Isotopic composition of cattle pancreatic stones: Biological and geochemical implications[1]

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Summary: Latitudinal variations of the O^{18}/O^{16}-ratios of carbonate and phosphate of cattle pancreatic stones parallel a similar pattern of oxygen isotope values in rain water. C^{13}/C^{12}-ratios were virtually identical for the 7 cases studied. Isotopic measurements of mammalian hard tissues may be used for studying short-term climatic variations through Quaternary.

Material and methods. Cattle pancreatolithiasis is a spontaneous animal equivalent of the human disease, chronic calcifying pancreatitis[2]. While pancreatic lesions differ in the 2 species[3], pancreatic stones are quite similar, as shown by chemical analyses[4,5], thermal analyses[6] and X-rays diffraction[7,8]. Stones of both species are mainly calcite. In man, chronic calcifying pancreatitis is related to alcoholism in some countries and to malnutrition in others[9]. In cattle, no etiological factor has been found, except that the occurrence of the disease seems to be associated with the existence of a siliceous under-soil[10]. All known characteristics of the cattle disease, such as epizootiology, lesions and chemical composition of stones, are identical from one country to another, though the incidence of the disease varies with under-soil composition.

The oxygen and carbon isotopic composition of stones from different areas was studied to assess the possibility of identifying the area in which stones originate or the times of the year of formation in cases in which the migration habits of the animals are known. Also we wanted to check if precipitation conditions remained constant during pancreatic stone formation. It has been shown that conditions did not remain constant in the case of the formation of biliary stones[11]. Isotopic analyses of pancreatic stones were undertaken on cattle, rather than on human specimens, because the former are more readily available. Also, cattle are more closely dependent upon local soil and climate conditions than man who travels and feeds on nonlocal products. Pancreatic stones were collected from 7 animals in 4 European countries: Denmark, England, Finland and France[12]. Oxygen and carbon isotopic composition of calcium carbonate (about 95% in weight[4]) and oxygen isotopic composition of calcium phosphate (about 1.5% in weight[4]) were determined. Samples were prepared and analyzed according

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Oxygen and carbon isotopic composition of pancreatic stones ($CaCO_3$)*

France		England		Denmark No. 1		Denmark No. 2		Denmark No. 3		Finland No. 1		Finland No. 2	
δO^{18}	δC^{13}	δO^{13}	δC^{13}	δO^{18}	δC^{13}	δO^{18}	δC^{13}	δO^{18}	δC^{13}	δO^{18}	δC^{13}	δO^{18}	δC^{13}
22.97	–15.21	21.95	–15.15	21.87	–15.21	21.88	–14.94	21.27	–15.10	16.49	–15.48	16.70	–14.98
23.11	–15.17	21.65	–15.19	21.87	–15.30	21.97	–15.21	21.09	–15.53	16.44	–15.40	16.76	–14.95
23.04	–14.89	22.14	–14.95	22.09	–15.18	21.88	–15.03	21.53	–15.05	16.56	–15.51	16.61	–15.11
23.08	–14.82	21.86	–15.25	21.91	–15.22	21.74	–15.03	21.53	–15.44	16.35	–15.23	16.85	–15.03
23.19	–15.50	22.15	–15.32	21.95	–15.40	21.92	–14.95	21.48	–15.36	16.44	–15.19	17.00	–15.14
22.94	–15.16	22.26	–15.38	21.85	–15.44	21.79	–15.33	21.44	–15.18	16.63	–15.40	16.80	–14.99
23.06	–15.18	21.85	–15.25	21.89	–15.34	21.72	–15.20	21.32	–14.90	16.42	–15.39	16.79	–15.09
22.87	–15.09	22.15	–15.00	22.26	–15.23	21.46	–15.37	21.26	–14.81	16.78	–15.12	16.70	–15.20
22.75	–15.00	22.15	–14.90	21.96	–15.20	21.90	–15.10	21.49	–15.19	16.48	–15.09	16.78	–15.08
Average		Average		Average		Average		Average		Average		Average	
23.00	–15.10	22.02	–15.15	21.96	–15.28	21.80	–15.13	21.38	–15.17	16.51	–15.31	16.78	–15.06
$\delta O^{18}(PO_4^{3-})$		$\delta O^{18}(PO_4^{3-})$		$\delta O^{18}(PO_4^{3-})$		$\delta O^{18}(PO_4^{3-})$		$\delta O^{18}(PO_4^{3-})$		$\delta O^{18}(PO_4^{3-})$		$\delta O^{18}(PO_4^{3-})$	
15.9		14.7		14.3		14.3		13.8		9.5		10.0	
Probable δO^{18} of rain water in the studied areas													
-7.0 ± 0.5		-8.0 ± 0.5		-9.0 ± 0.5		-9.0 ± 0.5		-9.0 ± 0.5		-12.5 ± 1.0		-12.5 ± 1.0	

