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Nestlé Products Technical Assistance Company Ltd. Biological Laboratory, Orbe (Switzerland)

Exposure of rats during 90 days to mineral water containing various amounts of sulphate

H. P. Würzner

With 4 figures and 4 tables

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The supply of hygienic innocuous and controlled drinking water is of great concern in many countries. Therefore National and International Standards were issued which regulate those constituents which have a direct effect on consumer health, or other constituents which, when present in excessive amounts, may discourage consumption.

For taste and other reasons large consumer groups use natural mineral waters as standard drinking water.

We were interested in the fixed limits for sulphate in drinking water standards, which are given as a maximum of 250 mg/l. The origin of this limit seems to be a twenty-year-old German claim that sulphate in excess of this limit causes intestinal damage. However, no experimental proof, to our knowledge, has been provided for this claim. In the following 90-day study we report on the observed effects of several waters with either low, medium or high sulphate content in the rat.

Experimental

In this investigation Sprague Dawley rats (Charles River SA, COBS, Elbeuf, France) received during 90 days drinking waters with different sulphate contents. The control group A received tap water containing 9 to 10 mg/l sulphate, the low sulphate group B received Bagats natural mineral water containing less than 10 mg/l sulfate. The medium sulphate group C got Vittel Grande Source containing 280 mg/l sulphate while the high sulphate group, D, received Vittel Hepar with 1.595 mg/l. Bagats, Vittel Grande Source and Vittel Hepar are natural bottled mineral waters and were provided by courtesy of the Société des Eaux Minérales de Vittel; Vosges, France. Of course, these waters were also different with respect to other ions and trace elements, but the aim of this investigation was to study the effects of different sulphate contents in their natural environment of other ions. More about overall composition of these natural mineral waters has been documented elsewhere (4, 5). The tap water was analysed in our laboratories.

Each experimental group consisted of 25 male and 25 female rats, which had been randomly alloted from a large pool of animals in order to have comparable starting body weights in the various groups (Table 1). The rats were housed singly in makrolon cages type III on soft wood bedding (Litalabo, Paris, France). The cage covers were made from stainless steel. The makrolon water bottles were provided with stainless steel caps and contained a volume of 100 ml. The experimental

animals lived in a barrier protected unit which was fully air conditioned with filtered air. Temperature was maintained at 23 °C ± 1° and relative humidity at 60% ± 5%. A day night cycle of 12 hours was adjusted on the automatic artificial illumination. Rats from control as well as all experimental groups received a standard pelleted diet (Usines Alimentation Rationelle, Villemoisson-sur-Orge, France) containing 20% protein. Food consumption and individual body weight were recorded weekly. Water consumption was measured daily by back weighing of individual drinking bottles. The bottles were afterwards washed in an automatic washing machine, rinsed with demineralised water and sterilised in an autoclave at 130 °C for half an hour. Always only freshly opened commercially bottled mineral water was used to fill the drinking bottles. At 90 days of the trial 20 male and 20 female rats of each group were fasted for 16 hours prior to blood sampling. Blood was drawn into heparinized tubes by puncture of the retrobulbar *venous plexus* under carbondioxyde-oxygen anaesthesia. Whole blood was immediately analysed for red blood cells, white blood cells, haemoglobin and haematocrit. Prothrombin time was determined from specially sampled sodium citrate treated blood. Additionally whole blood was centrifuged for analysis of plasma parameters such as blood urea nitrogen, glucose, triglycerides, total cholesterol, phospholipids and alkaline phosphatase. The animals were then killed after ether narcosis by opening the aorta. All rats were thoroughly inspected macroscopically and the weights of liver, kidneys, adrenals, brain and testis recorded. Standard tissue slices were taken from stomach, duodenum, ileum, caecum, colon, both kidneys, liver, adrenals, gonads, heart, lung, thyroid, pancreas, thymus, spleen, bladder and aorta and fixed in Bouin's fixative. After usual fixation and processing, tissues were embedded in paraffin (Paraplast, Sherwood Medical Industries, St. Louis, U.S.A.). Sections of 4 microns were cut from all organs and stained with haematoxylin eosin and additional sections from liver, kidneys and the intestinal tract with PAS and Alcian Blue. All haematological and biochemical parameters were checked with a quality control system utilising Labtrol, Enzatrol and Hematology References (*Merz* and *Dade*, Luzern, Switzerland).

