Acidic Drinking Water and Risk of Childhood-Onset Type 1 Diabetes

LARS C. STENE, PHD[1,2]
DAG HONGVE, MSC[3]
PER MAGNUS, MD, PHD[2]
KJERSTI S. RØNNINGEN, MD, PHD[2]
GEIR JONER, MD, PHD[1]

OBJECTIVE — To estimate the associations of acidity and concentration of selected minerals in household tap water with the risk of type 1 diabetes.

RESEARCH DESIGN AND METHODS — We designed a population-based case-control study with 64 cases of type 1 diabetes and 250 randomly selected control subjects. Acidity, color, and mineral content were measured in tap water from each participant's household.

RESULTS — Tap water pH 6.2–6.9 was associated with a fourfold higher risk of type 1 diabetes compared with pH ≥7.7 (OR 3.73, 95% CI 1.52–9.15). This result was similar after exclusion of individuals with the highly protective HLA-DQB1*0602 allele, but adjustment for maternal education, urban/rural residence, sex, and age tended to strengthen the estimated association. Higher tap water concentration of zinc was associated with lower risk of type 1 diabetes after adjustment for pH and other possible confounders, but the overall association was strictly not significant.

CONCLUSIONS — These results suggest the possibility that quality of drinking water influences the risk of type 1 diabetes. The possible mechanisms by which water acidity or mineral content may be involved in the etiology of type 1 diabetes remain unknown, but the mechanisms are most likely indirect and may involve an influence on survival of microorganisms in the water.

Diabetes Care 25:1534–1538, 2002

Type 1 diabetes results from an immune-mediated destruction of pancreatic β-cells, but the initiating causes are unknown. Both environmental and genetic factors are implicated in the etiology of the disease (1). The most important genetic contribution comes from the HLA complex, where different alleles are associated with varying degrees of both increased and reduced risk (1). For instance, the HLA DQB1*0602 allele is associated with strong and dominant protection against type 1 diabetes (2). A number of environmental factors, such as infant diet and virus infections, have been suggested as putative risk factors, but few or no specific environmental factors are generally accepted as causally related to type 1 diabetes (1,3). Previous studies have investigated the possible relation between quality of drinking water and type 1 diabetes, particularly the concentrations of nitrate or nitrite (4–8) and zinc (9,10), but all previous studies have used an ecological design in which only municipality or regional averages for quality of drinking water were measured.

During the planning of a case-control study of possible risk factors for type 1 diabetes in Vest-Agder county of Norway (11), ideas from the local branch of the Norwegian Diabetes Association about the relevance of well water led us to investigate this issue. We initially found that use of well water as the household source of drinking water was associated with significantly increased risk of type 1 diabetes compared with water from waterworks. This association was not attributable to socioeconomic status (as measured by maternal education) or urban-rural residence. Based on previous studies of water quality in Southern Norway (12), we hypothesized that the observed association was somehow related to acidic drinking water, which again is associated with leaching of components from soil minerals and plumbing systems and may influence the survival of microorganisms in water sources.

To our knowledge, no previous study has analyzed tap water from individual households in relation to type 1 diabetes. Therefore, the objective of this study was to estimate the associations of acidity and concentration of selected minerals in household tap water with the risk of type 1 diabetes before and after excluding children carrying the highly protective HLA-DQB1*0602 allele.

RESEARCH DESIGN AND METHODS

— The study was carried out in two phases. First, all cases of type 1 diabetes diagnosed in Vest-Agder county of Norway before the age of 15 from 1989 to 1998 using EURODIAB diagnostic criteria (13) and who were born between 1982 and 1998 were eligible. Control subjects were randomly selected from the official population registry among residents in Vest-Agder County born between 1982 and 1998. In May 1999, all participants were contacted by mail and received a questionnaire inquiring about sociodemographic factors, source of drinking water, and early nutrition (11). Venous blood samples for DNA extraction were taken from 70 patients who attended the outpatient clinic at Vest-Agder Central Hospital in Kristiansand. Remaining patients and all control subjects were sent two cytology brushes (Medscand Medical, Malmö, Sweden) for a self-administered buccal cell sample. After one reminder, 85 cases of type 1 diabetes

From the [1]Diabetes Research Center, Aker and Ullevål University Hospitals, Oslo, Norway; the [2]Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway; and the [3]Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway.
Address correspondence and reprint requests to Lars C. Stene, Division of Epidemiology, Norwegian Institute of Public Health, P.O. Box 4404 Nydalen, N-0403 Oslo, Norway. E-mail: lars.christian.stene@fhi.no.
Received for publication 22 January 2002 and accepted in revised form 23 May 2002.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

