A Renal Action of Glucagon (22854)

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In the course of a study on the fate of I^{131}-glucagon *in vivo* it was observed that a considerable portion of the protein bound I^{131} appeared rapidly in the urine as free iodide.* Furthermore, this excretion of inorganic I^{131} was 3 or 4 times greater than when an equivalent amount of inorganic I^{131} alone was injected. This suggested that glucagon had accelerated the excretion of the liberated iodide. For this reason a study was undertaken to determine the effect of glucagon not only on the renal excretion of I^{131}, but on a number of other electrolytes.

Procedure and methods. Adult mongrel dogs of both sexes were used in the experiments to be described. Unless otherwise specified, the animals were anesthetized with sodium pentobarbital (Nembutal) and diuresis induced by a constant infusion of 5% glucose solution at a rate of 3 to 5 ml/min. These conditions yielded a fairly constant urine flow of at least 2 ml/min after 2 to 3 hours of infusion. Electrolyte excretion was determined by analysis of urine samples collected at specific time intervals through an indwelling bladder catheter. The bladder was washed once with 10 ml of distilled water and the wash added to the urine sample. During the control period the urine samples were collected at 30 min intervals for 90 minutes followed by four 15 minute collections. Glucagon was then administered intravenously and the urine collected again at 15 minute intervals for 1 hour followed by three 30 minute samples. The total duration of the experiment was 5 hours. Chloride, inorganic phosphate, urinary reducing substance, and creatinine clearances were determined by standard methods(1). Sodium and potassium were analyzed on an internally compensated flame photometer.

Results. Table I summarizes the excretion data from the 19 experiments. The variability of the magnitude of the glucagon effect is obviously very great. However, the overall average increases in excretion of all electrolytes studied are very marked, ranging from 207 to 510% over control values. No consistent correlation between the magnitude of the effect and the dose of glucagon in this relatively small number of experiments is apparent.

The effect of glucagon on renal excretion of electrolytes was studied in 7 dogs using a single intravenous dose ranging from 30-300 γ/kg body weight of a 50% pure preparation.† Fig. 1 and 2 represent a typical experiment showing the effect on Cl^-,PO_4^{---}, Na^+ and K^+ excretion. The upper curves illustrate the cumulative output of electrolytes as a function of time. It is evident from these graphs that per unit time output of all electrolytes increases several fold shortly after administration of glucagon. As shown by the lower curve of Fig. 1 the urine flow expressed as ml/min remained fairly constant throughout the experiment. Although the experiments were designed to keep the urine flow as constant as possible, some fluctuation was unavoidable. It is unlikely, however, that these limited variations are responsible for changes in electrolyte output since it has been shown by Wesson and coworkers(2) that the excretion of Na^+ and Cl^- is independent of urine flow in the range from 1 to 6 ml/min. Furthermore, an increase in ion excretion was frequently observed while urine flow was decreasing. The pH of all urine samples was measured in several experiments; no signifi-

* Unpublished observations.

† A 50% pure glucagon preparation (Lot No. 208-158B-197) was kindly supplied by Dr. O. K. Behrens of the Lilly Research Laboratories, Indianapolis. The crystalline glucagon was prepared from such material according to the method of Staub *et al.* (*J. Biol. Chem.*, 1955, v214, 619.)

TABLE I. Effect of Glucagon on Renal Excretion of Electrolytes in the Dog. Summary of electrolyte excretion data showing avg changes and variability of results comparing control periods with experimental (glucagon) periods.

Glucagon,* γ/kg body wt	No. of dogs	Na		K		Cl		PO_4		I^{131}	
		Avg increase, %	Range, %	Avg increase, %	Range, %	Avg increase, %	Range, %	Avg increase, %	Range, %	Avg increase, %	Range, %
30–100	6	249	0 → 758	184	0 → 489	42	0 → 130	152	0 → 843	0	0
100–200	6 or 7†	581	85 → 855	243	43 → 676	459	0 → 1670	598	61 → 2163	205	0 → 754
200–300	5 or 6†	1069	0 → 4152	192	76 → 481	810	0 → 1450	529	16 → 1158	531	27 → 1241
Overall avg increase, %	17 or 19	510		207		409		399		252	

* Dose expressed as potency of the crystalline glucagon preparation.
† Electrolyte measurements incomplete in one animal.

cant change was observed after the administration of glucagon.

Endogenous and in some instances exogenous creatinine clearances were determined in all experiments as a measure of the glomerular filtration rate. Glucagon had no significant effect on the glomerular filtration rate, the variations being in most cases within ± 10%. This suggests that the polypeptide acts at the renal tubular level rather than at the glomerulus.