Results

During the whole of the 90-day study, control and treated rats did not show any disturbance of development, appearance or behaviour. No deaths occurred and no soft faeces or even diarrhea were noticed. The

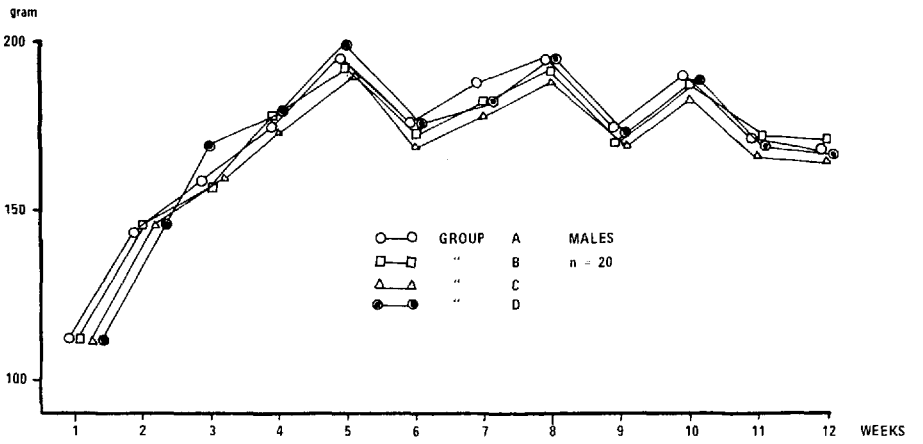

Fig. 1. Mean food consumption/rat/week.

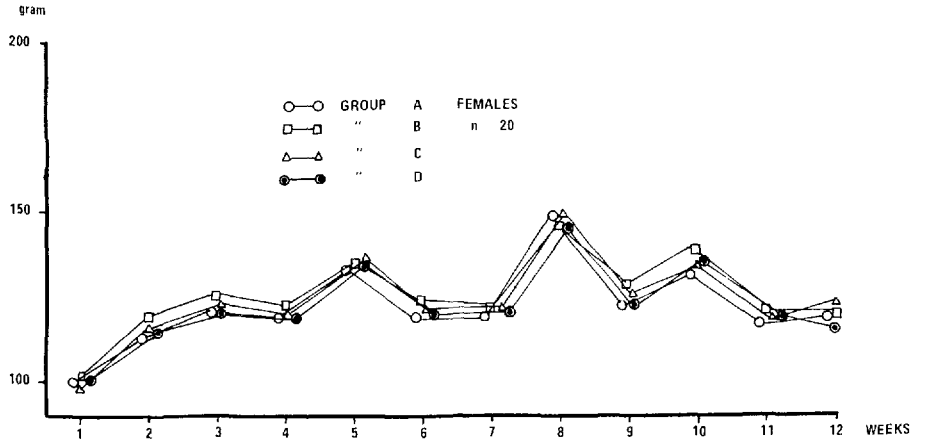

Fig. 2. Mean food consumption/rat/week.

water consumption (Diagram 3, 4) was comparable in control and treated groups and always remained within physiological limits. Urine output and analysis were done in several simultaneous experiments, in which increasing dosages of the various mineral waters were administered by gavage and compared to tap and distilled water. No striking differences were found in sediment, albumin or urinary glucose but the volume of urine measured 1, 2, 5 or 24 hours afterwards was always dependant on the volume of water administered orally. Thus increasing the water volume orally administered results in increased urinary output, wheras osmolarity decreases. Food consumption was equally equilibrated in control and treatment groups (Diagram 1, 2). Since generally body weight at 4 and 12 weeks (Table 1) also did not show significant differences between the control group and the groups treated with mineral water, the nitrogen balance study which was foreseen was dropped. A significant difference in

