FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol.9, No 1, 2002, pp. 34 - 38 UC 616.61-004

WEATHERED COAL DEPOSITS AND BALKAN ENDEMIC NEPHROPATHY

Gerald L. Feder[1], Calin A. Tatu[2,4], William H. Orem[3], Virgil Paunescu[4], Victor Dumitrascu[4], Diana N. Szilagyi[4], Robert B. Finkelman[3], Florin Margineanu[5], Francisc Schneider[6]

[1]Florida Community College at Jacksonville, Jacksonville, FL, U.S.A., [2]Forslys Group, Arad, Romania
[3]US Geological Survey, Reston, VA, USA, [4]Department of Immunology, Clinical Laboratory No.1, Timisoara, Romania
[5]Center of Hemodialysis, County Hospital, Drobeta Turnu Severin, Romania
[6]Department of Physiology, Western University, Arad, Romania

Summary. *The correlation between the geographic occurrence of Balkan endemic nephropathy (BEN) and low rank Pliocene lignites is striking. Comparison of mass spectra of methanol extracts from lignite samples collected in the vicinity of two endemic villages, with mass spectra of methanol extracts higher grade coals, shows the presence of many more potentially nephrotoxic compounds, and much higher total organic concentrations in the lignite extracts. The Pliocene lignite composition is dominated by highly functional aromatic compounds not found in the other coal samples. The geologic history of the endemic regions helps to explain the high concentrations of highly functional aromatic compounds found in the low grade lignites in this region. The Pliocene lignite area, and associated endemic villages in Bulgaria, Romania, Serbia, and Croatia, occur on the margins of the Balkan Peninsula's major Tertiary basins. The young age and lack of deep burial or tectonic effects on the lignites have resulted in the incomplete coalification of the original peats.*

Key words: *Balkan Endemic Nephropathy, lignite, drinking water and health, environmental toxins*

Introduction

Balkan Endemic Nephropathy (BEN) is a fatal kidney disease that occurs in geographically discrete areas of the Balkan Peninsula (1,2). The disease was first described in 1956, but may have existed for many centuries (3). The disease seems to occur only in rural villages located on alluvial valleys of tributaries of the lower Danube River (4). A common geologic feature of endemic villages is the proximity to distinctive low rank Pliocene lignite deposits and lignitic shales. It was hypothesized (4) that weathering of the lignites and associated shales yielded soluble organic compounds, that were transported by the local ground water flow system to the shallow water wells used by the villagers. Figure 1, is a diagrammatic figure showing the geologic and hydrologic setting of endemic home sites.

Geochemical analysis of water samples from endemic and control villages (4) indicated the presence of potentially carcinogenic and nephrotoxic organic compounds (including napthylamines, aniline, aminophenols, and polycyclic aromatic hydrocarbons-PAHs) in much higher concentrations compared to non-endemic villages.

The purpose of this paper is to describe more recent research on the possible relationship between Pliocene lignites and the etiology of BEN.

Method of study

Field investigations and coal samples collection

In May 1999 we performed a field trip in the main Romanian BEN endemic area (Drobeta Turnu Severin), located in SW Romania. Eight endemic villages and two non-endemic sites were visited on this occasion. The villages were selected based on the medical records showing their endemic character, which we obtained from the Drobeta Turnu Severin County Hospital. Location, elevation above sea level, proximal presence or absence of coal deposits and/or coal mines were noted for each investigated site, and an observation of the surroundings was performed.

Pliocene lignite samples were collected from Pietris mine (located in the endemic village of Pietris). Methanol extracts of the lignite were made, using approximately 5 g of coal and 5 ml of chromatographic purity methanol. The extraction was performed in clean glass tubes, the coal remaining in contact with the methanol for two days. After two days, 500 μl of methanol extract were transferred into another clean glass tube, and the macroscopic particulate matter was removed by spinning down in a centrifuge, at 2000 rpm, for 10 minutes. The supernate was transferred into the GC/MS analysis glass vials. The methanol extracts were subjected to GC/MS analysis on an HP 6890/5973 system (Agilent Technologies, Palo Alto, CA, USA), using for injection an HP 7683 Series automatic injector. We used a cus-