(94.4%) and 1,071 control subjects (72.7%) responded. The mean age at diagnosis was 6.9 ± 3.3 years. The buccal cell samples were returned to the National Institute of Public Health by mail for DNA extraction (14,15). The HLA-DQB1 locus was typed using PCR with biotinylated primers and reverse hybridization with sequence-specific oligonucleotide probes as described by Cinek et al. (16). Details on the genotyping may be obtained from the authors on request. Individuals were categorized as carrier or noncarrier of the highly protective DQB1*0602 allele (2).

After the initial detection of a significant association between use of well water and type 1 diabetes, a second phase of the study was performed in June of 2000. All respondents with type 1 diabetes and 350 subjects randomly selected among control respondents received a 125-ml plastic bottle with written information by mail. Participants were asked to fill the bottle with tap water from the kitchen and return the filled bottle by mail. A total of 74 type 1 diabetic patients (87%) and 294 control subjects (84%) returned the bottle with sufficient tap water for analysis. Of the 74 patients who returned a water sample, 8 had changed address of residence between the time of diagnosis and the time of collection of water samples, all within the same municipality of Vest-Agder county. We could not confirm that 2 patients and 44 control subjects had been born in Vest-Agder county. The analyses were restricted to those who were born in Vest-Agder and patients who had the same address of residence at the time of diagnosis and at the time of water sample collection (64 patients and 250 control subjects). Based on previous experience with concentrations and variation in parameters influencing water quality in the study region (12), we chose to analyze the water samples with respect to acidity (pH), color, and concentration of iron, aluminum, manganese, zinc, and copper. The water samples were analyzed at the Norwegian Institute of Public Health using conventional standardized methods (12). The water variables were categorized in four groups to allow for possible nonlinear relationships. We used logistic regression for statistical analysis. We assessed whether the strength of associations was similar in different age groups (ages 0–5.9, 6–9.9, 10–13.9, and 14–17.9) by stratified analysis and by formally testing the interaction term between age and the exposure variable. Written informed consent was obtained from all participants, and the study protocol was approved by the regional ethics committee and by the national data inspectorate.

Table 1—*Distribution of age, sex, maternal education, and rural-urban residence for cases with type 1 diabetes and randomly selected population control subjects in Vest-Agder County, Norway*

	Case subjects	Control subjects	OR (95% CI)
n	85	1,071	
Age (years)*	11.1 ± 3.6	8.5 ± 4.7	
Male sex	54 (63.5)	522 (48.7)	1.8 (1.2–2.9)
Maternal education ≥12 years†	33 (39.3)	521 (49.4)	0.7 (0.4–1.0)
Urban residence‡	38 (44.7)	462 (43.1)	1.1 (0.7–1.7)
HLA-DQB1*0602 positive§	1 (1.2)	320 (30.3)	0.03 (0.004–0.20)
Drinking water from private well	26 (30.6)	175 (16.3)	2.3 (1.4–3.7)

Data are means ± SD and *n* (%) unless otherwise indicated. *Age at data collection; †highest attained education at the time of giving birth to the index child (1 case of type 1 diabetes and 17 control subjects had missing information for maternal education); ‡Kristiansand municipality vs. the rest of Vest-Agder county; §two case subjects and 14 control subjects had missing data for HLA-type.

RESULTS

Characteristics of the whole data set

The characteristics of the whole data set are shown in Table 1. Nearly 31% of the control subjects but only 1 of the 85 cases (1.2%) carried the DQB1*0602 allele. In addition, there was a preponderance of boys compared with girls. The mothers of the control subjects tended to have more years of education than mothers of cases with type 1 diabetes, but this association was not significant. There was little difference in diabetes risk between urban and rural residents. Using well water or other private sources of tap water was significantly associated with risk of type 1 diabetes compared with using water from waterworks (OR 2.3). The latter estimated association was similar after adjustment for urban/rural residence or maternal education or after excluding children carrying the strongly protective HLA-DQB1*0602 allele (data not shown). There was a tendency that the association between well water and type 1 diabetes was stronger among the younger children, but the interaction between age and use of well water was not significant ($P = 0.16$, data not shown).

