given 6½ months after the initial hypoproteinemia, at which time their plasma protein levels were 6.7 and 5.8 g %. Control dogs excreted less than half the mercury; the hypoproteinemic dogs excreted over half of it within the first 3 days. It is suggested that the resistance to mercury toxicity seen in the hypoproteinemic animals may be due to increased extracellular volume and, more important, a relatively small binding of Hg++ by protein, permitting more rapid excretion and diminished cell susceptibility.

1. Havill, W. H., Lichty, J. A., Jr., and Whipple, G. H., *J. Exp. Med.*, 1932, v55, 627.
2. Lippman, R. W., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 682.
3. Holman, R. L. and Donnelly, G. L., *J. Exp. Med.*, 1942, v76, 511.
4. Gettler, A. O. and Lehman, R. A., *Am. J. Clin. Path.*, 1938, tech. suppl., v8, 161.
5. Cizek, L. J., and Zucker, M. B., *Am. J. Physiol.*, 1950, v162, 153.
6. Milnor, J. P., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 63.
7. Zeldis, L. J., Alling, E. L., McCoord, A. B., and Kulka, J. P., *J. Exp. Med.*, 1945, v82, 157.

Received November 26, 1951. P.S.E.B.M., 1951, v78.

Effect of Intravenous Injections of Desoxycorticosterone Glucoside upon Blood Glucose of Adrenalectomized Dogs.* (19245)

W. W. SWINGLE, JOSEPH PERLMUTT, ELLIOTT COLLINS,† PATRICK SEAY, EDWARD FEDOR, AND GEORGE BARLOW.

From the Section of Physiology, Biological Laboratory, Princeton University, Princeton, N. J.

It is well established that the oil soluble preparation of DCA, given intramuscularly in small doses, exerts little, if any, effect upon the intermediary metabolism of carbohydrate. On the other hand, those adrenal steroids oxygenated at C-11 are highly efficacious in this respect(1-5). However, within recent years several reports have appeared indicating that DCA, administered in large doses, induces glycosuria in the force-fed, partially depancreatized rat(6) and "restores glycogen production to normal from carbohydrate or protein in liver and muscle of adrenalectomized animals which are kept alive for some time" (7,8). Harrison and Harrison(9) concluded from their experiments that "the injection of adequate amounts of desoxycorticosterone acetate into fasted adrenalectomized rats does prevent the drop in concentration of blood sugar found in untreated adrenalectomized rats." Other investigators have reported some positive effects of large amounts of DCA on gluconeogenesis and blood sugar level of adrenalectomized rats(10) and the glucose requirements of adrenalectomized-eviscerated rats(11). In the course of a study on the efficiency of the water soluble glucoside of desoxycorticosterone‡ (DCG), in reviving adrenalectomized dogs from insufficiency, massive doses of this steroid were administered as a single injection by vein. Since the amounts employed were far larger than anything reported in the literature, and since fasted adrenalectomized animals receiving DCG, during revival from severe insufficiency, were only occasionally observed to exhibit sharp increases in blood glucose, it was considered worth while to study in more detail the possible correlation between DCG and blood glucose levels.

The present report is based upon a study of 16 adrenalectomized dogs and one intact animal. The type of dog employed, amount of DCG administered and other details of the

* This investigation was supported by funds from the Eugene Higgins Trust allocated to Princeton University, and also by a grant-in-aid from the Ciba Pharmaceutical Products Co., of Summit, N. J., and a grant from Sharp and Dohme, Inc., Philadelphia, Pa.

† Research Fellow of the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

‡ Supplied through the courtesy of Dr. E. Oppenheimer of the Ciba Pharmaceutical Products Co.

TABLE I. The effect of Massive Doses of DCG on Fasted Intact and Adrenalectomized Dogs.

Dog No.	Period of fasting, hr Pre-inj.	Post-inj.	Initial B. P., mm Hg	DCG dosage, mg	Blood glucose, mg % Pre-inj. period	Post-inj. period 3 hr	6 hr	12 hr	18 hr	24 hr
				Intact animal						
1	30	24	110	300	66	76	70.5	72.5	—	67.5
				Adrenalectomized in normal health						
2	30	24	103	300	62.5	72	70.5	62	—	66.5
3	30	24	115	600	68	69	66	66.5	—	68.5
4	30	24	105	600	66	68.5	72	71.5	—	66.5
				Adrenalectomized in mild insufficiency						
5	30	24	70	300	69	70.5	76	73.5*	71	72.5
6	30	24	71	300	79.5	76.5	81.5	—	80.5	87.5
				Adrenalectomized in severe insufficiency						
7	30	24	50	300	72.5	81.5	90	—	119.5	111.5
8	30	24	55	300	61.5	88	88.5	84 *	84	81
9	30	24	54	300	85	62.5	71.5	72.5	69	75
10	30	24	52	300†	64	65	62.5	74	72	76
11	30	24	46	300	64	66	—	65	70	65

* 16 hr sample. † 200 mg DCG 24 hr later.

experiment concerned solely with fasted animals, are shown in Table I. In order to eliminate exogenous sources of carbohydrate, all food was withheld for 30 hours prior to the initial injection of DCG and for 24 hours thereafter. Thus, the dogs were subjected to a prolonged fast of 54 hours. This is severe treatment for adrenalectomized animals maintained solely on a steroid presumably without effect upon gluconeogenesis. The experimental results are presented in Table I.

