In the liver preparations of each group of rats some nuclei contained osmiophilic material. Whether this was lipid is not clear because it was not possible to reproduce this picture with Sudan-Black stain. There was no microscopic evidence of liver injury in any of the animals.

The question arises as to whether there is any correlation between the described liver changes and the presence of increased levels of urinary acetone bodies. There apparently is no such correlation because a ketonuria is present, on the one hand, in niacin-injected rats which have high levels of liver glycogen and, on the other, in priscoline-injected rats which have low levels of liver glycogen. Evidence that priscoline modifies carbohydrate metabolism is brought forth by Sasson(10) who showed that the insulin requirements of some human diabetics is reduced following the administration of priscoline.

Summary. When large amounts of niacin or priscoline were given daily to rats fasted for 5 days, an excessive excretion of urinary acetone bodies was noted in most of the animals. Niacin-injected rats had higher levels of liver glycogen, priscoline-injected lower levels. Total liver lipid was not altered by either drug. There appeared to be no correlation between the amount of liver fat or liver glycogen and the presence of excess ketone bodies in the urine.

1. Janes, R. G., and Brady, J., *Am. J. Physiol.,* 1949, v159, 547.

2. Janes, R. G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 246.

3. Janes, R. G., and Boeke, L. S., *Am. J. Physiol.,* 1951, v167, 799.

4. Mirsky, A. I., and Nelson, W. E., *Am. J. Diseases Child.,* 1944, v67, 100.

5. Beringer, A., *Schweiz. med. Wchnschr.,* 1949, v79, 298.

6. Deuel, H. J., Jr., and Hallman, L., *J. Biol. Chem.,* 1941, v140, 545.

7. Roberts, S., and Samuels, L. T., *J. Biol. Chem.,* 1943, v151, 267.

8. Van Slyke, D. D., *J. Biol. Chem.,* 1917, v32, 455.

9. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.,* 1933, v100, 485.

10. Sasson, M. I., *N. Y. State J. Med.,* 1951, v51, 1315.

Received June 30, 1952. P.S.E.B.M., 1952, v80.

Intramuscular Cortisone Administration to Dogs. (19703)

OTAKAR V. SIREK AND CHARLES H. BEST.

From the Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

Most of the experimental studies dealing with the effects of cortisone administration to dogs refer to profound changes in electrolyte and water metabolism, but there is little information available as to the glycemia and the behavior of the glucose tolerance test curves during and after prolonged injection with cortisone. Mushett, Porter, and Silber (1) reported that daily subcutaneous treatment of 3 dogs with 10 mg/kg for a period of 2-3 weeks brought about a profound polyuria and polydipsia. Davis, Bass, and Overman(2) in an extensive study were able to demonstrate the remarkable influence of cortisone on the ionic balance of normal and adrenalectomized dogs. The present study was originally an investigation of the influence of cortisone upon the carbohydrate metabolism in normal dogs. However, it was found that large doses of cortisone may produce a picture which resembles diabetes insipidus rather than diabetes mellitus.

Material and methods. Five mongrel dogs, 3 males and 2 females of a weight between 9 and 12 kg were used in this study. The animals were confined in metabolic cages and a measured amount of commercial dog food was fed. The amount of urine, the specific gravity and the amount of consumed drinking water were measured every morning. Urine

TABLE I. Carbohydrate and Water Balance Before and After Treatment with Cortisone.

Dog No.	Before treatment*					Cortisone treatment, mg/day	After treatment				
	Wt, kg	Fasting blood sugar, mg %	Max (24 hr) Drinking water, cm³	Max (24 hr) Urine, cm³	Spec. grav.		Wt, kg	Highest fasting blood sugar, mg %	Max (24 hr) Drinking water, cm³	Max (24 hr) Urine, cm³	Spec. grav.
DS 11	12.7	78	760	620	1027	50 mg daily for 13 days Total: 650 mg	11	98	3000	2600	1007
DS 13	9	93	400	300	1040	200 mg 7 days 100 mg 7 days Total: 2100 mg	8.5	105	6200	5790	1007
DS 14	9	95	680	400	1035	100 mg 7 days 50 mg 7 days Total: 1050 mg	8	84	5580	5000	1008
DS 15	10.5	76	300	220	1050	50 mg 7 days 25 mg 6 days Total: 500 mg	9.5	99	420	390	1037
DS 16	12.6	90	180	210	1045	300 mg 4 days Total: 1200 mg	12.4	106	1000	900	1018

