Biochemical Pharmacology, Vol. 46, No. 12, pp. 2269–2276, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

EFFECT OF SODIUM ORTHOVANADATE ON THE HEPATOBILIARY CLEARANCE OF ROSE BENGAL IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

JOHN B. WATKINS III,* MARK E. BAUMAN and TIMOTHY M. BEATY
Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 47405, U.S.A.

(*Received* 13 *May* 1993; *accepted* 12 *August* 1993)

Abstract—Sodium orthovanadate is known to promote glucose uptake in muscle and adipose tissues and has been suggested as a possible oral hypoglycemic agent. In addition, insulin-dependent diabetes has been shown to alter the hepatobiliary clearance of several drugs in rats. This study has determined whether orthovanadate, like insulin, can reverse diabetes-induced changes in the biliary excretion of endogenous bile acids and in the hepatobiliary clearance of rose bengal. Six groups of male Sprague–Dawley rats were used: normal, insulin-treated normal, vanadate-treated normal, diabetic, insulin-treated diabetic, and vanadate-treated diabetic. Diabetes was induced by injection of streptozotocin (45 mg/kg, i.v.). One week later, insulin (2–4 U/day, s.c.) and sodium orthovanadate (877 ± 82 μmol/kg/day, p.o.) treatments were initiated. After 4 weeks, the clearance and biliary excretion of rose bengal (60 μmol/kg, i.v.) were determined for 3 hr. Bile flow rate, rose bengal excretion, and excretion of endogenous bile acids were unchanged in the two treated normal groups and in the insulin-treated diabetic rats. These parameters were increased in untreated diabetic and vanadate-treated diabetic rats as compared with normal. Pharmacokinetic analyses indicated that total and biliary clearances of rose bengal were increased in diabetic rats and that orthovanadate did not reverse these changes. However, liver weight and serum glucose concentrations were reduced by orthovanadate treatment. These data indicate that the oral insulinomimetic chemical sodium orthovanadate effectively reversed some, but not all, of the diabetes-induced alterations of hepatic function.

Key words: bile acids; bile production; biliary excretion; clearance, hepatobiliary; diabetes, insulin-dependent; orthovanadate, sodium; pharmacokinetics; rose bengal; streptozotocin

Vanadium, the 21st most abundant element in the earth's crust, is present in varying concentrations in almost all mammalian cells [1, 2]. Vanadium exists in body fluids predominantly in the 5^+ oxidation state as vanadate, VO_3^-, which is structurally similar to phosphate, as well as in the 4^+ state [2, 3]. In intracellular compartments such as hepatocytes and erythrocytes, vanadium exists practically in the 4^+ state only [3]. One mechanism for its *in vivo* actions may be its ability to mimic the structure of inorganic phosphate in important intracellular phosphoenzymes [4]. Other studies have examined the insulinomimetic properties of vanadate in adipocytes [5, 6], skeletal muscle [7], and hepatocytes [8]. In fact, oral administration of orthovanadate to diabetic rats prevents the decline in cardiac function resulting from diabetes [9]. Two possible mechanisms for the improvement in cardiac performance involve the role of vanadate as an endogenous regulator of certain enzymes, particularly Na^+,K^+-ATPase [10] and adenylate cyclase [11], or of cellular calcium fluxes [12]. In addition, a number of reports indicate that oral administration of orthovanadate could also partially normalize blood glucose concentrations in streptozotocin-induced diabetic rats [9, 13–16] or in insulin-resistant diabetic *ob/ob* mice [17]. Moreover, oral vanadate therapy over 2 months reduces glycosylated hemoglobin levels, activates glycolysis, and depresses gluconeogenesis in streptozotocin-induced diabetic rats [18]. All of these studies provide evidence that dietary orthovanadate can be somewhat effective as an oral agent for treating diabetes.

Insulin-dependent diabetes has been shown to induce changes in hepatic uptake, biotransformation, and biliary excretory function [19]. Several studies indicate that bile flow changes in response to diabetogen treatment; flow is depressed for 1–2 weeks after treatment with alloxan or streptozotocin [20–23], but returns to normal 3–4 weeks after induction of diabetes [23–27]. Two examples of the temporary impairment of hepatobiliary function immediately after streptozotocin injection are the decreased biliary excretion of both sulfobromophthalein [22] and rose bengal (Watkins JB III and Sanders RA, unpublished results). Several studies in which experiments were performed 4–5 weeks following streptozotocin-induced diabetes in order to minimize abnormalities that may be due to diabetogen toxicity have demonstrated alterations in the biliary excretion of several organic anions (acetaminophen, rose bengal, indocyanine green, bromcresol green, bilirubin), an organic cation (procainamide ethobromide) and two neutral organic compounds (ouabain, digoxin) [23, 25–28]. In particular, maximal biliary excretion rate and total clearance of rose bengal were increased 390 and 65%, respectively, in the diabetic rat [26], whereas

* Corresponding author. Tel. (812) 855-3201; FAX (812) 855-4436.

non-biliary clearance of rose bengal was not altered by insulin-dependent diabetes (Watkins JB III and Sanders RA, unpublished results).