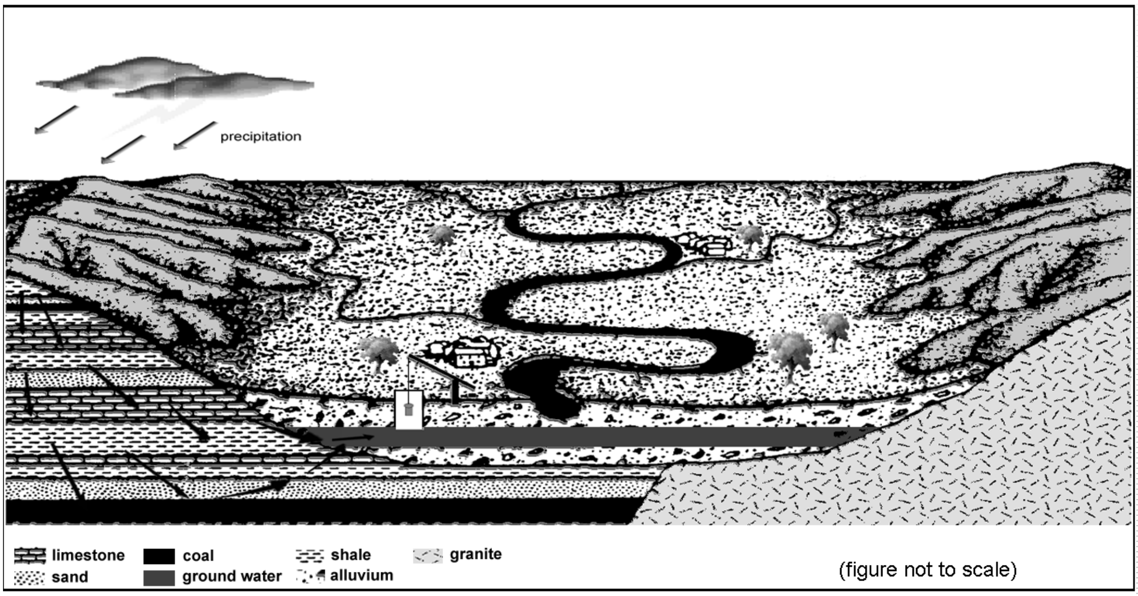

Fig. 1. Diagrammatic cross section of alluvial valley in the vicinity of an endemic home site, showing the geologic and hydrologic setting. (Map scale is approximately 1 centimeter equals 300 meters).

tom method, with the following specifications and conditions: carrier: helium 5.6 purity, constant flow, 1.0 ml/min; column: HP-5MS, 30 m × 0.25 mm × 0.25 μm; injection: splitless, 2 μl; oven: 60°C (3 min), 8°C/min 280°C (1 min); detector: 5973 MSD, 280°C. Mass spectra interpretation was helped by the use of NIST/NIH/EPA 1998 mass spectral library.

Methanol extracts from two other coal samples (see "Results and Discussions" for description) were analyzed for comparison under the same conditions.

Results and discussions

The setting of the endemic villages

All the endemic sites we visited during the field trip are located at elevations below 250 m, and occur in both narrow and wide alluvial valleys surrounded by soil covered hills. Most villages have a river flowing through, with many intermittent tributaries within the village. These streams are tributaries of the Danube River. Field observations and discussions with the villagers revealed the presence of Pliocene lignite deposits in the surrounding hills and of coal mines located in close proximity (less than 1 km) to several endemic villages (Pietris, Erghevitza, Livezile, Prunisor, Husnicioara). Figure 2. shows the locations of Pliocene lignite deposits, and endemic villages in Romania.

GC/MS analyses

The analysis of the methanol extract of the Pietris mine Pliocene lignite sample revealed a unusual organic composition of this coal. The mass spectrum is presented in figure 3, in comparison with spectra of methanol extracts of a subbituminous and a bituminous coal sample, obtained from Tebea-Brad and Paroseni-Petrosani mines, respectively. Tebea-Brad mine is located 180 km north from the Drobeta Turnu Severin BEN endemic area; the villages around the coal mine also have a population dependent on well/spring water for drinking and cooking purposes but no BEN cases have been described here. Paroseni-Petrosani coal mining complex is located 100 km NNE from the endemic area and again, no BEN cases have been diagnosed in the villages around. It is also interesting to note the tint of

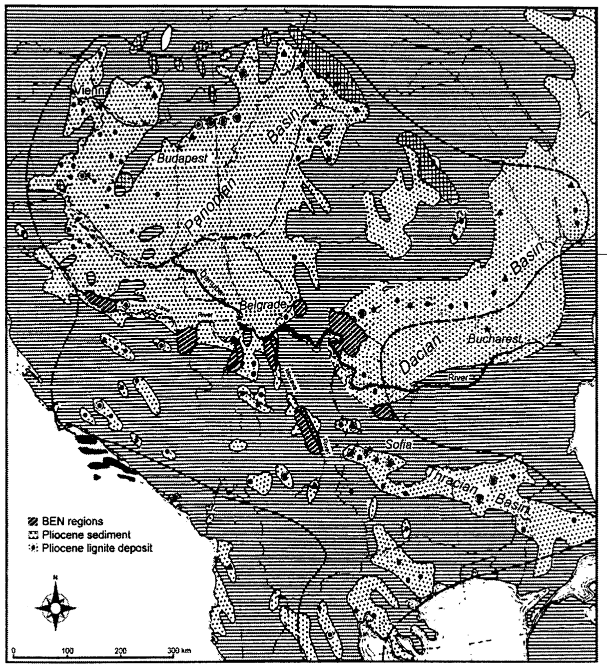

Fig. 2. Map showing the overlap between the Balkan Peninsula Tertiary basins margins and Balkan endemic nephropathy areas. Map also shows the present location of Pliocene lignite deposits. Adapted from Jasko (5).

the three methanol extracts, the Pliocene lignite giving a dark yellow color, compared to the faint yellow color of the subbituminous coal extract and the almost transparent bituminous coal extract.