Examination of the data reveals that of the 11 fasted dogs, receiving a single minimum dose of 300 mg of DCG intravenously, only 2 animals (No. 7 and 8) showed marked increases in blood glucose. These dogs exhibited severe insufficiency symptoms, such as, low arterial pressure of 50 and 55 mm Hg, spasticity and muscular weakness at the time DCG was injected. The blood glucose of one animal (No. 7) rose from the pre-injection level of 72.5 mg to 119.5 mg % during an 18-hour interval. The second dog (No. 8) showed an elevation in glucose from 61.5 mg to 84 mg % during the same time interval.

Three other adrenalectomized animals with equally severe symptoms (No. 9, 10 and 11) revealed either slight or no changes in blood glucose following injection. The maximum increase observed in these dogs, during the 24-hour observation period, was 12 mg % (dog 10). This animal had received a total of 500 mg of DCG in 2 injections: 300 mg at the time of crisis and 200 mg 24 hours later. The post-injection observation period for this animal was extended an additional 12 hours with no further significant alterations in the blood glucose level.

The other types of animal used in this experiment, *i.e.*, intact and adrenalectomized, but maintained free from symptoms of insufficiency, and those dogs, presenting only mild symptoms (Table I), failed to show any appreciable or sustained rise in blood glucose, following DCG treatment. It seemed evident that injections of massive doses of the glucoside by vein to fasted, adrenalectomized dogs is without effect upon the blood sugar of most of the animals. An occasional dog suffering from severe insufficiency may exhibit a significant rise in blood glucose following DCG treatment. However, the writers do not attribute the sharp increases in glucose in these exceptional cases to an effect of the DCG *per se*, but consider the sugar elevation to be due to the marked general improvement of the animal's condition, *e.g.*, the increased hydration, increased blood flow through organs and tissues, and opening up of previously closed capillary beds. This interpretation of the effects of DCG upon the blood glucose of adrenalectomized dogs is in line with a suggestion originally made by Russell(11) some years ago to account for

TABLE II. Effect of Massive Doses of DCG on Non-Fasted Adrenalectomized Dogs in Insufficiency.

Dog No.		Adrenal insufficiency	24 hr	48 hr
1	B. P., mm Hg	50	57	80
	Glucose, mg %	72	87.5	89.5
	DCG dosage, mg	300	200	100
2	B. P., mm Hg	54	86	89
	Glucose, mg %	66	83	88
	DCG dosage, mg	250	100	100
3	B. P., mm Hg	59	62	72
	Glucose, mg %	67.5	86	88.7
	DCG dosage, mg	300	200	50
4	B. P., mm Hg	65	67	87
	Glucose, mg %	82	99.5	110
	DCG dosage, mg	300	200	200
5	B. P., mm Hg	78	84	98
	Glucose, mg %	76.5	80	93
	DCG dosage, mg	300	150	50
6	B. P., mm Hg	78	91	114
	Glucose, mg %	76	75	80
	DCG dosage, mg	300	150	50

certain apparent effects of DCA upon glucose utilization of her adrenalectomized-eviscerated rats.

In contrast to the equivocal results obtained by administering DCG to fasted adrenalectomized dogs, are the blood sugar changes which follow such injections in the non-fasted animal exhibiting insufficiency. During the first 24-48 hours of the revival period, the dogs reveal striking and unmistakable elevations in blood glucose. The arterial pressure(12) and blood sugar record of 6 such animals are recorded in Table II.

The dogs were permitted to develop mild to severe symptoms of adrenal insufficiency. None of the animals had eaten food for approximately 12 hours before the initial blood samples were taken. Examination of the blood pressure data will give some idea of the severity of the symptoms presented by these animals at the beginning of the experiment.

Immediately following injection of DCG, the usual diet was given and at the end of 24 hours the blood pressure and blood samples were taken. As shown in Table II, a significant elevation of the blood glucose occurred in 5 of the 6 animals. There was no direct correlation between the rise in arterial pressure and the increased blood glucose. One dog showed a sharp rise in blood pressure but a negligible change in the sugar level (No. 6, Table II) at the end of the first 24 hours. Other dogs (No. 2 and 4) revealed rises in sugar and also elevations in the arterial pressure. As a general rule, however, the blood pressure does not return to normal levels before 72-96 hours after injection. The dogs listed in Table II were eager for food and displayed great improvement in activity and vigor in spite of the still lowered blood pressure. The marked effect of DCG upon the blood sugar in these cases was probably due to the increased appetite and improved alimentary absorption of glucose, and not to any direct action of the steroid upon carbohydrate metabolism.