Many changes induced by diabetes are reversible with insulin administration, but whether the purported insulinomimetic agent sodium orthovanadate can reverse changes in hepatobiliary function is unknown. One study has examined the effects of vanadate on bile production, but excretion of endogenous and exogenous chemicals was not determined [29]. Therefore, the present study was designed to determine whether oral sodium orthovanadate administration, like insulin therapy, would reverse diabetes-induced alterations in hepatobiliary function. Diabetes was induced 4–5 weeks before experimentation by administration of streptozotocin. Biliary excretion of the xenobiotic rose bengal and endogenous bile acids was determined in normal and diabetic rats treated with either insulin or sodium orthovanadate. Rose bengal was chosen as the test xenobiotic because the entire change in total clearance caused by diabetes results from altered biliary clearance. Pharmacokinetic parameters were calculated to detect alterations in rose bengal disposition in the different treatment groups.

MATERIALS AND METHODS

Chemicals. A glucose diagnostic kit, 3α-hydroxysteroid dehydrogenase, β-nicotinamide adenine dinucleotide, rose bengal, sodium orthovanadate, streptozotocin, taurocholate, Trizma base and urethane were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest quality available. Deionized water was used for all studies.

Animals. Six groups of male Sprague–Dawley rats (Harlan Sprague–Dawley, Inc., Indianapolis, IN) identified as normal, normal plus insulin, normal plus orthovanadate, diabetic, diabetic plus insulin, and diabetic plus orthovanadate were housed in stainless steel cages in groups of three or four, under a 12-hr light–dark cycle in a temperature-controlled (21–28°) room. The animals were provided Purina Laboratory Rodent Chow No. 5012 (St. Louis, MO) and water *ad lib.* until they were used. All housing and treatments were in compliance with the Public Health Service Guide for the Care and Use of Laboratory Animals.

Rats, weighing 250–275 g and under light halothane anesthesia at the time of dosing, were injected with streptozotocin at 45 mg/kg, i.v., to induce insulin-dependent diabetes. Streptozotocin was dissolved in freshly prepared 0.01 M sodium citrate, pH 4.5, immediately before injection into a saphenous vein. Blood glucose levels were measured periodically throughout the treatment period beginning 3 days after streptozotocin injection and ending on the day of surgery, using the hexokinase diagnostic kit available from the Sigma Chemical Co. A minimum serum glucose concentration of 400 mg/dL was required for consideration as a diabetic rat. There was no mortality associated with this dose of streptozotocin or with the prolonged untreated diabetes and markedly elevated hyperglycemia. One week after injection of streptozotocin, treatment with sodium orthovanadate (0.4 mg/mL in water adjusted to pH 7 with 1.75 g ascorbic acid/L) was available in the drinking water for 18–24 days. To make the taste more palatable, 10 g Tang instant breakfast drink was added per liter. The diabetic rats consumed an average daily dose of 877 ± 82 μmol/kg body weight/day of sodium orthovanadate. The insulin-treated diabetic group received 2–4 U/day, s.c., protamine zinc iletin I insulin (Eli Lilly & Co., Indianapolis, IN) for 22–24 days. Insulin dosage was adjusted periodically as needed to achieve normoglycemia. Normal rats (200–225 g) consumed an average 472 ± 17 μmol/kg/day, p.o., of sodium orthovanadate as a 0.8 mg/mL solution or were treated with 2 U insulin/day. The difference in orthovanadate dosage between normal plus orthovanadate and diabetic plus orthovanadate resulted primarily from the smaller volume of the 0.8 mg/mL solution consumed by the normal plus orthovanadate group. Diabetic rats consumed three to seven times the volume of the normal plus orthovanadate rats. Higher concentrations of sodium orthovanadate caused severe morbidity or death and were not used.

Biliary excretion studies. Immediately after anesthetization with urethane (1.2 g/kg, i.p.), a femoral artery was cannulated with PE-50 tubing for subsequent collection of blood samples in which serum drug concentrations were measured. The bile duct was isolated through a midline abdominal incision and was cannulated with PE-10 tubing. Body temperature was maintained at 37° with a heat lamp to prevent hypothermic alteration of biliary excretion. A 15-min bile sample was obtained prior to drug administration in order to determine basal bile flow and bile acid excretion rates. Bile flow was continuous, and samples were collected every 15 min for 60 min, and then every 30 min for 120 min after rose bengal administration. Approximately 25 min after injection of the anesthetic (10 min for narcosis and surgery and a 15-min pre-drug bile collection period), rose bengal (60 μmol/kg, i.v.; 4 mL water/kg) was injected into a saphenous vein in less than 20 sec. Blood samples (200–250 μL) were collected at 2, 5, 10, 20, 30, 45, 60, 75, 90, and 120 min after injection. Blood volume was maintained by infusion of an equal volume (250 μL) of isotonic saline.