Tap water analyses

The associations between water parameters and type 1 diabetes are shown in Table 2. Tap water acidity was strongly associated with the risk of type 1 diabetes, and the overall trend was highly significant. The OR for the category with pH between 6.2 and 6.9 was 3.73 compared with the category with the highest pH (lowest acidity). The risk seemed to level off to some extent in the category with the lowest pH. After exclusion of individuals carrying the HLA-DQB1*0602 allele, the estimated ORs for the different categories of pH were slightly higher than the unadjusted (OR 1.06, 5.25, and 3.50, respectively, for the three categories with decreasing pH compared with the category with the highest pH). The strength of the association was similar in all age groups (data not shown).

Water color or any of the minerals analyzed in this study was not significantly associated with type 1 diabetes. All results were essentially unchanged after adjustment for maternal education, urban-rural residence, source of drinking water, age, and sex (data not shown), except for a more consistent but nonsignificant inverse association with zinc (see below).

Among the population control subjects, acidity (lower pH) was associated with higher concentrations of zinc, copper, and manganese and with higher color count (all $P < 0.001$, Spearman's rank correlation, data not shown). Well water compared with water from water works was associated with higher concentrations of zinc, copper, as well as with lower pH and lower color count ($P < 0.001$; Mann-Whitney test, data not shown). Because zinc has been associated with lower risk of type 1 diabetes in previous studies (9,10) and lower tap water pH was associated with increased zinc concentration, we estimated the association between tap water zinc concentration and type 1 dia-

Table 2—*Association of acidity, color, and concentrations of selected minerals in household tap water with type 1 diabetes in Vest-Agder county, Norway*

	Case subjects*	Control subjects*	OR (95% CI)
N	64	250	
pH			
<6.2	12	28	2.18 (0.97–4.90)
6.2–6.9	11	15	3.73 (1.52–9.15)
7.0–7.6	18	83	1.10 (0.56–2.17)
≥7.7	23	117	1.00 (reference)
Test for trend†			$P = 0.003$
Color			
<4 units	16	74	1.00 (reference)
4–9.9 units	14	33	1.96 (0.86–4.48)
10–14.9 units	20	95	0.97 (0.47–2.01)
≥15 units	13	43	1.40 (0.61–3.18)
Test for trend			$P = 0.82$
Iron (mg/l)			
<0.02	15	52	1.00 (reference)
0.02–0.039	17	70	0.84 (0.39–1.84)
0.04–0.079	25	85	1.02 (0.49–2.11)
≥0.8	7	43	0.57 (0.21–1.51)
Test for trend			$P = 0.14$
Manganese (mg/l)			
<0.01	14	55	1.00 (reference)
0.01–0.019	25	83	1.18 (0.57–2.47)
0.02–0.029	15	75	0.79 (0.35–1.76)
≥0.03	10	37	1.06 (0.43–2.64)
Test for trend			$P = 0.43$
Aluminum (mg/l)			
<0.1	17	66	1.00 (reference)
0.1–0.174	14	63	0.86 (0.39–1.90)
0.174–0.24	19	87	0.85 (0.41–1.76)
≥0.25	14	34	1.60 (0.70–3.63)
Test for trend			$P = 0.35$
Zinc (μg/l)			
<15	16	67	1.00 (reference)
15–44.9	35	117	1.25 (0.65–2.43)
45–74.9	5	29	0.72 (0.24–2.16)
≥75	8	37	0.90 (0.35–2.31)
Test for trend			$P = 0.57$
Copper (μg/l)			
<2.0	11	47	1.00 (reference)
2.0–49.9	29	117	1.06 (0.49–2.29)
50–99.9	7	24	1.25 (0.43–3.62)
≥100	17	62	1.17 (0.50–2.74)
Test for trend			$P = 0.31$
Magnesium (mg/l)			
<0.70	19	74	1.00 (reference)
0.70–0.84	18	85	0.83 (0.40–1.69)
0.85–0.99	15	55	1.06 (0.50–2.78)
≥1.00	12	34	1.38 (0.60–3.15)
Test for trend			$P = 0.27$

Data are *n* unless otherwise indicated. *Data on pH was missing for seven control subjects, data on color was missing for one case and five control subjects, and data on magnesium was missing for two control subjects. †Tests for trend were Wald test from logistic regression model with the explanatory variable entered as a continuous variable.

betes after adjusting for pH. Use of well water, urban/rural residence, maternal education, age, and sex were also adjusted for in multiple logistic regression analyses (Table 3). The results showed that higher tap water concentration of zinc was relatively strongly associated with lower risk of type 1 diabetes after adjustment for pH and other possible confounders. The group with highest zinc concentration had a significantly lower risk of type 1 diabetes compared with those with lowest zinc concentration, but the test for overall trend was only borderline significant ($P = 0.10$), probably due to large random variation. The estimated ORs for the associations between tap water pH and risk of type 1 diabetes was higher after adjustment for zinc and other potential confounders (Table 3). After exclusion of individuals carrying the HLA-DQB1*0602 allele, the adjusted associations of pH and zinc with type 1 diabetes was essentially unchanged (data not shown).