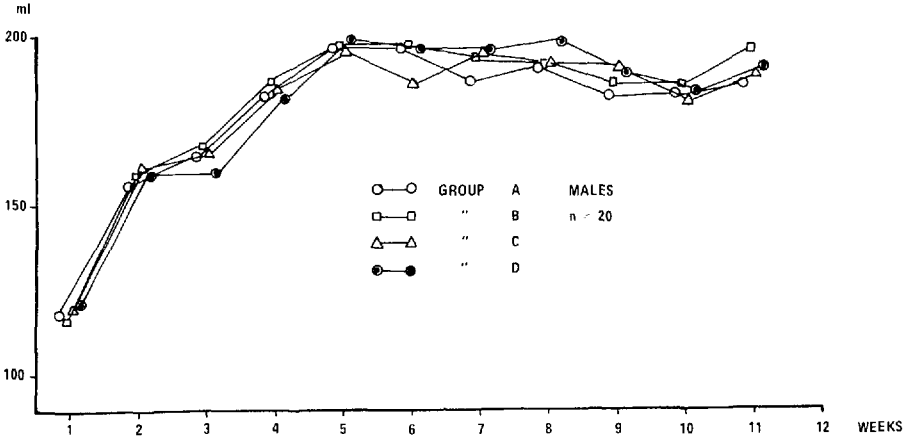

Fig. 3. Mean water consumption/rat/week.

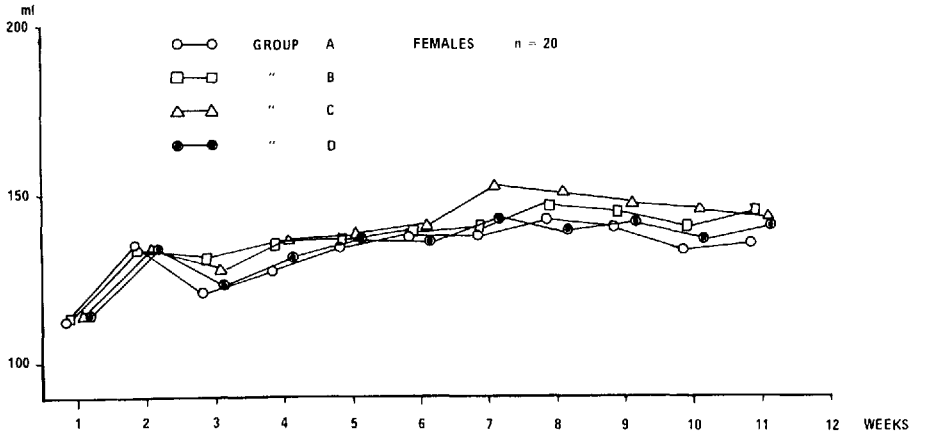

Fig. 4. Mean water consumption/rat/week.

body weight was only noticed at 12 weeks in males of group C, using the Student t-test. Food conversion efficiency, calculated at 4 weeks was consequently not different in either sex or in any of the treatments (Table 1).

Results of plasma biochemistry (Table 2) did not yield significant differences in either sex, when treatments B, C and D were compared to the controls A. However, blood urea nitrogen values in males and females indicate a trend to lower values in the Vittel Grande Source and Vittel Hepar treatments. This observation became significant after 10 months in the surviving 10 rats/group which were killed only after this chronic treatment. Results of most haematological parameters (Table 3) were also comparable in treated and control rats, with a possible exception of white blood cells in tap water treated males. Later when blood sampling and

Table 1. Body weight and food conversion efficiency.
(Mean value ± S.E.M.)

Group	n	Initial body weight	Body weight 4 weeks	Body weight 12 weeks	Food conversion efficiency 4 weeks
			Males		
A	20	75.9±1.2	284.9±3.6	458.8±7.0	0.35
B	20	75.7±1.2	281.4±4.7	445.8±6.6	0.34
C	20	75.9±1.2	276.9±3.5	438.3±6.5*	0.33
D	20	75.3±1.2	282.3±5.1	451.9±9.3	0.33
			Females		
A	20	69.1±1.5	181.2±2.3	261.5±3.2	0.25
B	20	68.6±1.6	192.1±3.9	269.3±5.8	0.25
C	20	69.1±1.5	188.8±3.9	262.2±5.8	0.25
D	20	68.5±1.7	188.6±3.9	266.8±5.9	0.26

* = $P < 0.05$.