Bile flow rate was measured gravimetrically assuming a density of 1.0 and expressed as microliters per minute per gram liver. Bile acid concentration was determined enzymatically by the hydroxysteroid dehydrogenase method of Paumgartner *et al.* [30] with sodium taurocholate (10–150 μmol/mL) as the standard. Rose bengal concentrations in serum and bile were determined spectrophotometrically at 550 nm after appropriate dilution of the samples with deionized water using a standard curve (0.25 to 15 nmol/mL). Biliary excretion was calculated as the product of biliary concentration times bile flow rate and expressed as nanomoles per minute per gram liver.

Pharmacokinetics and statistics. Plasma concentration versus time data were fit by PCNONLIN (Statistical Consultants, Lexington, KY) to the following biexponential equation: Concn =

$Ae^{-\alpha t} + Be^{-\beta t}$ where A and α are the Y-intercept and rate constant for the distribution phase and B and β are the Y-intercept and rate constant for the elimination phase. The area under the curve and area under the moment curve (which was calculated with the integrated equation of the model) were determined, permitting calculation of the steady-state volume of distribution (dose times area under the moment curve divided by the area under the curve squared) and total clearance (dose divided by area under the curve) for each rat [31]. Total excretion into bile was quantified, and biliary clearance was calculated as cumulative excretion divided by area under the curve. Non-biliary clearance, which represents primarily renal clearance, may be calculated as the difference between total and biliary clearance.

Means and standard errors were calculated for all data. Significant differences were determined using a one-way analysis of variance and Duncan's least significant difference test to compare the means. Bartlett's test was used to be certain of homogeneity of variance. Asterisks (*) indicate that the value of any group was different from that of untreated normal rats at $P < 0.05$, whereas a dagger (†) indicates that the value in a treated diabetic group was significantly different from the untreated diabetic rats at $P < 0.05$.

RESULTS

Liver weight to body weight ratio, basal bile flow and bile acid excretion rates, and serum glucose concentrations were elevated in the diabetic and in the orthovanadate-treated diabetic groups (Table 1). These parameters returned to normal in the insulin-treated diabetic rats. When the increase in liver weight is considered for the diabetic rats and bile flow is calculated per g liver, there was no difference in basal bile flow rate (1.46 ± 0.12 in normal vs $1.34 \pm 0.10\ \mu L/min/g$ in diabetic rats). However, bile flow rate was augmented in the orthovanadate-treated diabetic rats by either 49 or 20% over normal when expressed per kg body weight or per g liver, respectively. Insulin and orthovanadate treatments did not affect any of these parameters in the normal rats. Finally, diabetes-induced changes in liver weight and serum glucose concentrations were reduced significantly by orthovanadate treatment when compared with diabetic rats, but not completely to normal levels.

Figure 1 shows the decrease in serum rose bengal concentration with time in the six experimental groups. Concentrations were similar in the three normal groups and the insulin-treated diabetic rats. Serum disappearance was more rapid in the diabetic and orthovanadate-treated diabetic rats, and serum rose bengal concentrations were not different between these two groups.

Figure 2 illustrates biliary excretion versus time profiles for rose bengal (top panel) and bile acids (middle panel), and bile flow rate versus time (bottom panel). Bile flow rate and the biliary excretion of bile acids and rose bengal were increased in both the diabetic and orthovanadate-treated diabetic groups throughout most of the 3-hr

Table 1. Effect of orthovanadate treatment on selected parameters

	Body weight (g)	Liver weight (g)	Liver weight: Body weight Ratio	Basal bile flow: (μL/min/kg)	Basal bile flow: (μL/min/g)	Bile acid excretion (nmol/min/kg)	Serum glucose (mg/dL)
Normal	325 ± 5	11.8 ± 0.44	3.63 ± 0.09	53.2 ± 4.1	1.46 ± 0.12	2700 ± 220	110 ± 29
Normal + insulin	336 ± 4	12.8 ± 0.78	3.81 ± 0.19	60.8 ± 3.1	1.59 ± 0.08	2980 ± 336	125 ± 12
Normal + orthovanadate	331 ± 4	12.9 ± 0.45	3.89 ± 0.11	58.6 ± 4.9	1.50 ± 0.13	2360 ± 273	121 ± 7.2
Diabetic	303 ± 20	15.6 ± 0.41*	5.15 ± 0.04*	68.5 ± 3.9*	1.34 ± 0.10	5570 ± 849*	866 ± 75*
Diabetic + insulin	406 ± 8*†	14.2 ± 0.58*†	3.50 ± 0.07†	49.9 ± 2.7†	1.43 ± 0.08	2200 ± 233†	147 ± 25†
Diabetic + orthovanadate	290 ± 11*	13.3 ± 0.35*†	4.59 ± 0.03*†	79.6 ± 3.8*	1.74 ± 0.09*†	4810 ± 536*	453 ± 60*†

Values are means ± SEM for five to seven rats.
* Significantly different from normal rats at $P < 0.05$.
† Significantly different from untreated diabetic rats at $P < 0.05$.