CONCLUSIONS — We found a strong association between acidity in drinking water and risk of type 1 diabetes and a tendency that higher tap water concentration of zinc was associated with lower risk of type 1 diabetes. These associations were independent of presence of the highly protective HLA-DQB1*0602 allele and possible confounders. An advantage of this study compared with all previous studies of quality of drinking water and type 1 diabetes is that water quality was measured in the households of individual study participants. Previous studies of water quality and type 1 diabetes have only measured water quality at the municipality or regional level (4–10). Analyzing individual household tap water samples takes into account possible variation in quality of drinking water due to different water pipes as well as use of filters or other purification devices in the households. Due to the relatively small sample size, we were not able to stratify the data according to more strictly defined high-risk HLA genotypes or to do formal tests of interactions with HLA genotype.

The major limitation that the current study shares with most previous ones is that some people may have changed place of living between the time of relevance for the etiology of type 1 diabetes and the time of data collection. However, the most relevant time window for environ-

Table 3—*Association of tap water pH and zinc concentration with risk of type 1 diabetes in multiple logistic regression analysis adjusting for potential confounders*

	Crude OR (95% CI)	Adjusted OR (95% CI)*
pH		
<6.2	2.18 (0.97–4.90)	2.89 (0.83–10.0)
6.2–6.9	3.73 (1.52–9.15)	5.56 (1.83–16.9)
7.0–7.6	1.10 (0.56–2.17)	1.49 (0.73–3.05)
≥7.7	1.00 (reference)	1.00
Test for trend†	$P = 0.003$	$P = 0.002$
Zinc (μg/l)		
<15	1.00 (reference)	1.00
15–44.9	1.25 (0.65–2.43)	1.01 (0.46–1.70)
45–74.9	0.72 (0.24–2.16)	0.38 (0.15–1.55)
≥75	0.90 (0.35–2.31)	0.28 (0.07–0.75)
Test for trend	$P = 0.57$	$P = 0.10$

*Adjusted for tap water zinc concentration (in pH analysis) or pH (in zinc analysis) and maternal education, use of well water, urban/rural residence, sex, and birth year. †Tests for trend were Wald test from logistic regression model with the explanatory variable entered as a continuous variable.

mental exposures are unknown, and it is hard to envisage that individuals have moved to areas with different water quality depending on their subsequent development of diabetes. It is likely that there is variation between individuals with respect to the amount of tap water they drink and that some individuals drink only a small amount of tap water. However, because of the generally good quality of the tap water (17), most people in Norway use tap water for both drinking and cooking. We were not able to take possible differences in water quality over time into account. Most of the minerals measured in this study are also provided by diet, which we did not measure in the present study.

Acid water in itself is unlikely to be causally related to type 1 diabetes, but may be a marker of some other factor. Acidity of ground water is associated with concentration of a number of minerals (12). Furthermore, the physicochemical speciation, and therefore the bioavailability and toxicity of certain elements, may change dramatically over rather narrow pH ranges, as is the case, for example, for aluminum in the pH range of 4.5–5.5 (18). However, none of the minerals analyzed in this study was significantly associated with type 1 diabetes. There were indications of a relatively strong association between zinc concentration and type 1 diabetes, but the overall association was only borderline significant. An inverse association between zinc concentration and risk of type 1 diabetes has been found in two previous ecological analyses (9,10). In one study from Sweden (9), a low groundwater concentration of zinc in the area of residence was associated with increased risk of type 1 diabetes. Zhao et al. (10) found indications of a nonmonotonous association of zinc and magnesium concentrations in the water with incidence of type 1 diabetes. A possible protective effect of zinc is also supported by studies in experimental animals (19). Others have studied nitrate or nitrite concentrations at municipality level in relation to type 1 diabetes, but most studies have not found any significant association (4–8,10). We did not measure nitrate in the present study. From previous studies in Southern Norway, concentrations of nitrate in Norwegian drinking water is known to be very low, usually <10% of what is found in other European countries (12,17).