*Period of observation 4-6 days.

sugar was determined by "Clinitest Tablettes" (Ames Co., Inc.) and blood sugars by the method of Miller and Van Slyke(3). Fasting blood sugar specimens were usually taken every second day during the experiment and every morning during the first 4 days of cortisone treatment. Glucose tolerance tests were carried out using the method of intravenous injection; 1 g per 1 kg of body weight were injected and samples drawn after 3, 10, 20, 40, 60 and 90 minutes. Cortisone (Cortone Acetate "Merck") was administered intramuscularly and the daily total dose was divided into several injections of 50 mg each. In one dog 300 mg a day was to be injected; 150 mg was given intramuscularly in 3 injections, the remaining 150 mg was administered intraperitoneally.

Results. It is obvious from Table I that a significant elevation of the fasting blood sugar following cortisone treatment was not observed in any of our 5 dogs. Fig. 1 shows a glucose tolerance test curve before and after treatment with 300 mg of cortisone a day for 4 days. Both curves are normal. Similar glucose tolerance test curves were obtained also from the other 4 dogs. It is further obvious from Table I that a daily dose of 50 mg and more maintained for several days was sufficient to produce a remarkable polydipsia and polyuria which reached in some instances 10 times the normal values (Fig. 2 and 3). Our electrolyte balance study in such polyuric conditions needs further investigation; however, we are able to say that the concentration of sodium and chlorine in urine is decreased. Hence, all the cardinal symptoms of diabetes insipidus such as polydipsia, polyuria, low specific gravity and low concentration of NaCl in urine have been observed. The test of water deprivation to a "point of discomfort" was performed in 2 polyuric dogs. The dogs were fed the usual diet but water was withheld. The urine showed a specific gravity lower than 1010. After 9 hours the experiment was discontinued because of the dehydration of the animals. Dog DS 11 was treated with pituitrin for one day; 5 I.U. were injected subcutaneously at 10 A.M. and 6 P.M. The posterior pituitary hormone was able to restore almost normal conditions as far

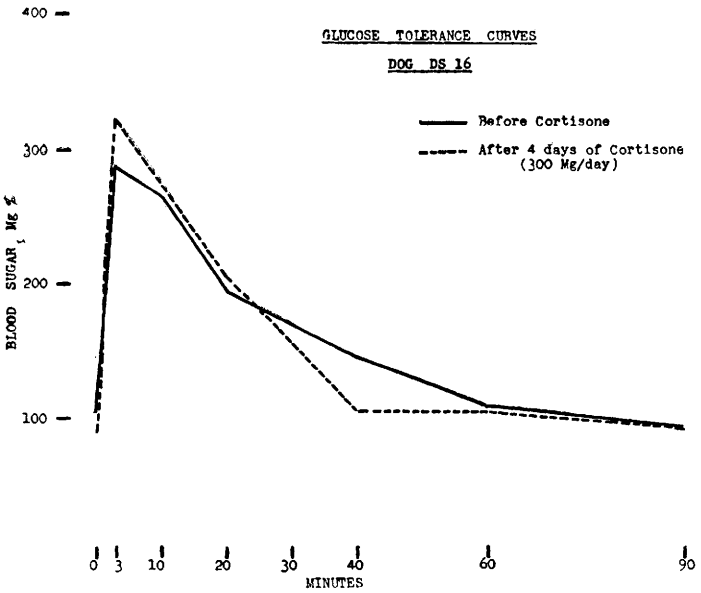

FIG. 1. Glucose tolerance curve before and after 1200 mg of cortisone.