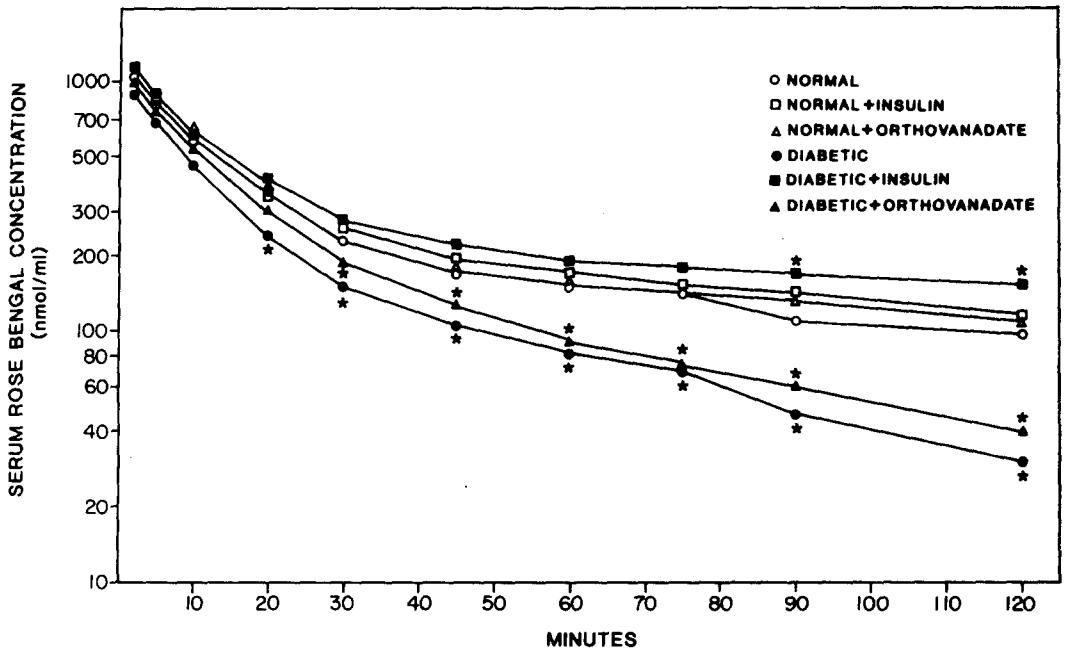

Fig. 1. Effect of sodium orthovanadate on the serum concentration of rose bengal in normal and streptozotocin-induced diabetic rats. Diabetes was induced 4–5 weeks before experimentation to induce insulin-dependent diabetes by i.v. injection of streptozotocin (45 mg/kg). Sodium orthovanadate (mean ± SEM daily oral consumption of 877 ± 82 μmol/kg/day, p.o., for diabetic rats and 472 ± 17 μmol/kg/day, p.o., for normal rats) was initiated 1 week after induction of diabetes and continued for 18–24 days. Insulin-treated rats received 2–4 U of protamine zinc insulin per day. Symbols represent means ± SEM for five to seven rats. Key: (*) significant difference from untreated normal at $P < 0.05$.

experiment. These changes were reversed completely by insulin treatment. Bile flow, bile acids excretion, and rose bengal elimination were similar in all three normal groups as well as the insulin-treated diabetic rats.

The pharmacokinetic analysis presented in Table 2 indicates that total and biliary clearances were elevated in the diabetic and in the orthovanadate-treated diabetic rat groups. Clearance values were similar in the three normal groups and the insulin-treated diabetic rats. Steady-state volume of distribution and terminal half-life were not significantly different among the groups.

DISCUSSION

Vanadate ions can be considered low-molecular-weight analogs of phosphate capable of mimicking many of the rapid actions of insulin. When administered orally to diabetic hyperglycemic rats, vanadate is absorbed into the circulation, stimulates glucose transport and metabolism, and helps shift intracellular metabolism from a catabolic to a partial anabolic state and may even promote normoglycemia [32]. Vanadate appears to restore tissue responsiveness to insulin and activates new synthesis of key enzymes involved in carbohydrate metabolism. Although the mechanism by which vanadate mimics insulin is ill-defined, much data support a theory that vanadate activates glucose metabolism by an insulin-independent mechanism or by skirting the early events of the insulin-dependent cascade [1, 2, 32].

Shechter [32] noted the attractiveness of obtaining an orally active insulinomimetic agent that uses an alternative pathway, and recommended that further studies were needed to elucidate the level of vanadate toxicity over chronic treatment and to search for new agents that could be coadministered with vanadate or insulin to achieve normoglycemia. Ideally, however, any new oral hypoglycemic agent must be able to reverse almost all of the complications of diabetes. Although short-term vanadate treatment clearly reverses cardiac problems in streptozotocin-induced diabetic rats [9], helps normalize glucose tolerance and protects pancreatic islet cells from destruction by streptozotocin [33], the present study is the first to examine whether oral orthovanadate therapy normalizes diabetes-induced alterations in hepatobiliary function. The present data indicate that the excretion of rose bengal and endogenous bile acids (Fig. 2) was increased in untreated diabetic and vanadate-treated diabetic rats as compared with normal or insulin-treated diabetic rats. The increased total and biliary clearances of rose bengal in diabetic rats were not reversed by orthovanadate therapy (Table 2). Moreover, oral sodium orthovanadate did not completely reverse diabetes-induced alterations of basal bile flow rate or excretion of endogenous bile acids.