δO^{18} are given versus SMOW, δC^{13} versus PDB-1. * Reported measurements refer to both small stones (less than 1 mm in diameter) and large stones (1–4 mm in diameter). δO^{18} rain water values are evaluated from current literature and from Dansgaard temperature/δO^{18} relationship[19]. They refer obviously to a 'probable range' of values taking into account the local meteorological conditions and the possibility of yearly variations. The different sign of δO^{18} of carbonate and phosphate and δO^{18} of rain water is the effect of the carbonate/water and phosphate/water fractionation factor (α) which causes a large O^{18}-enrichment in the solid phase compared with the $\delta O^{18}(H_2O)$ of the solution from which the solid phase is precipitated.

to the techniques developed by Epstein et al.[13] for carbonate and by Longinelli[14–16] for phosphate. The standard reproducibility of the measurement was ± 0.1 per mil (1σ) for carbonate (both oxygen and carbon) and ± 0.2 per mil (1σ) for phosphate (oxygen). Isotopic analyses were carried out on single stones and analyses of phosphate were made on groups of 10–20 stones because of the relatively low phosphate content. The isotopic compositions are reported in the δ-notation:

$$\delta = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000.$$

R is the isotopic ratio (O^{18}/O^{16} or C^{13}/C^{12}). The isotopic values are reported versus SMOW-standard as defined by Craig[17] for the oxygen and versus PDB-1 Chicago standard for the carbon.

Results and discussion. The results obtained exhibit 2 main features (table). First, $\delta_{C_{13}}$ of the carbonate is essentially identical for all the samples. It is rather difficult to interpret this. We do not know the C^{13}/C^{12}-ratios of the food and consequently cannot advance hypotheses concerning biological fractionation effects.

Second, oxygen isotopic composition of carbonate is also fairly constant among stones from one single case but varies significantly from area to area, along with the phosphate isotopic composition. Moreover, these differences between areas exhibit a striking latitudinal pattern. 2 conclusions can be drawn from the $\delta_{O_{18}}$-values. First, in each case physical chemical conditions probably remained constant during the precipitation of the stones, because there is a lack of variability, within the experimental error, for the carbonate isotopic ratios even when stones of different size were measured (table). Second, variability between areas seems to be closely related to the $\delta_{O_{18}}$ of average rain water in the same areas. The oxygen isotopic composition of pancreatic stones reflects that of the water of the animal's environment, which probably is not far from the value of average rain water. A similar relationship was previously found in the case of the oxygen isotopic composition of some mammal bones[18]. Recent unpublished measurements on bones confirm the previous findings and those reported here. This seems reasonable since temperature and oxygen isotopic composition of the water in a solution are the only variables which can affect the final isotopic composition of calcium carbonate and calcium phosphate precipitated under isotopic equilibrium conditions or when biological fractionation factors remain constant. In our case, the temperature of precipitation was likely to be constant through time. The isotopic composition of the water in the body fluids is not known, but it should reflect the isotopic composition of the environmental water taken in by the organism, even if relative humidity and environmental temperature may be important as they affect vapor loss during breathing. Differences caused by isotopic fractionations during vital processes should cause constant or quasi-constant isotopic effects through time. It follows that the minor differences between average $\delta_{O_{18}}$ of the 3 cases from Denmark could reflect similar differences in the average isotopic composition of local water.

Since it is well-known that the average isotopic composition of rain water depends mainly on local climatic conditions[19], and since the oxygen isotopic composition of some mammalian hard tissues seems to reflect that of rain water, it may be possible to use isotopic measurements of mammalian hard tissues for studying short-term climatic variations. For instance, isotopic study of fossil bones of known age could provide a $\delta_{O_{18}}$ versus time curve, whose gradients could reflect variations of average rain water $\delta_{O_{18}}$ and, consequently, climatic variations in their area of origin. These isotopic measurements could then represent a completely independent tool to compare paleoclimatic curves obtained in continental areas (e.g. studying bones of Quaternary age from food refuse in caves) with sections of 'paleotemperature' curves obtained from oceanic sediment cores.

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On the in vitro behaviour of mouse submaxillary gland cells. II. Metabolic differences between male and female C3H mice[1]

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Summary. Submaxillary gland cells from female C3H mice were isolated, cultivated in vitro and their metabolic properties compared with those of male derived cells. From the results it can be concluded that these cells retain their metabolic differences when grown in vitro.

It is now well established that the sexual dimorphism of murine submaxillary glands is related to differences in the cytological differentiation of the tubular portion of the gland[2]. In addition, biochemical sex-linked differences, which are due mainly to specific properties of regulatory enzymes of the glycolytic pathway, have been found[3–6]. Moreover cells from male C3H submaxillary glands have been isolated and cultivated in vitro and their biochemical and morphological characteristic have been described[7]. Because these cells retain some biochemical properties related to their enzymatic content and their responsiveness to dibutyrylcyclic-AMP, it is conceivable that sex-related biochemical differences may be genetically determined and so independent of environmental conditions. The aim of this study was to verify this hypothesis. The cells from submaxillary glands of female mice grown in vitro and their metabolic properties compared with those of male derived cells.

Materials and methods. Submaxillary glands were randomly collected from 3-month-old male and female C3H/He mice (I.R.E. colony), removed aseptically, minced and resuspended in Hank's balanced salt solution. The cells were isolated and cultivated in vitro as previously described[7]. Cells were harvested from the growth medium