Haglund et al. (9) suggested that low groundwater zinc concentration could contribute to zinc deficiency, which again may affect immune function or otherwise be diabetogenic. Zhao et al. also discussed the possibility that deficiencies in zinc or magnesium could be involved in the etiology of type 1 diabetes via insufficient protection against free radicals or other biological mechanisms in the host. We question these explanations, however, because the contribution to the total intake of zinc and magnesium from drinking water is negligibly small compared with a normal dietary intake. The median contribution of zinc and magnesium from 2 l of drinking water is ~2–3% of the daily dietary intake in a normal western diet, and the relative contribution of the other measured minerals from drinking water is even less (20). We can only speculate about the possible mechanism by which acidity or mineral content of drinking water may be linked to type 1 diabetes. Confounding by other salts or minerals or other factors not measured in the present study cannot be excluded. One possibility is that acidity or mineral content of the tap water influence survival of water borne microorganisms (21). Perhaps our observed highest risk of type 1 diabetes for pH levels ~6.2–6.9 is a reflection of the optimal level for some microorganisms that may be relevant in the etiology of type 1 diabetes, such as enteroviruses or rotaviruses (22,23). Enteroviruses are known to survive in water at relatively acidic pH levels (24). A combination of many factors, such as minerals and salts, presence of suspended solids, and temperature, seems to influence the survival of viruses in drinking water sources, but the critical question is of course whether significant amounts of enteroviruses enter drinking water sources at all. We did not measure content of any microorganisms in our water samples, but it has been suggested that standard indicators of bacteriological quality of drinking water may not be representative of the virological quality (25). Enteroviruses are known to be resistant to several disinfectants but not to 0.3–0.5 ppm chlorine (26). Unfortunately, we did not measure chlorine content in our water samples, nor did we have information on chlorination practices at the various water works in Vest-Agder county. However, well water is usually not disinfected. Geological, hygienic, and other characteristics that may be area specific or change over time will influence the complex interactions between pH, minerals and salts, and microorganisms of the tap water, and our observed associations are not necessarily generalizable to other geographical areas or countries. Variations in purification methods between water works and between surface water and ground water sources may also influence our results.

In conclusion, we found a strong association between acidity of household tap water and risk of type 1 diabetes and an indication that high zinc concentration in tap water was associated with lower risk of type 1 diabetes, independent of HLA-DQB1*0602 carrier state and possible confounders. Although there are many possible sources of error, these results suggest the possibility that quality of drinking water influences the risk of type 1 diabetes. The mechanisms by which water acidity or mineral content may be involved in etiology of type 1 diabetes remain unknown, but the mechanisms are most likely indirect and may involve an influence on survival of microorganisms in the water. Future studies should investigate whether drinking water is a significant source of infections with, for example, enteroviruses.

Acknowledgments— L.C.S. and G.J. were supported by a grant from the Norwegian Foundation for Health and Rehabilitation (Grant no. 1997/156), grants from the Norwegian Diabetes Association, and by a grant from the Research Council of Norway (148359/330). Funding was also kindly provided by TINE Norwegian Dairies and Novo Nordisk Pharma A/S.

The authors would like to thank Eva Andruchow for laboratory analyses of water samples and Liv Paltiel, Elisabet Witsø, and Hang Lu for assisting with DNA extraction and HLA genotyping. We are grateful for assistance and support from Unni Hinna, Jorunn Ulriksen, Per Vesterhus, and Torleif Trydal at Vest-Agder central Hospital and the Norwegian Childhood Diabetes Study Group. Special thanks to Jens Kjos-Hansen and the Vest-Agder branch of the Norwegian Diabetes Association.