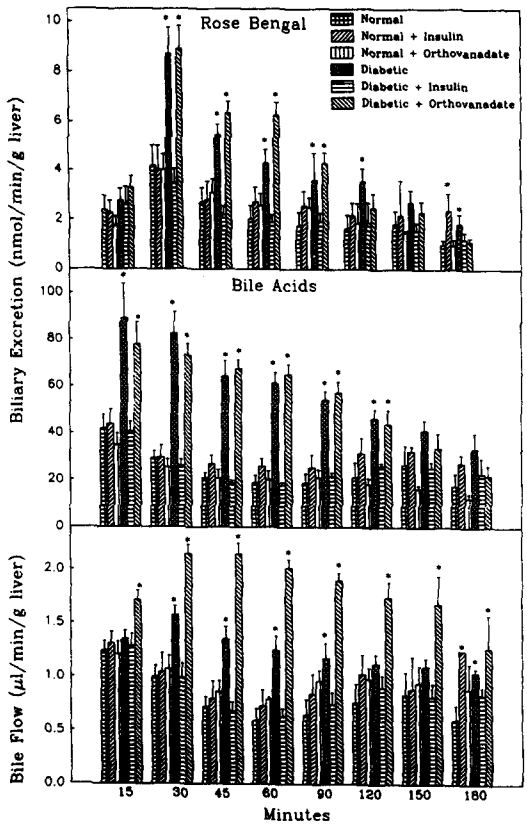

Fig. 2. Effect of sodium orthovanadate on bile flow rate and the biliary excretion of bile acids and rose bengal in normal and streptozotocin-induced diabetic rats. Diabetes was induced 4–5 weeks before experimentation to induce insulin-dependent diabetes by i.v. injection of streptozotocin (45 mg/kg). Sodium orthovanadate (mean ± SEM daily oral consumption of 877 ± 82 µmol/kg/day, p.o., for diabetic rats and 472 ± 17 µmol/kg/day, p.o., for normal rats) was initiated 1 week after induction of diabetes and continued for 18–24 days. Insulin-treated rats received 2–4 U of protamine zinc insulin per day. Symbols represent means ± SEM for five to seven rats. Key: (*) significant difference from untreated normal at $P < 0.05$.

One other study that examined the effect of vanadate, a potent vasoconstrictor, on hepatic hemodynamics and bile production has demonstrated that vanadate increases hepatic vascular resistance in a dose-dependent manner [29]. These workers also showed that there was no change in bile production until hepatic oxygen consumption was diminished. They concluded that the vanadate-induced reduction in bile flow rate was due to hypoxia caused by the direct action on vascular smooth muscle resulting in decreased vascular perfusion and not to any inhibition of Na^+,K^+-ATPase, an enzyme involved in production of the so-called bile acid-independent fraction of bile secretion. The lack of a reduction in bile flow in the present study may be interpreted as demonstrating that the dose of orthovanadate was too low to compromise hepatic perfusion. Finally, vanadyl ions, produced intracellularly from vanadate, are less effective inhibitors of Na^+,K^+-ATPase [1].

It is clear from this (Table 1 and Fig. 2) and other studies [34–36] that uncontrolled diabetes increases bile acid formation. In fact, recent work has demonstrated that an alternative biosynthetic pathway of cholic acid via 3α,7α-dihydroxy-5β-cholestane is accelerated by diabetes, and that 2 weeks of oral vanadate treatment partially cancels the increased cholic acid production in diabetic rats similarly to insulin therapy [36]. Although the mechanism by which vanadate alters bile acid metabolism is not fully understood, vanadate does not increase the serum concentration of insulin and is not functioning via an insulin-dependent pathway [37].

Recent work has demonstrated that the hypoglycemic action of vanadate is both dose and time dependent. Pederson *et al.* [33] indicate that the glucose-lowering effect could be seen as early as 3 days after onset of therapy, but that complete normalization did not occur until after 3 weeks. Sekar *et al.* [18] noted that normoglycemia was achieved 5 days after the onset of oral vanadate therapy and could be sustained for 2 months, but that hyperglycemia returned rapidly upon discontinuation of therapy. However, in our work and in other studies [36, 38], vanadate-treated rats remained somewhat hyperglycemic in spite of a clear significant reduction in serum glucose concentrations from the levels seen in the untreated diabetic rats. Most studies having good normoglycemic responses from oral orthovanadate therapy did not have diabetic rats with as high a glucose level as in the present study (glucose concentrations of 250–400 mg/dL versus 866 ± 75 in Table 1). Moreover, our criteria for use as a diabetic rat is a blood sugar concentration of greater than 400. It may be that the effectiveness of vanadate diminishes with increasing severity of the disease. Also, the addition of Tang Breakfast Drink to the vanadate solution could have contributed to the remaining hyperglycemia.

