Observations on Sodium Retention Related to Insulin Treatment of Experimental Diabetes

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SUMMARY

Streptozotocin (STZ)-diabetic rats regularly retained sodium (Na^+), and tended to retain potassium (K^+) as well, in response to insulin. Diabetic patients have also been reported to exhibit antinatriuresis and antikaliuresis early in the course of insulin therapy. Insulin-related Na^+ retention can occur without a marked reduction in blood glucose level and does not appear to be attributable to preexisting Na^+ depletion, mineralocorticoid effect, or suppression of glucosuria. The decrease in urinary Na^+ excretion (UNaV) in the rats incident to insulin administration was appreciably greater than the decrease in chloride (Cl^-) or water excretion. The significance of this observation is uncertain. It may be, in part, a consequence of the nephrotoxicity of STZ. Insulin-related Na^+ retention may be closely related pathogenetically to the Na^+ retention of refeeding and may reflect a direct renal action of insulin or, less likely, an alteration of renal tubular metabolism in response to insulin-mediated changes in systemic metabolism. DIABETES *24*:645-49, July, 1975.

When blood glucose concentration falls abruptly in a previously uncontrolled diabetic patient, either because treatment with insulin is started or modified or because diet is more faithfully observed, the transition from poor to good or improved control may be accompanied by retention of Na^+ and water. The frequency with which insulin-related Na^+ retention occurs is unknown, but in its milder manifestations it is probably common. However, case reports documenting this phenomenon are difficult to find in the medical literature.[1,2] The latest edition of a standard text on diabetes devotes one short paragraph to a description of the peripheral edema that may accompany insulin administration but cites no supporting references.[3]

Rarely, the antinatriuresis associated with insulin therapy can be extreme and can cause severe edema. For example, the investigator has seen a young woman who gained 46 lb. and became massively edematous during the ten days following her hospitalization for treatment of diabetic ketoacidosis.

Atchley et al. performed balance studies on two diabetic subjects in whom ketosis was precipitated by withdrawal of insulin.[4] In both subjects, striking reductions were observed in urinary volume and in the rate of urinary Na^+ excretion during the first seventy-two to ninety-six hours after resumption of insulin therapy. Saudek et al. have also studied the antinatriuresis incident to treatment with insulin and have presented evidence that it is unrelated to preexisting Na^+ depletion.[5] Nevertheless, its pathogenesis remains unclear.

Experiments were undertaken in STZ-diabetic rats in an effort to quantitate the Na^+ retention induced by insulin therapy and to clarify its pathogenesis.

MATERIALS AND METHODS

Sixteen male Sprague-Dawley rats, between 250 and 350 gm. body weight, were studied initially. Diabetes was induced by the intraperitoneal injection of 75 mg./kg. of STZ,* and the animals were placed in metabolic cages. During the twenty-four hours following STZ injection, 5 per cent glucose in water was provided as the drinking fluid. Thereafter, ordinary tap water was given ad libitum until the eleventh day of the experiment. One day after administration of STZ, daily intraperitoneal injections of U-40 NPH insulin were started and were continued for thirteen consecutive days. The initial dose was 10 U./100 gm. body weight/day. Subsequent doses ranged from 1 to 5 U./100 gm./day.

After thirteen days of treatment, insulin was withheld for two days, days 15 and 16 of the experiment,

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and was then resumed for eight more days in a fixed dose of 10 U./100 gm./day. Rats 5, 10, and 14 died before completion of the experiment. All data concerning them have therefore been omitted.

Daily morning weights were recorded for each rat throughout the twenty-four days of the experiment. Starting on the eleventh experimental day, i.e., ten days after injection of STZ, the rats were placed on a Na^+-free diet† and isotonic saline was substituted for tap water as the drinking fluid. This permitted performance of Na^+ balance studies from day 11 through day 24, the last day of the experiment. Since the K^+ content of the diet was known and food consumption was measured, K^+ balances were also estimated. Morning blood specimens for plasma glucose were obtained by orbital puncture at weekly intervals.