Summary and conclusion. (A) Three types of fasted adrenalectomized dogs were studied: (1) those maintained in normal health; (2) those exhibiting mild insufficiency symptoms; and (3) animals in severe adrenal crisis. When these dogs were injected intravenously with massive doses of DCG, only occasional blood glucose changes were observed. These infrequent rises in glucose were associated with marked improvement in the circulation of the animal, increased hydration, increased vigor and muscular activity. (B) A fasted, intact dog, injected with 300 mg DCG, did not show any significant changes in blood sugar level over a 24-hour period. (C) Adrenalectomized dogs, presenting severe symptoms but allowed food *ad libitum* during revival from insufficiency, exhibit sharply increased blood glucose levels in the recovery period following DCG administration.

1. Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinol.*, 1940, v26, 309.

2. Wells, B. B., *Proc. Staff Meet., Mayo Clin.*, 1940, v15, 294.

3. Kendall, E. C., *J.A.M.A.*, 1941, v116, 2394.

4. Olson, R. E., Thayer, S. A., and Kopp, L. T., *Endocrinol.*, 1944, v35, 430.

5. Ingle, D. J., *Ann. N. Y. Acad. Sci.*, 1949, v50, 576.

6. ———, PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 329.

7. Sass-Kortsak, A., Wang, F. C., and Verzar, F., *Am. J. Physiol.*, 1949, v159, 256.

8. Wang, F. C., and Verzar, F., *Am. J. Physiol.*, 1949, v159, 263.
9. Harrison, H. E., and Harrison, H. C., PROC. SOC. EXP. BIOL. AND MED., 1939, v42, 506.
10. Wells, B. B., and Kendall, E. C., *Proc. Staff Meet., Mayo Clin.*, 1940, v15, 565.
11. Russell, J. A., *Am. J. Physiol.*, 1943-44, v140, 98.
12. Parkins, W. M., *Am. J. Physiol.*, 1934, v107, 518.

Received November 26, 1951. P.S.E.B.M., 1951, v78.

Augmented Excretion of Urine Gonadotrophins During ACTH Administration. (19246)

RICHMOND W. SMITH, JR. (Introduced by O. H. Gaebler.)
From the Section of Endocrinology, Department of Medicine, Henry Ford Hospital, Detroit, Mich.

Observations on the effects of ACTH on renal excretion of pituitary gonadotrophin have been made by the author during the past two years. Recently, Sohval and Soffer(1) reported the results of a similar investigation in 22 patients receiving ACTH and/or cortisone. They called attention to the limited data on the subject, specifically those of Mason *et al.*(2) and Sprague *et al.*(3), who observed no change in urinary gonadotrophin titers of 2 young women. Sohval and Soffer (1) found enhanced titers in 9 patients of their series but they stated that the mechanism of this change remained unknown. The present report concerns the results of gonadotrophin analysis of 73 urine samples from 9 patients receiving ACTH. Methods of gonadotrophin isolation and bioassay were employed which differed from those used in the above-mentioned investigations.

Materials and methods. Pertinent data on the case material is presented in Table I where dosage, route and duration of the hormone administration are summarized with the gonadotrophin titers. All patients received ACTH and Case 5 received, in addition, a short course of oral cortisone therapy. Case 3 received 15 mg of ACTH intravenously over a 6-hour period while all others received the hormone intramuscularly in divided doses. Urine samples, collected over periods of from 12 to 24 hours, were preserved under toluene. Gonadotrophins were isolated by adsorption on activated kaolin(4). The gonadotrophins were dried under a stream of nitrogen, sealed in a test tube and kept at 3°C until assayed. At this time the powdered material was dissolved in isotonic saline and the bioassay carried out as reported by Lloyd *et al.*(5). This method employs 21-day-old female white mice of 8 to 10 g, using 2 mice at each of 4 dilutions per gonadotrophin sample. Reassay at the same or different dilutions was carried out when confirmation was desired or the end point was not obtained initially. In these instances, a total of 16 mice per sample were required. Volumes of 0.25 cc of gonadotrophin solution were injected subcutaneously twice daily for 3 days and the animals sacrificed 24 hours following the last injection. Throughout the assay period the solutions of gonadotrophins were kept at 3°C. Both ovarian and uterine weights were recorded but only the latter were used to determine the titers. Mice were assayed in lots of 50 of which 5 to 7 served as saline-injected controls. A positive result was recorded when the average of the uterine weights of the 2 gonadotrophin-injected animals at any level exceeded the average of the saline-injected controls by 100%. Toxicity of the gonadotrophin solutions was encountered infrequently and only 9 of more than 800 mice used in the study were lost. One group of 5 mice received a total of 4.1 mg of ACTH per mouse in 6 divided doses over a 3-day period to determine the direct effects of this hormone on the uterine, ovarian and adrenal weights (Table II).

Results. In all cases augmented gonadotrophin excretion of varying degree was observed during the period of ACTH adminis-