Table 2. Plasma chemistry.
(Means value ± S.E.M.)

Group	n	Blood-urea-nitrogen	Glucose	Tri-glycerides	Cholesterol (total)	Alkaline phos-phatase	Total protein
		mmol/l	mmol/l	mmol/l	mmol/l	mU/ml	g/l
				Males			
A	20	6.29±0.25	6.86±0.15	1.09±0.08	2.39±0.13	59.0±2.7	69±0.6
B	20	6.24±0.32	6.80±0.11	0.96±0.06	2.11±0.10	56.8±2.6	68±0.8
C	20	5.63±0.26	6.48±0.13	1.08±0.09	2.27±0.11	54.0±2.5	68±0.5
D	20	5.79±0.23	6.82±0.23	0.98±0.07	2.20±0.08	58.1±3.5	66±0.6
				Females			
A	20	6.80±0.32	6.85±0.13	0.70±0.07	2.36±0.11	37.6±2.5	72±0.9
B	20	6.42±0.18	6.86±0.16	0.77±0.09	2.33±0.10	34.8±2.5	70±0.7
C	20	6.29±0.32	6.53±0.11	0.80±0.07	2.38±0.10	38.3±2.6	72±0.9
D	20	5.80±0.20	7.21±0.21	0.71±0.09	2.37±0.12	37.1±2.0	69±1.0

analysis of haematological parameters was repeated at 6, 7 and 10 months on the surviving rats, no difference in white cell counts were detectable any more. Therefore the higher leucocyte count in the male rats of treatment group A at three months have to be considered as an artefact. Anyway the values were not pathological and remained fully in the physiological range. Organ weights of dissected animals (Table 4) were surprisingly homogenous and did not indicate any atrophy or hypertrophy. This interpretation was confirmed by the follow-up histopathological evaluation of these organs.

There was no change at the light-microscopic level in the stomach in any animal. The small intestine was examined in longitudinal and cross sections at the level of the duodenum, jejunum and ileum on sections stained HE and alcian blue PAS. Special attention was drawn to the length

Table 3. Haematological results.
(Mean value ± S.E.M.)

Group	n	Red blood cells (10^6/mm^3)	Haemoglobin (g%)	Haematocrit (%)	White blood cells (10^3/mm^3)	Prothrombin-time (s)
				Males		
A	20	7.37±0.08	15.9±0.2	51±0.5	10.9±0.5	12.7±0.1
B	20	7.26±0.13	15.4±0.1	49±0.5	7.8±0.6	12.3±0.1
C	20	7.29±0.10	15.8±0.2	50±0.6	7.1±0.4	12.2±0.1
D	20	7.29±0.11	15.6±0.2	50±0.5	7.1±0.4	12.2±0.1
				Females		
A	20	6.76±0.09	15.5±0.1	49±0.4	4.8±0.3	12.6±0.2
B	20	5.88±0.38	15.0±0.1	47±0.5	4.1±0.3	12.3±0.1
C	20	6.57±0.10	15.2±0.1	48±0.4	4.5±0.2	11.9±0.1
D	20	6.56±0.10	14.8±0.1	47±0.3	3.6±0.2	12.2±0.2

Table 4. Absolute organ weights.
(Mean value ± S.E.M.)

Group	n	Liver g	Kidney g	Adrenals mg	Brain g	Testis g
				Males		
A	20	12.0±0.3	2.8±0.1	52±2	1.6±0.02	3.6±0.0
B	20	11.5±0.3	2.8±0.1	55±2	1.6±0.03	3.4±0.0
C	20	11.0±0.3*	2.8±0.1	56±2	1.6±0.02	3.5±0.1
D	20	11.2±0.3	2.9±0.1	58±2	1.5±0.02	3.5±0.1
				Females		
A	20	6.5±0.1	1.7±0.1	72±2	1.4±0.02	
B	20	6.5±0.1	1.7±0.0	71±3	1.5±0.01	
C	20	6.5±0.1	1.7±0.1	70±3	1.4±0.02	
D	20	6.4±0.1	1.7±0.1	73±2	1.5±0.01	

* = $P < 0.05$

of villi, number of villi, the size of epithelial cells, the cytoplasm – nucleus ratio, the number of goblet cells, the mitotic activity as an indication of cell turnover, the resorptive surface and the lymphocytic activity. There was no difference detectable at the light microscopic level between the groups or individual animals. No pathologic change in occurred the small intestine at any level in any animal.