References

1. Atkinson MA, Maclaren NK: The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med* 24:1428–1436, 1994
2. Pugliese A, Gianani R, Moromisato R, Awdeh ZL, Alper CA, Erlich HA, Jackson RA, Eisenbarth GS: HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM. *Diabetes* 44:608–613, 1995
3. Åkerblom HK, Knip M: Putative environmental factors in type 1 diabetes. *Diabete Metab Rev* 14:31–67, 1998
4. Kostraba J, Gay E, Rewers M, Hamman R: Nitrate levels in community drinking waters and risk of IDDM. *Diabetes Care* 15: 1505–1508, 1992
5. Virtanen SM, Jaakkola L, Rasanen L, Ylonen K, Lounamaa R, Åkerblom HK, Tuomeilehto J: Nitrate and nitrite intake and the risk for type 1 diabetes in Finnish children. *Diabet Med* 11:656–662, 1994
6. Parslow RC, McKinney PA, Law GR, Staines A, Williams R, Bodansky HJ: Incidence of childhood diabetes mellitus in Yorkshire, northern England, is associated with nitrate in drinking water: an ecological analysis. *Diabetologia* 40:550–556, 1997
7. Van Maanen JMS, Albering HJ, Van Breda SGJ, Curfs DMJ, Ambergen AW, Wolffenbuttel BHR, Kleinjans JCS, Reeser HM: Nitrate in drinking water and risk of childhood diabetes in the Netherlands (Letter). *Diabetes Care* 22:1750, 1999
8. Casu A, Carlini M, Contu A, Bottazzo GF, Songini M: Type 1 diabetes in Sardinia is not linked to nitrate levels in drinking water. *Diabetes Care* 23:1043–1044, 2000
9. Haglund B, Ryckenberg K, Selinus O, Dahlquist G: Evidence of a relationship between childhood-onset type I diabetes and low groundwater concentration of zinc. *Diabetes Care* 19:873–875, 1996
10. Zhao HX, Mold MD, Stenhouse EA, Bird SC, Wright DE, Demaine AG, Millward BA: Drinking water composition and childhood-onset type 1 diabetes mellitus in Devon and Cornwall, England. *Diabet Med* 18:709–717, 2001
11. Stene LC, Ulriksen J, Magnus P, Joner G: Use of cod liver oil during pregnancy associated with lower risk of type I diabetes in the offspring. *Diabetologia* 43:1083–1092, 2000
12. Weideborg M, Hongve D, Andruchow E: Trace metals in Norwegian drinking water sources. *Verh Internat Verein Limnol* 24:2180–2184, 1991
13. EURODIAB ACE Study Group: Variation and trends in incidence of childhood diabetes in Europe. *Lancet* 355:873–876, 2000
14. Richards B, Skoletsky J, Shuber AP, Balfour R, Stern RC, Dorkin HL, Parad RB, Witt D, Klinger KW: Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. *Hum Mol Genet* 2:159–163, 1993
15. Parad RB: Buccal cell DNA mutation analysis for diagnosis of cystic fibrosis in newborns and infants inaccessible to sweat chloride measurement. *Pediatrics* 101: 851–855, 1998
16. Cinek O, Wilkinson E, Paltiel L, Saugstad OD, Magnus P, Rønningen KS: Screening for the IDDM high-risk genotype: a rapid microtitre plate method using serum as source of DNA. *Tissue Antigens* 56:344–349, 2000
17. Flaten TP: A nation-wide survey of the chemical composition of drinking water in Norway. *Sci Total Environ* 102:35–73, 1991
18. Driscoll CT, Postek KM: The chemistry of aluminum in surface waters. In *The Environmental Chemistry of Aluminum*. 2nd ed. Sposito G, Ed. Boca Raton, FL, CRC Press, 1996, p. 363–418
19. Ohly P, Kohle C, Abel J, Seissler J, Gleichmann H: Zinc sulphate induces metallothionein in pancreatic islets of mice and protects against diabetes induced by multiple low doses of streptozotocin. *Diabetologia* 43:1020–1030, 2000
20. National Research Council: *Recommended Dietary Allowances*. 10th ed. Washington, DC, National Academy Press, 1989
21. Gerba CP, Rose JB: Viruses in source and drinking water. In *Drinking Water Microbiology: Progress and Recent Developments*. McFeters GA, Ed. New York, Springer-Verlag, 1990, p. 380–395
22. Rewers M, Atkinson M: The possible role of enteroviruses in diabetes mellitus. In *Human Enterovirus Infections*. Rotbart HA, Ed. Washington, DC, ASM Press, 1995, p. 353–385
23. Honeyman MC, Coulson BS, Stone NL, Gellert SA, Goldwater PN, Steele CE, Couper JJ, Tait BD, Colman PG, Harrison LC: Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. *Diabetes* 49:1319–1324, 2000
24. Salo RJ, Cliver DO: Effect of acid pH, salts, and temperature on the infectivity and physical integrity of enteroviruses. *Arch Virol* 52:269–282, 1976
25. Bosch A: Human enteric viruses in the water environment: a minireview. *Int Microbiol* 1:191–196, 1998
26. Zeichhardt H, Grunert H-P: Enteroviruses: polioviruses, coxsackieviruses, echoviruses and enteroviruses 68–71. Vol. 2, section 8. In *Armstrong and Cohen Infectious diseases*. London, Mosby 1999