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

(Received November 6, 1978)

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 allotted 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