There was no pathologic change in the large intestine. Single colon worms (heterakis sp.) were present in 2 C males. There was no microscopic change in the exocrine or endocrine pancreas.

In the liver no unusual change of difference between groups was observed. Kidney tubular calcification at the level of the corticomedullary junction was present in a minimal to pronounced degree in a majority of females of all groups. It was not present in males. This observation is well known in rats and is not related to the experimental conditions. Arteriosclerosis of intrarenal arteries was observed in a minimal degree in a majority of males and females of all groups without difference between the groups. Other findings were in most instances related to early development of progressive nephropathy and were not related to experimental conditions.

Special attention was drawn to the width of the 3 cortical layers of the adrenals especially the glomerulosa and to possible focal glandular activation or hyperplasia. There was no concentric of focal hyperplasia in the adrenal cortex of any animal. Coincidental findings in single animals consisted of focal extracapsular nodular hyperplasia as is frequently seen in this species and of focal increase in lipid in cells of the fasciculata. No group difference was detectable. Thus adrenal cortex has not been influenced by the experimental conditions.

Minimal degree of arteriosclerosis of intramural coronary arteries was present in some animals of all groups without any group difference. The changes consisted of reorientation of smooth muscle cells in the media and proliferation of these cells in the intima with collagen and elastic fiber

formation. There were no foam cells or further progressed lesions. Focal, minimal or mild mononuclear myocarditis and myocardiolysis were present in 2 C and 1 D male. This change was observed with some frequency in males and is not related to the experiment.

No change was detected in sections of the major arteries stained HE and elastica-van Gieson.

No difference in activity of thyroids an parathyroids between experimental and control groups and no pathologic change were observed.

Discussion

The absorption, utilization and metabolic fate of sulphate has been investigated by several workers (*Dziewiatkowsky, Benesch* and *Benesch* (6); *Crevier* and *Belanger* (7); *Button, Brown, Michels* and *Smith* (8)). It was also shown by *Michels* and *Smith* (9), *Arnaud* and *Welsch* (10) that not only the organic sulphur from proteins and amino acids is utilised, but also sulphur from inorganic sources in the form of sulphate participates in the constitution of the necessary body pool. Exogenous supply of sulphate increases the body sulphate pool. This provides besides the incorporation in chondroitin sulphate the amount for further glutathion synthesis as required by certain detoxification processes. *Arnaud* and *Welsch* (10) showed by fractionation of urinary radioactive sulphur compounds that indeed 30–40% of the compounds were conjugated. It appeared that there is no difference in sulphate utilisation, when sulphate is either provided via the drinking water or in the diet. The drinking water may, however, provide only a small amount of the dietary sulphate in the case of a low sulphated water (Bagats), or two- to threefold the dietary intake in high sulphated waters (Grande Source and Hepar). Absorption and incorporation in cartilage of dietary sulphate is rapid. Recently, *Froesch, Humbel* and *Labhart* (11) isolated a peptide IGF (insulin like growth factor) which is probably responsible for this and which can be transmitted by serum of growth hormone stimulated donors. Growth hormone itself appears to have no direct influence on this mechanism.

There was no evidence of any symptomatic intestinal disorder in high as well as in low sulphate drinking water treatments. According to the water consumption data of the rats and an assumed mineral water consumption of one liter/day in an adult subject, it can be estimated that the rats in this study had about a ten times higher water consumption. This further extends the safety margin already established in the high sulphate treatment (Hepar). In connection with the extensive macroscopic and microscopic inspection of the intestinal tract it is justified to conclude that there was no toxic or other deleterious effect attributable to the high sulphate administration and it appears that there is no scientific reason to limit the sulphate content of drinking water to 200 mg/l.

