigorous and rapid evaluation procedure.

Material and methods. Male guinea pigs weighing 350-400 g were used. The animals were sacrificed under light ether anesthesia and exsanguinated from the jugular vein with heparin as the anti-coagulant. Plasma corticoids were determined by the method of Porter and Silber(2). Highly purified corticotrophin (100 USP units/mg) was dissolved in the various menstrua tested *viz.*, 15% gelatin; 4% phosphorylated hesperidin plus 15% gelatin; saline. All injections were given subcutaneously.

Results. Fig. 1 presents distribution of plasma corticoid values of a group of 50 untreated animals. Average value is 43.4 μg/100 ml plasma. These values were further analyzed to determine whether a seasonal variation in plasma steroid concentration existed, but no significant difference was found in values of animals sacrificed in January, June, or September (Fig. 1). The base values are in agreement with those of Done, *et al.* (3) and Boulouard(4) who used a procedure similar to ours for sacrificing their animals. Sobel, *et al.*(5) have also found that the stress produced by ether anesthesia and surgery, such as exposure of the carotid artery

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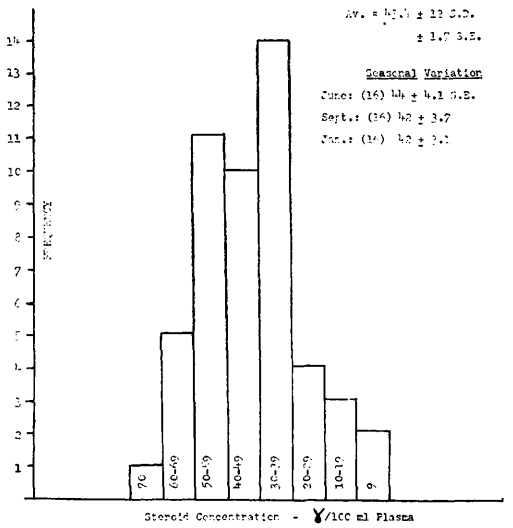

FIG. 1. Distribution of plasma 17-OHCS values of untreated male guinea pigs, 350-400 g body wt.

was insufficient to induce increased release of ACTH, as measured by urinary corticoid output. However, Eik-Nes(6) reported the average plasma steroid value of guinea pigs to be 24 μg/100 ml when a special neck clamp was used for sacrifice whereas animals anesthetized before bleeding had a higher concentration of plasma corticoids.

The marked diurnal variation in plasma 17-OHCS content of humans prompted us to examine this phenomenon in the guinea pig. Our data indicate that at least within the 8-hour period (8 a.m.-5 p.m.) no variation in blood steroid content is evident, in contrast to Migeon's(7) data which demonstrates that in the human a marked decrease in plasma corticoids occurs between these times. Our observations agree with the findings of Schweitzer (personal communication) who noted that the eosinophil count of the guinea pig does not vary during this time period, and also with those of Lazo-Wasem and Hier(8) who collected 8 hr. guinea pig urine samples from 8 a.m.-12 M and could detect no difference in steroid output during this time.

Since the procedure for determining corticoids in plasma measures only unconjugated steroids, representative plasma samples of different steroid content were examined to determine whether any glucuronide conjugated steroid was present. We noted that after enzymatic hydrolysis with β-glucuronidase a small but consistent amount of glucuronide conjugated Porter-Silber chromogen is present in most samples examined. This is in marked contrast to the large amount of conjugated steroid found in human plasma. The significance of this data is admittedly questionable and does not preclude the presence of conjugates other than glucuronides. Péron and Dorfman(9) report that all urinary corticoids of guinea pigs are present in the free form, but Wyngaarden(10) has noted that C^{14}-hydrocortisone administered to guinea pigs results in excretion of a small percentage of urinary metabolites as glucuronides. This has also been noted by Silber & Porter(11).

The possibility of establishing a quantitative relationship between plasma corticoid content and dose of administered corticotrophin was next considered. The data presented in Table I show that a maximum increase in plasma corticoid occurs 3 hours after subcutaneous injection of corticotrophin dissolved in gelatin, with a return to normal values between 8 and 12 hours later. This phenomenon is the same regardless of dose administered, although a difference in rate of decay is evident.

With effective delaying agents it is possible to alter this pattern so that 12 hr after administration of the hormone, increased amounts of plasma corticoids still persist. Table II demonstrates that addition of phosphorylated hesperidin to corticotrophin in gelatin, results in maintenance of an increased concentration of plasma corticoids 12 hr after administration of the hormone, whereas after a similar dose of corticotrophin dissolved in gelatin alone, the steroid values have returned to normal. This is attributed to ability of phosphorylated hesperidin to effect both a

TABLE I. Plasma Levels of 17-OHCS following Corticotrophin Injection.

Dose, USP units	Hr after ACTH inj.* 0	3	8	12
1.5	49 ± 2 (5)	144 ± 14 (5)	61 ± 4 (5)	41 ± .9 (5)
7.0	49 ± 2 (5)	183 ± 10 (6)	131 ± 16 (8)	35 ± 3 (8)

( ) No. of animals.
* ACTH dissolved in 15% gelatin.

TABLE II. Effect of Retarding Agents on Steroidogenic Effect of ACTH.