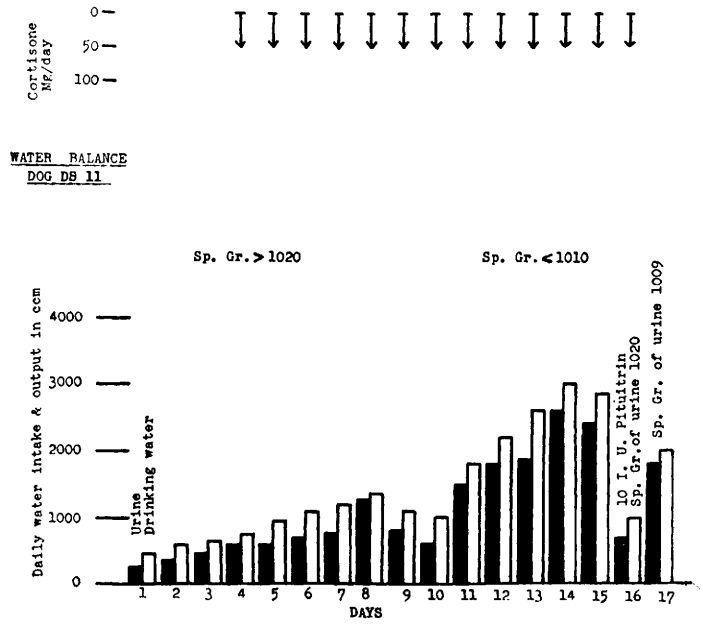

FIG. 2. Development of polydipsia and polyuria during cortisone treatment. Pituitrin was able to restore almost normal water balance.

as thirst, polyuria and specific gravity of urine were concerned (Fig. 2). Dogs DS 11 and DS 16 were followed for a certain time also after the cortisone treatment was stopped, while the dogs DS 13, 14 and 15 were sacrificed at the end of the injection period. The pancreas, kidney and hypophysis were examined histologically. In the dogs which were kept alive all the above mentioned symptoms of diabetes insipidus disappeared and normal conditions were established within 6-7 days.

Paraffin sections of the pituitary and of representative blocks (Bouin or formalin fixation) of the liver, kidney, pancreas, and ad-

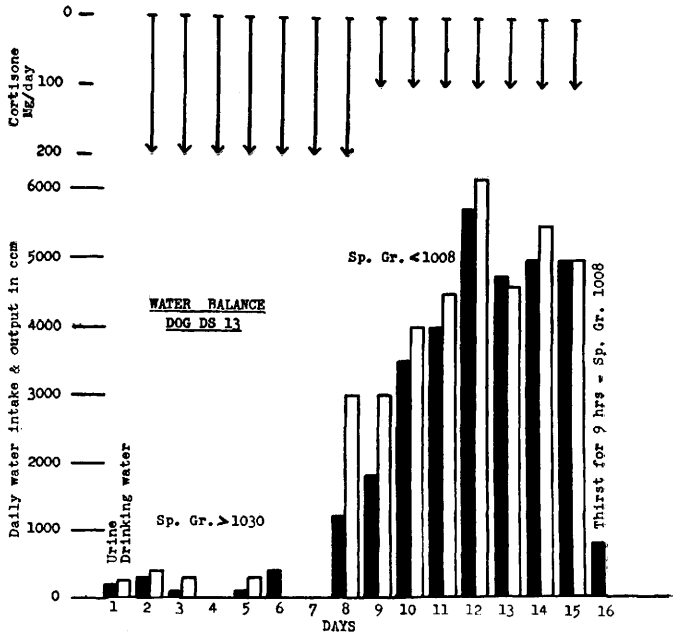

FIG. 3. Development of polydipsia and polyuria during cortisone treatment. Thirst for 9 hr had no remarkable effect upon the diuresis, and the specific gravity of the urine remained low.

renals were prepared in the usual manner and stained with hematoxylin and eosin. Sections of pancreas were specially stained to demonstrate the β-granules in the islets of Langerhans and examined by phase-contrast microscopy. Frozen sections of adrenals, kidneys and livers were stained by Wilson's technic(4) to demonstrate deposits of lipid. With one exception (see below) the tissues were free of pathological lesions, aside from terminal renal congestion and minimal fatty change in the liver. Deposits of stainable fat were present in the tubules of the kidneys from 2 of the dogs. Abundant lipid was present in the adrenal cortices of all animals, particularly in the zona reticularis. The intensity of β-cell granulation of the islets of Langerhans fell within normal limits.*

Discussion. The normal blood sugar values found in our experimental animals throughout the experiment on one hand and the production of the syndrome complex of diabetes insipidus on the other hand provide a good demonstration of the considerable overlapping between the group of "glucocorticoids" and "mineralocorticoids." The maintenance of a normal blood sugar level and the normal glucose tolerance test curves after large doses of cortisone are features quite similar to the response obtained by Fajans, Conn, *et al.*(5) with large doses of ACTH. An amount as high as 345 mg of ACTH per day injected for 4-6 days produced a typical diabetic glucose tolerance curve only in one out of 3 mongrel dogs.