The same effects were observed in 12 dogs in which crystalline glucagon was used instead of the 50% pure preparation. No effects were observed in one animal which received a heat inactivated preparation of glucagon and in another animal to which insulin was administered. For these reasons it is unlikely that the described effects can be attributed to an impurity in the glucagon preparation or to a non-specific protein effect.

To rule out anesthesia as a factor influencing renal function after glucagon administration, several experiments under varying conditions were repeated in a trained, unanesthetized female dog. Fig. 3 summarizes the effect of glucagon on the excretion of Na^+ under these conditions. All other electrolytes tested behaved in a similar manner. In the experiment illustrated by curves "A", 5% glucose was infused as usual and the urine flow was approximately 2 ml/min. Another experiment was performed on a different day with no water load as is evident by the very low urine flow illustrated by the lower "B" curve. The results provide evidence that neither anesthesia nor moderate diuresis is a necessary condition for the effect of glucagon on electrolyte excretion.

It is known that large amounts of glucagon cause prolonged hyperglycemia with occasional glycosuria(3). It is conceivable, therefore, that the high blood sugar levels and/or glycosuria might affect the pattern of electrolyte excretion. To test this possibility, plasma glucose levels of 300-500 mg % were established by a priming dose of glucose (1 g/kg) and infusion of 12.5-15% glucose solution. These conditions caused consistent glycosuria as shown by the lower curve of Fig. 4.