A second group of seventeen male Sprague-Dawley rats ranging from 280-330 gm. in body weight were given standard laboratory chow and were placed in metabolic cages. After a control period of four days each rat received 75 mg./kg. of STZ intraperitoneally. Thirteen of the rats developed overt diabetes, two others probably became diabetic also but died within two days after STZ administration, and two failed to become diabetic.

The latter four animals have therefore been excluded from further consideration. Twenty-four hours after STZ injection, nine of the diabetic rats were started on insulin and four were sacrificed immediately after being anesthetized and subjected to abdominal aortic puncture for blood chemistries (Na^+, K^+, Cl^-, pH, pCO_2, glucose). Blood chemistries were similarly obtained in four control rats. U-100 NPH insulin was given subcutaneously every morning in the following doses: 10 U./100 gm. body weight/d. for two days; 5 U./100 gm./d. for two days; 2 U./100 gm./d. for three days. Daily weights and daily urinary volumes, pH values and rates of Na^+, K^+, Cl^-, titratable acid, and ammonium (NH_4^+) excretion were determined throughout the control and experimental periods. Each twenty-four-hour urine specimen was collected under mineral oil and preserved with a small amount of phenyl mercuric nitrate. Plasma electrolytes, pH, pCO_2, and glucose were determined in representative rats during the period of insulin therapy.

Plasma glucose concentration was determined by the Glucostat method (Worthington Biochemical, Freehold, N.J.) or by the Beckmann glucose analyzer. Urinary glucose concentration was assayed by Technicon AutoAnalyzer. Plasma and urinary Na^+ and K^+ concentrations were determined by an internal lithium standard flame photometer and plasma and urinary Cl^- concentration by the Buchler-Cotlove chloridometer. Plasma beta-hydroxybutyrate was determined spectrophotometrically (B-hydroxybutyrate dehydrogenase supplied by Boehringer, New York). Keto-Diastix reagent strips (Ames, Elkhart, Ind.) were used to test qualitatively for urinary ketones. Urinary pH was determined with a glass electrode (Corning, Medfield, Mass.), and plasma pH, pCO_2, and pO_2 were measured with electrodes manufactured by Instrumentation Laboratory, Boston. Plasma bicarbonate (HCO_3^-) was calculated from the Henderson-Hasselbalch equation, with the use of a pK of 6.1 and the measured values for pH and pCO_2. Urinary titratable acid was determined by titration to pH 7.4 with 0.1 N NaOH, NH_4^+ by the method of Conway and O'Malley.[6]

RESULTS

Thirteen nonketonuric STZ-diabetic rats (mean blood glucose 421 mg. per 100 ml.) showed a 10 per cent reduction in blood glucose levels and a 30-35 per cent decrease in UGV‡ with treatment. Resumption of insulin therapy after forty-eight hours of insulin deprivation was uniformly accompanied by a significant reduction ($P<0.005$) in UNaV that was disproportionate to the reduction in blood glucose concentration. The decrease in UNaV generally began forty-eight to seventy-two hours after restarting insulin and continued for three to six days. In every instance, the days of maximum antinatriuresis were days 20,21, and 22 of the experiment (corresponding to the fourth, fifth, and sixth days after reinstitution of insulin treatment).§

For eight of the thirteen rats studied, it was possible to get complete balance data from day 15, the day of insulin withdrawal, through day 24, the last experimental day. Figure 1 and tables 1 and 3 relate to the rats for which complete balance data exist.

Figure 1 illustrates the changes in mean values of UNaV, UKV,‡ UClV,‡ UGV, and V‡ after resumption of insulin therapy. For each rat, the mean UNaV

†Sodium-deficient test diet, Hartroft formula, supplied by Biol. Res. Prod., Chagrin Falls, Ohio.