A diabetes insipidus-like picture has also been produced in dogs by 11-desoxycorticosterone as reported by Ragan, Ferrebee, Phyfe, Atchley, and Loeb(6) and by Mulinos, Spingarn, and Lojkin(7). Britton and his coworkers(8-10) and Mulinos and associates(7), on the basis of their results, developed a theory that the adrenal cortex and the posterior pituitary are physiological antagonists in relation to certain phases of renal function. Thus, according to this theory, the increased water exchange of our dogs can be explained to a certain degree by a preponderance of adrenocortical activity to an extent which cannot be counterbalanced by the posterior pituitary, the end result being a diabetes insipidus-like picture.

* We are greatly indebted to Dr. W. Stanley Hartroft for making the histological examinations.

Summary. A syndrome closely resembling diabetes insipidus was produced in 4 mongrel dogs by daily injections of cortisone in doses of 50 to 300 mg a day. The period of hormone injection varied from 4 days to 14 days in different dogs. The polydipsia reached values as high as 6200 cc per day and the polyuria reached values as high as 5790 cc per day. Frequent fasting blood sugar determinations and glucose tolerance tests did not reveal any changes in carbohydrate metabolism.

1. Mushett, C. H., Porter, C. C., and Silber, R. H., *Fed. Proc.*, 1951, v10, 366 (part 1).

2. Davis, A. K., Bass, A. C., and Overman, R. R., *Am. J. Physiol.*, 1951, v166, 493.

3. Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, 1936, v114, 583.

4. Wilson, W., *Bull. Internat. Assn. of Med. Museums*, 1950, No. xxxi, 216.

5. Fajans, S. S., Conn, J. W., Johnson, D. V., and Christman, A. A., *Endocrinology*, 1951, v49, 225.

6. Ragan, C., Ferrebee, J. W., Phyfe, P., Atchley, D. W., and Loeb, R. F., *Am. J. Physiol.*, 1940, v131, 73.

7. Mulinos, M. G., Spingarn, C. L., and Lojkin, M. E., *Am. J. Physiol.*, 1941, v137, 102.

8. Silvette, H., and Britton, S. W., *Am. J. Physiol.*, 1938, v123, 630.

9. Britton, S. W., and Corey, E. L., *Science*, 1941, v93, 405.

10. Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1941, v133, 511.

Received June 30, 1952. P.S.E.B.M., 1952, v80.

Agglutination of a Pure Strain of Mammalian Cells (L Strain, Earle) by Suspensions of Vaccinia Virus.* (19704)

WILLIAM F. SCHERER. (Introduced by J. T. Syverton)

From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis.

It is the purpose of this paper to record experimental results which demonstrate the ability of suspensions of vaccinia virus to agglutinate altered fibroblastic cells (L strain, Earle)(1). Heretofore, the reported evidence for agglutination of mammalian cells by viruses was limited to hemagglutination.

A variety of viruses have in common the ability to agglutinate erythrocytes(2-4). These viruses fall into two groups(5).† The first group is made up of the viruses of vaccinia, variola and ectromelia. These viruses possess hemagglutinins which are separable from the infectious viral particle. The second group includes the viruses of mumps, Newcastle disease and influenza. These viruses have hemagglutinins which are inseparably associated with the viral particle. It has been shown that viruses from the second group adsorb to cells other than erythrocytes. For example, influenza virus adsorbs to cells from ferret lung(6), and the virus of Newcastle disease has been shown to react with tumor cells to inhibit tumor growth(7). However, in those studies it was not possible to demonstrate that cells were agglutinated by the viruses.

During a study of the host cell-virus relationships between each of a variety of viruses and a strain of mouse cells cultivated *in vitro* (L strain, Earle), it was noted that a hemagglutinating virus which has a soluble hemagglutinin, vaccinia virus, caused cellular agglutination. This strain of cells has been maintained *in vitro* by Earle and coworkers since 1940(1). While being cultivated *in vitro,* the cells developed the capacity to produce sarcomas upon inoculation into mice(8). Since 1948, this strain of cells has had as its parent cell, a single cell(9). Cells from this strain may be considered as altered fibroblastic cells (10).

Materials and methods. Viruses. Three viruses were employed. Vaccinia virus was

* Aided by a grant from the American Cancer Society, on recommendation of the Committee on Growth, National Research Council.

† For a review of the subject of hemagglutinins and hemagglutination, the reader is referred to reference 5.