Combination of sodium orthovanadate with ascorbic acid, as done in the present and another study [38], could have beneficial effects by reducing oxidative stress in diabetes [39]. In contrast, reducing agents like ascorbate can stimulate the oxidation of NADH by V^{5+}, suggesting that the biological effects of vanadium may be mediated via oxidative mechanisms [40]. For example, the insulinomimetic effect of vanadate can be augmented by hydrogen peroxide [41]. However, consumption of the vanadate-ascorbate solution by normal rats caused no apparent changes in hepatobiliary function (Table 1, Figs. 1 and 2). Nevertheless, the effects of ascorbate on free radical production are complex, and the relative pro- or anti-oxidant action of ascorbate may depend on doses and routes of administration of ascorbate and vanadate, as well as the severity of diabetes. Further work is clearly necessary before ascorbate–vanadate interactions are understood completely and before unequivocal therapeutic benefits to diabetics can be demonstrated.

Higher doses of orthovanadate, which were

Table 2. Pharmacokinetics of rose bengal disposition

	Cl_{total} (mL/min/kg)	$Cl_{biliary}$	Vd_{ss} (mL/kg)	Half-life (min)
Normal	1.65 ± 0.27	0.28 ± 0.06	149 ± 17	92.4 ± 17
Normal + insulin	1.31 ± 0.09	0.30 ± 0.08	151 ± 22	100 ± 18
Normal + orthovanadate	1.29 ± 0.11	0.28 ± 0.05	155 ± 19	116 ± 13
Diabetic	2.36 ± 0.47*	1.22 ± 0.31*	191 ± 63	127 ± 49
Diabetic + insulin	1.22 ± 0.21†	0.21 ± 0.04†	147 ± 23	112 ± 22
Diabetic + orthovanadate	2.54 ± 0.24*	1.20 ± 0.13*	124 ± 39	54.3 ± 18

Values are means ± SEM for five to seven rats.
* Significantly different from untreated normal rats at $P < 0.05$.
† Significantly different from untreated diabetic rats at $P < 0.05$.

administered in an attempt to achieve greater reductions in hyperglycemia, were not tolerated by the diabetic rats in our study. In fact, severe diarrhea and death were noted when the concentration was 0.8 mg/mL [9], but this toxicity was prevented when the solution was buffered to pH 7.0 [38]. Apparently, at neutral pH some vanadate exits as vanadyl ion (4^+), which is significantly less toxic than vanadate (5^+) and still capable of diminishing the diabetic state in the rat by substituting for insulin and/or enhancing the effects of endogenous insulin [42]. Unfortunately, more recent reports indicate that oral vanadium in all forms (metavanadate, orthovanadate or vanadyl sulfate) elicited toxicity that ranged from decreased weight gain and increased serum concentrations of urea and creatinine to death [43]. Clearly then, the use of vanadium salts as adjuncts to insulin therapy for insulin-dependent diabetic patients must carefully balance therapeutic versus toxic actions of the agents. Moreover, no studies to date have ascertained the effect of combined therapies (insulin plus vanadate) on hepatic function.

The lack of effective reversal of diabetes-induced changes in hepatobiliary function by oral sodium orthovanadate, as well as its toxicity, suggests that its use as an independent oral hypoglycemic agent is probably limited. However, there is the potential for low dose vanadate plus insulin therapy to prove beneficial and, therefore, continued study is warranted. More recent studies have noted that peroxovanadium compounds [44, 45], selenite [46], molybdate and tungstate [47], zinc ion [48] and chromium [49] can all exert insulin-like effects *in vitro*. Further research is needed to establish the therapeutic efficacy of these insulinomimetic chemicals *in vivo* and to elucidate the detailed mechanism by which these agents act. Development of an orally active insulinomimetic agent capable of utilizing an alternative pathway to insulin is an attractive goal, and efforts to develop promising compounds as new drugs for managing diabetes mellitus should continue.

Acknowledgements—This work was supported by grants from the Indiana Affiliate of the American Diabetes Association and the national American Diabetes Association. The authors are deeply indebted to Mae Bay and Ruth Sanders for outstanding technical assistance.