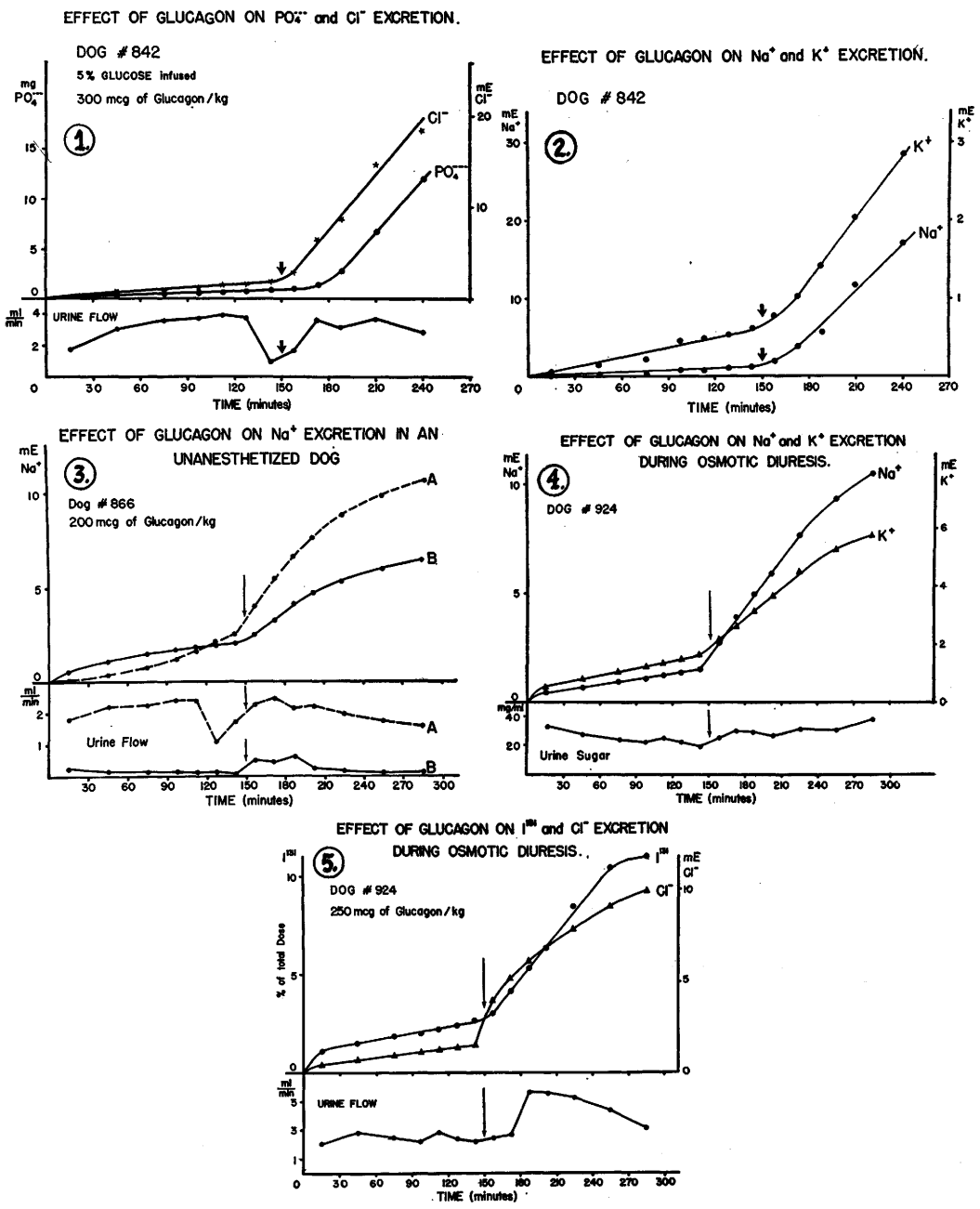

FIG. 1-5.

It was found (Fig. 4 and 5) that even in animals with pre-existing hyperglycemia and glycosuria, glucagon has a marked enhancing effect on the urinary excretion of I^{131}, Cl^-, Na^+ and K^+. Consequently it appears that hyperglycemia and glycosuria *per se* are not responsible for the described electrolyte changes.

Summary and conclusions. It has been shown that glucagon has a marked enhancing effect on renal excretion of I^{131}, Na^+, K^+, Cl^- and PO_4^{---} in both the anesthetized and con-

scious dog. This action of glucagon, which is independent of its hyperglycemic effect, appears to be due to a direct action on the kidney tubules. The physiologic significance of these effects remains to be clarified.

We wish to thank Dr. C. G. Mackenzie and Dr. J. H. Holmes for their interest and advice.

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Received September 28, 1956, P.S.E.B.M., 1957, v94.

Some Factors Which Influence Vesiculase Action. (22855)

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A previous publication(1) described coagulation of proteins of the seminal vesicle secretion by vesiculase, an enzyme of prostatic origin. In experiments conducted at room temperature, coagulation was prevented by ethylenediamine tetraacetic acid (versene), and certain other metal chelating agents. Versene inhibition could be reversed by addition of Mn^{++} ions, and somewhat higher concentrations of Ca^{++} ions, but not by Mg^{++} ions. Heavy metal ions, *e.g.*, Hg^{++}, also abolished coagulation. This paper describes experiments which give further insight into the role of metal ions in the coagulation of semen. The influence of temperature and of a number of substances on the over-all coagulation process has also been studied.

Methods. Turbidity measurements were made with a Coleman Junior spectrophotometer using cuvettes of 0.8 cm light path. Other spectrophotometric measurements were made with Beckmann model D.U. spectrophotometer using quartz cells of 1 cm light path. Protein was determined spectrophotometrically according to Warburg and Christian(2) or by the method of Lowry *et al.*(3). Nitrogen was estimated by the Kjeldahl technic. Dry weights were determined by heating to constant weight at 105°. Total phosphorus was estimated after digestion with sulfuric acid, subsequent clarification with H_2O_2 and hydrolysis in 1 N acid for 10 minutes at 100°, by determination of inorganic phosphorus according to Gomori(4).

Materials. Crystalline proteolytic enzymes, takadiastase and hyaluronidase were of commercial origin. Five times recrystallized soy bean trypsin inhibitor was obtained from Nutritional Biochemicals Corp. 2,3 dimercaptopropanol was purchased from Mann Research Laboratories. Spermine hydrochloride was generously donated by Dr. E. A. Evans, Jr. Heparin (60 units/mg) was obtained from Parke, Davis and Co. Acetone-desiccated proteins of seminal vesicle secretion were prepared as described elsewhere(1). For coagulation studies, the acetone powders were extracted with isotonic NaCl, and a small insoluble residue removed. Ultraviolet absorption spectra of these saline extracts were identical in either phosphate or tris (hydroxymethyl) aminomethane (Tris) buffers, and also in 6.4 M urea, at pH 7. The ratio of absorbancy at 280 mμ to that at 260 mμ ranged from 1.24 to 1.53 (mean 1.38). Mean absorbancy at 280 mμ was 2.64/mg nitrogen/ml and 0.43/mg dry weight/ml. Phosphorus content of acetone dried preparations varied from 0.02% to 0.03%. Crude preparations were obtained by homogenizing fresh coagulating glands in isotonic NaCl, followed by centrifugation at 5,000 x g for 20 minutes at 2°. Partially purified preparations were obtained by fractionation of crude extracts

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