‡UKV = daily urinary K^+ excretion; UClV = daily urinary Cl^- excretion; UGV = daily urinary glucose excretion; V = daily urinary volume.

§It should be noted that UNaV on days 20-22 was significantly less than that on days 11-14, the last days of the initial course of insulin therapy.

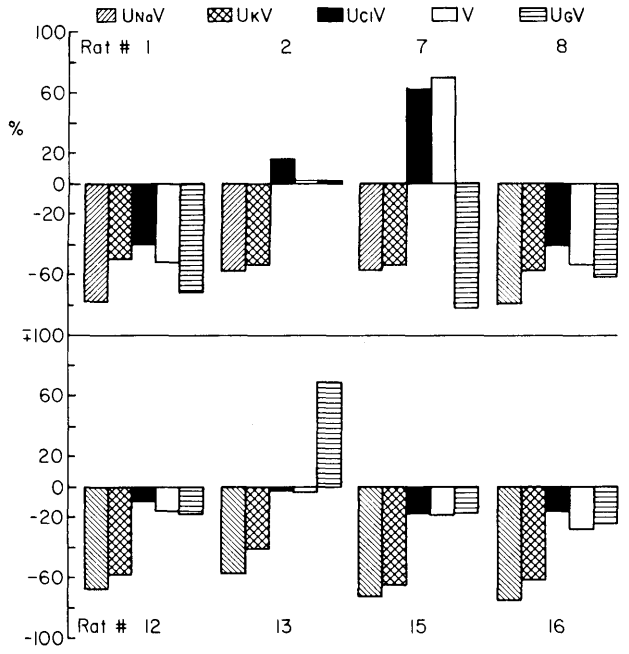

FIG. 1. Effects of insulin therapy on sodium, potassium, chloride, water, and glucose excretion in streptozotocin-diabetic rats.

during the natriuretic period (defined as the interval from start of insulin withdrawal to onset of Na^+ retention) is arbitrarily assigned a value of zero, and the mean decrease in UNaV during maximal antinatriuresis (days 20-22) is plotted downward from zero as a negative percentage. Mean values for UKV, UClV, UGV, and V are expressed in an analogous way. Wherever UClV, UGV, and V increase during antinatriuresis, the increments are plotted upward from zero as positive percentages. Figure 1 illustrates that during antinatriuresis the decrease in UNaV, which ranged from 60 to 80 per cent, was disproportionately greater than the decrease in UClV or V. Further, the fall in UNaV was associated with a comparable fall in UKV. Six of the rats represented in figure 1 showed a 20-80 per cent mean decrease in UGV during days 20-22.

TABLE 1

Relationship of weight gain to positive sodium balance during antinatriuresis (days 20-22)

Rat No.	Cumulative Na^+ Bal. in mEq. (days 20-22)	Weight Change in gm. (days 20-22)	Weight Change in gm. (days 11-14)
1	+ 27.2	+ 22	+ 11
2	+ 68.3	+ 1	− 37
7	+ 63.5	+ 20	+ 12
8	+ 29.2	+ 20	− 6
12	+ 114.7	+ 21	+ 1
15	+ 75.4	+ 5	− 19

One rat (13) actually showed a 70 per cent increase in UGV despite a simultaneous 60 per cent decrease in UNaV. This paradoxical finding is unexplained.

Table 1 shows that the Na^+ retention that occurred on days 20-22 was always accompanied either by accelerated weight gain or by reversal of an earlier pattern of weight loss. For unknown reasons, the increase in weight was not proportional to the amount of Na^+ retained. In table 1 the weight changes during days 20-22 are compared with those during days 11-14, the last four days of the initial thirteen-day course of insulin. During that earlier interval, the diabetes, though not well regulated in most of the animals, was fairly stable.

Table 2 refers to a second group of STZ-diabetic rats, which showed significant reduction in UNaV within twenty-four hours after insulin administration, thus supporting our original observations of insulin-related Na^+ retention.