REFERENCES

1. Nechay BR, Mechanisms of action of vanadium. *Annu Rev Pharmacol Toxicol* **24**: 501–524, 1984.
2. Nechay BR, Nanninga LB, Nechay PSE, Post RL, Grantham JJ, Macara IG, Kubena LF, Phillips TD and Nielsen FH, Role of vanadium in biology. *Fed Proc* **45**: 123–132, 1985.
3. Bruech M, Quintanilla ME, Legrum W, Koch J, Netter KJ and Fuhrmann GF, Effects of vanadate on intracellular reduction equivalents in mouse liver and the fate of vanadium in plasma, erythrocytes and liver. *Toxicology* **31**: 283–295, 1984.
4. Willsky GR, White DA and McCabe BC, Metabolism of added orthovanadate to vanadyl and high-molecular-weight vanadates by *Saccharomyces cerevisiae*. *J Biol Chem* **259**: 13273–13281, 1984.
5. Dubyak GR and Kleinzeller A, The insulin-mimetic effects of vanadate in isolated rat adipocytes: Dissection from effects of vanadate as a (Na^+-K^+)ATPase inhibitor. *J Biol Chem* **255**: 5306–5312, 1980.
6. Duckworth WC, Solomon SS, Lieonieks J, Hamel FG, Hand S and Peavy DE, Insulin-like effects of vanadate in isolated rat adipocytes. *Endocrinology* **122**: 2285–2289, 1988.
7. Rossetti L and Laughlin MR, Correction of chronic hyperglycemia with vanadate, but not with phlorizin, normalizes *in vivo* glycogen repletion and *in vitro* glycogen synthase activity in diabetic skeletal muscle. *J Clin Invest* **84**: 892–899, 1989.
8. Gil J, Miralpeix M, Carreras J and Bartrons R, Insulin-like effects of vanadate on glucokinase activity and fructose 2,6-bisphosphate levels in the liver of diabetic rats. *J Biol Chem* **263**: 1868–1871, 1988.
9. Heyliger CE, Tahiliani AG and McNeill JH, Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. *Science* **227**: 1474–1477, 1985.
10. Aiton JF and Cramb G, The effects of vanadate on rabbit ventricular muscle adenylate cyclase and sodium pump activities. *Biochem Pharmacol* **34**: 1543–1548, 1985.
11. Grupp G, Grupp I, Johnson CL, Wallick ET and Schwartz A, Effect of vanadate on cardiac contraction