Hr after inj.	Plasma 17-OHCS, μg/100 ml 15% gelatin	15% gelatin + 4% P.H.*
0	43 ± 2	43 ± 2
3	184 ± 12	200 ± 14
8	132 ± 8	198 ± 15
12	35 ± 2	124 ± 9
24	41 ± 3	46 ± 5

Dose of corticotrophin = 10 USP units.
* Phosphorylated hesperidin.

slow release of corticotrophin into the blood stream and protection against inactivation of the hormone at site of injection. These data are in accord with those reported for ACTH in phosphorylated hesperidin plus gelatin in the rat, using adrenal ascorbic acid depletion as a measure of effectiveness(12).

Table III presents data comparing the effect of corticotrophin dissolved in gelatin, and in saline on plasma steroid concentration of the guinea pig. It is apparent that the effect of ACTH in gelatin is superior to that of the hormone dissolved in saline. This is in accord with clinical results in the human.

Finally, quantitation of response was examined. When the plasma steroid concentration obtained 3 hours after injection of ACTH in 15% gelatin is plotted against the log of the dose of corticotrophin administered, a straight line is obtained indicating the suitability of this parameter for assay purposes (Table IV). The statistics for the dose response data indicate excellent precision and sensitivity.

TABLE III. Guinea Pig Plasma Steroid Values after Administration of Corticotrophin* in Saline Solution and in 15% Gelatin Solution.

	μg/100 ml plasma 15% gelatin	Saline
Exp. 1	189	78
	156	107
	173	94
	153	97
	162	103
Avg	166 ± 6.6	96 ± 5.9
Exp. 2	160	133
	147	122
	170	102
	159	98
	133	110
Avg	154 ± 6.3	113 ± 6.4

* 1.5 USP units ACTH administered subcut. Blood samples obtained 3 hr after inj.

Conclusion. The plasma corticosteroid content of the guinea pig offers a suitable means for evaluating the potency of adrenocorticotrophic hormone, and also the menstrua which may be useful in extending the physiological or pharmacological effect of this hormone.

Summary. The response of the guinea pig to exogenous adrenocorticotrophin was determined using plasma steroid concentration as criteria of response. Average steroid concentration for 50 untreated male guinea pigs was 43 ± 1.7. A graded response was obtained after corticotrophin administration which when plotted yielded a straight line with a slope adequate to determine differences between a 1½ to 3-fold increment in dose. Comparison of corticotrophin administered subcutaneously in a gelatin menstruum with that administered in water showed a 2-3 time greater activity when gelatin is employed as a menstruum than when the hormone is dissolved in water. This is in accord with findings in the rat using the adrenal ascorbic acid depletion as a measure of activity, and clinically in the human. Small amounts of glucuronide conjugated corticoids were also found in the plasma.

TABLE IV. Relationship between Dose of Corticotrophin* and Response Elicited.

No. pigs	Dose, USP units	Response, γ 17-OHCS/100 ml plasma
14	.50	82 ± 6.7
14	.75	114 ± 4.3
14	1.50	145 ± 5.9
	b = 193 ± 19.4	
	s = 21.09	
	λ = .109	

* Corticotrophin dissolved in 15% gelatin and administered subcut. Animals sacrificed 3 hr after inj.

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Composition of Renal Lymph and Its Significance.* (24633)

S. J. LEBRIE AND H. S. MAYERSON

Dept. of Physiology, Tulane University School of Medicine, New Orleans, La.

Various concepts of the function of renal lymph have been evolved, based largely on indirect evidence(1). The present report deals chiefly with electrolyte concentrations in renal lymph, preliminary data from a study designed to obtain more direct and definitive evidence of its function.

Procedure. Healthy mongrel dogs of both sexes were anesthetized with sodium pentobarbital (25 mg/kg). A polyethylene catheter was inserted into jugular vein *via* 15 gauge needle and utilized for plasma sampling and administration of fluids and drugs. The thoracic duct was cannulated and thoracic duct lymph collected in most experiments. Both ureters were catheterized at their junction with the bladder and urine collected separately from each kidney. Femoral arterial pressure was recorded with a mercury manometer. A left flank incision was employed to expose the kidney. The fat at both poles was usually ligated to engorge the capsular lymphatics and permit their cannulation with #50 polyethylene tubing. Renal lymph was collected in small glass tubes capped with one-hole stoppers to prevent evaporation and volumes estimated to the nearest .05 ml. During the prolonged period of exposure the kidney was covered with saran wrap to prevent evaporation. Sodium and potassium were analyzed with a Baird flamephotometer using internal standards, and chlorides by the method of Schales and Schales(2). Serum, renal lymph, and thoracic duct lymph proteins were analyzed with a Spinco paper electrophoresis apparatus while total protein was determined by the falling drop method(3). Heparin prevented clotting in blood and thoracic duct lymph samples.

Results. Concentrations of Na, Cl, and K in plasma, thoracic duct lymph and renal lymph are given in Table I. Na concentrations of plasma and thoracic duct lymph are similar (averages = 145.7 ± .77 and 145.6 ± 2.6 meq/l. Renal lymph, however, has an average concentration of 162.1 ± 2.8 meq/l, 11.3% higher than the average plasma value, a difference which is significant (P value = <.001). The average value for thoracic duct lymph Cl of 121.3 ± 2.5 meq/l is 9.8% higher than average plasma Cl value of 110.5 ± 4.3 meq/l. This difference is not statistically significant. However, the average Cl concentration of renal lymph is 140.2 ± 3.8 meq/l, 26.9% higher than the average plasma Cl concentration; a significant difference (P value = <.001). There is no apparent correlation between lymph flows and Na concentration as judged by data from 14 dogs or Cl concentration from 6 dogs. The mean K values for all 3 fluids are similar: 4.03, 3.95, and

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