TABLE 2

Control values for sodium excretion compared with sodium excretion after start of insulin therapy

Rat No.	UNaV (mEq./d.) Mean of 4 Control Days	UNaV (mEq./d.) First Day of Insulin Rx
9	2.83	2.30
10	3.81	1.24
11	2.13	1.88
15	3.05	1.65
16	3.10	2.51
17	2.24	1.24
18	2.85	2.60
19	3.13	1.58
20	2.39	2.30
$\bar{X}$	2.84	1.92
S.E.	0.52	0.53
P	<0.01	

DISCUSSION

Possible Mechanisms of Insulin-Related Na^+ Retention

The Na^+ retention manifested by the STZ-diabetic rats was almost certainly related to the initiation or resumption of insulin therapy because it invariably began within twenty-four to seventy-two hours after the first insulin injection. In many of the rats Na^+ retention was accompanied by K^+ retention. Saudek et al. have recently described antinatriuresis without appreciable changes in plasma Na^+ concentration in six insulin-treated diabetic patients.[5] In five of the six, K^+ retention was also evident. Simultaneous retention of K^+ and Na^+ suggests that the Na^+ retention is not mediated by aldosterone. However, our data are not conclusive and do not unequivocally exclude a mineralocorticoid effect.

Suppression of glucosuria does not account for the increased Na^+ retention since, in several of the rats, insulin treatment was associated with significant decrease in UNaV but little or no change in UGV. It therefore seems unlikely that insulin-related Na^+ retention is obligatorily linked either to increased glucose reabsorption or to cessation of glucose-induced osmotic diuresis.

Table 3 demonstrates that the antinatriuresis and increment in positive Na^+ balance initiated by insulin therapy cannot be ascribed to preexisting Na^+ depletion. In response to insulin, the two rats in positive balance (7 and 15) retained Na^+ to about the same extent as the rats in negative balance. The fact that in the face of a positive balance rats 7 and 15 retained additional Na^+ indicates that hypovolemia is not required to cause insulin-related Na^+ retention.

Our data permit no inferences regarding the role of glucagon suppression in the pathogenesis of the Na^+ retention accompanying insulin therapy.

Insulin-related Na^+ retention may be similar pathogenetically to the Na^+ retention that occurs with the refeeding of normal fasting subjects.[7] In several ways, the amelioration of diabetes produced by insulin administration resembles the termination of a fast by carbohydrate. In both situations, plasma insulin concentration rises, plasma glucagon falls, and hepatic gluconeogenesis and the mobilization and oxidation of fatty acids are suppressed. Lemann et al. have shown that glucose refeeding of healthy fasting men rapidly changes the pattern of urinary electrolyte and acid excretion.[8] Filtered Na^+, K^+, and Cl^- are more completely reabsorbed, excretion of HCO_3^- decreases, and excretion of calcium (Ca^{++}) and magnesium (Mg^{++}) increases without any change in the rate of phosphaturia. It is probable that significant modifications of renal tubular cell metabolism are required to achieve these complex alterations in electrolyte and hydrogen ion (H^+) excretion. If insulin-related Na^+ retention is based on analogous changes in renal tubular metabolism, such changes could result from a direct renal action of insulin or could represent a tubular response to insulin-induced changes in extrarenal metabolism. These two possibilities are, of course, not mutually exclusive. There is some evidence that at physiologic concentrations insulin can cause antinatriuresis by a direct effect on the renal tubules. Nizet et al. perfused isolated dog kidneys with homologous blood containing insulin at concentrations of approximately 100 μU./ml., while GFR and RBF were held constant, and observed decreases in Na^+ and K^+ excretion of 20-25 per cent.[9] Thus, it is possible that insulin stimulates a specific component of renal Na^+ reabsorption, perhaps by facilitating the entry of circulating glucose into certain tubular cells, and that this action may be the principal cause of insulin-related Na^+ retention.