and adenylate cyclase. *Biochem Biophys Res Commun* **88**: 440–447, 1979.
12. Takeda K, Temma K and Akera T, Inotropic effects of vanadate in isolated rat and guinea-pig heart under conditions which modify calcium pools involved in contractile activation. *J Pharmacol Exp Ther* **222**: 132–139, 1982.
13. Challiss RAJ, Leighton B, Lozeman FJ, Budohoski L and Newsholme EA, Effects of chronic administration of vanadate to the rat on the sensitivity of glycolysis and glycogen synthesis in skeletal muscle to insulin. *Biochem Pharmacol* **36**: 357–361, 1987.
14. Meyerovitch J, Farfel Z, Sack J and Shechter Y, Oral administration of vanadate normalizes blood glucose levels in streptozotocin-treated rats: Characterization and mode of action. *J Biol Chem* **262**: 6658–6662, 1987.
15. Brichard SM, Okitolonda W and Henquin J-C, Long term improvement of glucose homeostasis by vanadate treatment in diabetic rats. *Endocrinology* **123**: 2048–2053, 1988.
16. Pugazhenthi S and Khandelwal RL, Insulin-like effects of vanadate on hepatic glycogen metabolism in nondiabetic and streptozotocin-induced diabetic rats. *Diabetes* **39**: 821–827, 1990.
17. Brichard SM, Bailey CJ and Henquin J-C, Marked improvement of glucose homeostasis in diabetic *ob/ob* mice given oral vanadate. *Diabetes* **39**: 1326–1332, 1990.
18. Sekar N, Kanthasamy S, William S, Subramanian S and Govindasamy S, Insulinic actions of vanadate in diabetic rats. *Pharmacol Res* **22**: 207–217, 1990.
19. Watkins JB III and Sanders RA, The effects of diabetes on hepatobiliary function. In: *Biliary Excretion of Drugs and Other Chemicals* (Eds. Siegers C-P and Watkins JB III), Vol. 8, Chap. 5–6, pp. 475–496. Fischer, Stuttgart, 1991.
20. Carnovale CE, Marinelli RA and Rodriguez-Garay EA, Bile flow decrease and altered bile composition in streptozotocin-treated rats. *Biochem Pharmacol* **35**: 2625–2628, 1986.
21. Carnovale CE, Marinelli RA and Rodriguez-Garay EA, Toxic effect of streptozotocin on the biliary secretion of nicotinamide-treated rats. *Toxicol Lett* **36**: 259–265, 1987.
22. Carnovale CE and Rodriguez-Garay EA, Reversible impairment of hepatobiliary function induced by streptozotocin in the rat. *Experientia* **40**: 248–250, 1984.
23. Garcia-Marin JJ, Villanueva GR and Esteller A, Diabetes-induced cholestasis in the rat. Possible role of hyperglycemia and hypoinsulinemia. *Hepatology* **8**: 332–340, 1988.
24. Kirkpatrick RB and Kraft BG, Effect of streptozotocin-induced diabetes on bile acid sulfation in male rat liver. *Am J Physiol* **247**: G226–G230, 1984.
25. Watkins JB III and Dykstra TP, Alterations in biliary excretory function by streptozotocin-induced diabetes. *Drug Metab Dispos* **15**: 177–183, 1987.
26. Watkins JB III and Noda H, Biliary excretion of organic anions in diabetic rats. *J Pharmacol Exp Ther* **239**: 467–473, 1986.
27. Watkins JB III and Sherman SE, Long-term diabetes alters the hepatobiliary clearance of acetaminophen, bilirubin and digoxin. *J Pharmacol Exp Ther* **260**: 1337–1343, 1992.
28. Tunon MJ, Gonzalez P, Garciapardo LA and Gonzalez J, Hepatic transport of bilirubin in rats with streptozotocin-induced diabetes. *J Hepatol* **13**: 71–77, 1991.
29. Thomsen OO and Larsen JA, Comparison of vanadate and ouabain effects on liver hemodynamics and bile production in the perfused rat liver. *J Pharmacol Exp Ther* **221**: 197–205, 1982.
30. Paumgartner G, Horak W, Probst P and Grabner G, Effect of phenobarbital on bile flow and bile salt excretion in the rat. *Naunyn Schmiedebergs Arch Pharmacol* **270**: 98–101, 1971.
31. Gibaldi M and Perrier D, *Pharmacokinetics*, 2nd Edn. Marcel Dekker, New York, 1982.
32. Shechter Y, Insulin-mimetic effects of vanadate: Possible implications for future treatment of diabetes. *Diabetes* **39**: 1–5, 1990.
33. Pederson RA, Ramanadham S, Buchan AMJ and McNeill JH, Long-term effects of vanadyl treatment on streptozotocin-induced diabetic rats. *Diabetes* **38**: 1390–1395, 1989.
34. Uchida K, Takase H, Kadowaki M, Nomura Y, Matsubara T and Takeuchi N, Altered bile acid metabolism in alloxan diabetic rats. *Jpn J Pharmacol* **29**: 553–562, 1979.
35. Subbiah MTR, Yunker RL, Hassan AS and Thibert P, Abnormal bile acid pool and composition in neonates of spontaneously diabetic Wistar BB rats and its change during development. *Biochim Biophys Acta* **794**: 355–360, 1984.
36. Kimura K, Ogura Y and Ogura M, Biosynthesis of cholic acid accelerated by diabetes: Its mechanism and effect of vanadate administration. *Biochim Biophys Acta* **1123**: 303–308, 1992.
37. Ogura Y, Suzuki T, Yamamoto Y and Ogura M, Effects of vanadate on the metabolism of bile acids in diabetic rats. *Biol Chem Hoppe Seyler* **372**: 345–349, 1991.
38. Paulson DJ, Kopp SJ, Tow JP and Peace DG, Effects of vanadate on *in vivo* myocardial reactivity to norepinephrine in diabetic rats. *J Pharmacol Exp Ther* **240**: 529–534, 1987.
39. Young IS, Torney JJ and Trimble ER, The effect of ascorbate supplementation on oxidative stress in the streptozotocin diabetic rat. *Free Radic Biol Med* **13**: 41–46, 1992.
40. Stankiewicz PJ, Stern A and Davison AJ, Oxidation of NADH by vanadium: Kinetics, effects of ligands and role of H_2O_2 or O_2. *Arch Biochem Biophys* **287**: 8–17, 1991.
41. Heffetz D, Bushkin I, Dror R and Zick Y, The insulinomimetic agents H_2O_2 and vanadate stimulate protein tyrosine phosphorylation in intact cells. *J Biol Chem* **265**: 2896–2902, 1990.
42. Ramanadham S, Mongold JJ, Brownsey RW, Cros GH and McNeill JH, Oral vanadyl sulfate in treatment of diabetes mellitus in rats. *Am J Physiol* **257**: H904–H911, 1989.
43. Domingo JL, Gomez M, Llobet JM, Corbella J and Keen CL, Oral vanadium administration to streptozotocin-diabetic rats has marked negative side-effects which are independent of the form of vanadium used. *Toxicology* **66**: 279–287, 1991.
44. Fantus IG, Kadota S, Deragon G, Foster B and Posner BI, Pervanadate [peroxide(s) of vanadate] mimics insulin action in rat adipocytes via activation of the insulin receptor tyrosine kinase. *Biochemistry* **28**: 8864–8871, 1989.
45. Leighton B, Cooper GJS, DaCosta C and Foot EA, Peroxovanadates have full insulin-like effects on glycogen synthesis in normal and insulin-resistant skeletal muscle. *Biochem J* **276**: 289–292, 1991.
46. Ezaki O, The insulin-like effects of selenate in rat adipocytes. *J Biol Chem* **265**: 1124–1128, 1990.
47. Goto Y, Kida K, Ikeuchi M, Kaino Y and Matsuda H, Synergism in insulin-like effects of molybdate plus H_2O_2 or tungstate plus H_2O_2 on glucose transport by isolated rat adipocytes. *Biochem Pharmacol* **44**: 174–177, 1992.
48. Shisheva A, Gefel D and Shechter Y, Insulin like effects of zinc ion *in vitro* and *in vivo*: Preferential effects on desensitized adipocytes and induction of

normoglycemia in streptozotocin-induced rats. *Diabetes* **41**: 982–988, 1992.
49. Singh RE, Rastogi SS, Gupta RK, Sharma VK and Singh RG, Can a diet rich in chromium and other minerals modulate blood sugar and blood lipids in noninsulin dependent diabetes mellitus? *Trace Elem Med* **9**: 157–162, 1992.