The lowering effect of BUN plasma blood urea nitrogen values in treatments D (Hepar) is of interest since this effect became significant in older animals. Whether this is due to the role that urea plays in rat urine concentration after consumption of high protein diets and whether this effect can be expected also to occur in humans, remains to be investigated.

In conclusion all three treatments low, medium and high sulphate containing drinking waters, did not cause any gastrointestinal problems in

this 90-day study. Growth, food consumption and water intake were well promoted by the treatments. Hematological and blood chemistry parameters as well as extensive histological evalution gave convincing evidence that continuous high inorganic sulphate administration is well tolerated and safe.

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Summary

Male and female SPF Sprague Dawley rats received either tap water (control), Bagats (low sulphate), Vittel Grande Source (medium sulphate) or Hepar (high sulphate) as exclusive drinking water *ad libitum* during a 90-day study; additionally they received the sulphate contained in a standard, commercial, pelleted rat diet which was also fed *ad libitum.* All treatments were well tolerated and promoted the expected growth. The determination of several hematological and blood chemistry parameters gave comparable results in both sexes of treated and control animals. Observation of the animals during the treatment period did not show any diseases or ill effects. Organ weights were in the physiological range and macroscopic inspection showed a completely normal pattern. Subsequent microscopic evaluation of the organs did not indicate any deleterious effects of the treatments. An observation of interest was a trend towards lower blood urea nitrogen (BUN) values with high sulphate intake. This observation, however, only became significant in later stages of surviving rats (6 months treatment). Taking into account the ten times higher liquid intake of the rat compared to man and the continuous administration of these low, medium and high sulphate containing drinking waters, we may consider that the consumption of drinking water containing sulphate in excess of 200 mg/l as being safe.

This study therefore provides evidence that low, medium and high sulphate content in drinking water do not cause any gastro-intestinal disturbance and the hematological examinations, blood chemistry and the histopathological evaluation confirmed the absence of any deleterious effects.

Zusammenfassung

Während 90 Tagen erhielten männliche und weibliche SPF-Sprague-Dawley-Ratten *ad libitum* entweder Leitungswasser (Kontrolle), Bagats (niedriger Sulfatgehalt), Vittel Grande Source (mittlerer Sulfatgehalt) oder Hepar (hoher Sulfatgehalt) als ausschließliches Trinkwasser. Zusätzlich wurde ein kommerzielles, pelletiertes Standardfutter *ad libitum* verabreicht, in dem ebenfalls Sulfat enthalten war. Alle Trinkwasser wurden gut vertragen und förderten das erwartete Wachstum. Die Bestimmung von mehreren hämatologischen und klinisch-chemischen Bestimmungen ergab vergleichbare Resultate in beiden Geschlechtern von behandelten und Kontrolltieren. Die Beobachtung der Tiere während der Versuchsperiode ergab keinerlei Krankheiten oder pathologische Effekte. Die Organgewichte im physiologischen Bereich und die makroskopische Inspektion waren normal. Die mikroskopische Untersuchung der Gewebe ergab keinerlei Anhaltspunkte für irgendeinen schädlichen Effekt der Behandlung. Eine interessante Beobachtung war hingegen ein gewisser Trend zu erniedrigten Blutharnstoffwerten bei höherer Sulfateinnahme. Diese Beobachtung wurde nur in späteren Stadien in überlebenden Ratten (6 Monate Behandlung) statistisch signifikant. Wenn man die zehnfach höhere Flüssigkeitsaufnahme der Ratten im Vergleich zum Menschen und eine

kontinuierliche Behandlung mit Trinkwasser niedrigen, mittleren und hohen Sulfatgehaltes in Betracht zieht, kann die Aufnahme von sulfathaltigem Trinkwasser über 200 mg/l als gefahrlos gelten. Diese Studie bekräftigt daher, daß ein niedriger, mittlerer und hoher Sulfatgehalt im Trinkwasser keine Beeinträchtigung des Magen-Darm-Kanals verursacht, und die hämatologischen, klinisch-chemischen und histopathologischen Untersuchungen bestätigen die Abwesenheit schädlicher Effekte.

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Author's address:

Dr. *H. P. Würzner,* Biological Laboratory (Nestec), 1350 Orbe (Switzerland)