TABLE 3

Cumulative sodium balance data before and during the antinatriuretic period (days 20-22)

Rat No.	Cumulative Na^+ Bal. in mEq. (days 11-19)	Cumulative Na^+ Bal. in mEq. (days 20-22)
1	− 6.1	+ 27.2
2	− 14.6	+ 68.3
7	+ 19.0	+ 63.5
8	− 10.2	+ 29.2
12	− 7.8	+ 114.7
15	+ 4.1	+ 75.4
13*	− 11.5	+ 33.0
16*	− 1.9	+ 71.2

*Sodium balance data available only from day 15 on.

Possible Implications of Selective Na^+ Retention

In quantitative terms, the selective retention of Na^+ relative to Cl^- caused by insulin treatment was highly significant. In our original study, UClV during antinatriuresis exceeded the sum of UNaV and UKV by 10-35 mEq./d./rat. This suggested that some cation other than Na^+ or K^+ was excreted with Cl^- in order to maintain electroneutrality of the urine. It seemed likely that the only cation that could be excreted in sufficient quantity to fill a urinary cationic gap of this magnitude was H^+ in the form of NH_4^+. Any stimulus to the renal excretion of large amounts of H^+ might be expected to generate a metabolic alkalosis. However, studies of insulin treatment in a second group of mildly ketonemic, STZ-diabetic rats have failed to document the development of metabolic alkalosis.

It is worth noting that STZ is a nephrotoxic drug and has been reported to cause both acute renal failure and a variety of disturbances of tubular function.[10,11] Hence it is conceivable that selective Na^+ retention relative to Cl^- is an artifact resulting from unequal inhibitory effects of STZ on the reabsorption of these ions. Nevertheless, the central thesis that the Na^+ retention is related to insulin therapy seems valid since retention occurred only when the effects of insulin were superimposed on those of STZ.

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REFERENCES

[1]Griep, A.H.: Insulin edema: report of a case. J. Indiana State Med. Assoc. *48*:872, 1955.

[2]Kirtley, W.R.: Rapid correction of metabolism defect may cause edema in diabetes: report of a case. J. Indiana State Med. Assoc. *48*:1290-91, 1955.

[3]Ellenberg, M., and Rifkin, H.: Diabetes Mellitus: Theory and Practice. New York, McGraw-Hill, 1970, p. 645.

[4]Atchley, D.W., Loeb, R.F., Richards, D.W., Jr., Benedict, E.M., and Driscoll, M.E.: On diabetic acidosis: a detailed study of electrolyte balances following withdrawal and reestablishment of insulin therapy. J. Clin. Invest. *12*:297-326, 1933.

[5]Saudek, C.D., Boulter, P.R., Knopp, R.H., and Arky, R.A.: Sodium retention accompanying insulin treatment of diabetes mellitus. Diabetes *23*:240-46, 1974.

[6]Conway, E.J., and O'Malley, E.: Microdiffusion methods. Ammonia and urea using buffered absorbents (revised methods for ranges greater than 10 μg. N). Biochem. J. *36*:655-61, 1942.

[7]Weinsier, R.L.: Fasting—A review with emphasis on the electrolytes. Am. J. Med. *50*:233-40, 1971.

[8]Lemann, J., Jr., Lennon, E.J., Piering, W.R., Prien, E.L., Jr., and Ricanati, E.S.: Evidence that glucose inhibits net renal tubular reabsorption of calcium and magnesium in man. J. Lab. Clin. Med. *75*:578-85, 1970.

[9]Nizet, A., Lefebvre, P., and Crabbe, J.: Control by insulin of sodium, potassium, and water excretion by the isolated dog kidney. Pflügers Arch. *323*:11-20, 1971.

[10]Schreibman, P.H., DeKoliren, L.G., and Arky, R.A.: Metastatic insulinoma treated with streptozotocin. Ann. Intern. Med. *74*:399-403, 1971.

[11]Sadoff, L.: Nephrotoxicity of streptozotocin. Cancer Chemotherapy Rep. *54*:457-59, 1970.