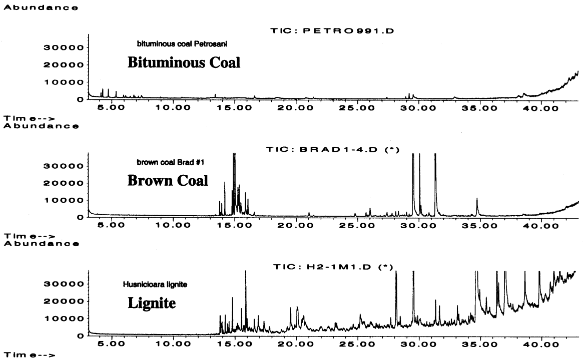

Fig. 3. Mass spectra of two days methanol extract of a Pliocene lignite sample from the endemic village of Pietris (A), of a subbituminous coal from Tebea-Brad mine (B) and of a bituminous coal from Paroseni-Petrosani mine(C).

The mass spectra of the Pliocene lignite shows an abundance of peaks not present in the other two coal samples. These represent aromatic hydrocarbons mainly in the benzene and naphthalene category, rich in functional groups: methoxy, acetyl, keto, hydroxy. Some of these compounds could be nephrotoxic/carcinogenic and have a sufficient hydrophilicity to be leached into the groundwater used by the villagers. Other compounds encountered in the Pliocene lignite extract belong to the terpane/steranic groups or represent functional derivatives of these. They are most likely generated by fungi degrading the original peat structure and reveal the poor quality of the coal, which even macroscopically resembles in many ways the fossil wood.

In contrast, the subbituminous and bituminous coal samples, although collected from two different Romanian coal fields, located many kilometers apart, give methanol extracts with similar mass spectra, the dominant compounds being monocyclic and polycyclic aromatic hydrocarbons. Compared to the Pliocene lignite, these compounds are poorer in functional groups, the most representative being: xylenes, benzene and methylbenzene, naphthalene, azulene. Alkanes like undecane and dodecane are also present in both coal samples, but not in the Pliocene lignite sample. These compounds are practically insoluble in water and their lack of functional groups with potential toxicity could explain why rural populations using well/spring water and living in the proximity of these coal deposits do not develop the disease. On the other hand, the Pliocene lignite deposits found in the hills around the town of Drobeta Turnu Severin contain many potentially toxic compounds that are leachable into the ground water, in consequence posing a health hazard to the villagers. Even if present in low concentrations into the well/spring water, in the time frame of several decades they could lead to BEN.

The geological history of the endemic regions

A map comparison (figure 2) between the present location of the BEN endemic sites and the corresponding Tertiary geographic features of the same regions, shows that BEN areas are located in places corresponding to the southeastern and southern margins of the Tertiary Panonian Basin (present day Croatia, Bosnia and Serbia along Sava River and the terminal part of Morava River, around its confluence point with Danube River, and Romania –the Caras Severin endemic area) and the southwestern and southern shores of the Dacian Basin (present day Romania – the Drobeta Turnu Severin endemic area - and Bulgaria – Vratza region -, respectively). Another endemic region is located in southwestern Serbia, around Morava River; this region also corresponds closely with the Tertiary location of a smaller interior basin – probably a shallow swampy area. A recently described endemic area in Kosovo is also spatially correlated with Tertiary marshlands that are sufficiently large to leave a significant amount of Pliocene sediment (5). A specific flora was growing on these places during the Tertiary, in a subtropical, warm and humid climate, as revealed by extensive paleobotanical investigations (6,7,8). Marshland vegetation including water grass and species of plants and trees (*Taxodium, Sequoia, Glyptostrobus*, etc.) was usually found at the edges of shallow lakes and lagoons. The dead vegetation was buried by sediment and decayed mainly under anaerobic conditions. Peat and lignite formation in these areas was a common event during the last part of Tertiary (Neogene). The locations of many of the lignite deposits that have been identified in present day Central and South-Eastern Europe correspond to the marshy limits of the Pliocene Basins (5,8). Coal formations occurring here vary in thickness from several centimeters up to several meters and are layered into several horizons separated by strata of organic rich shales and sediments. In Romania, as in the former Yugoslavia, most if not all of the endemic villages lay over or are adjacent to Tertiary sediments that have (in an 80-100 m column) 10 to 15 Pliocene lignite layers (8). Depending on location, one layer or another can be missing but overall, the presence of the Tertiary (mainly Pliocene) age coal with a particular degree of alteration is an individualizing and unifying factor for the BEN endemic foci from Romania and the former Yugoslavia. Frequently, open pit or underground lignite mines are located in the vicinity of the endemic villages. However, the Bulgarian endemic region (in the Vratza district, northwestern Bulgaria) apparently it is not very closely linked to such coal deposits, although north and south from the endemic region, extensive coal regions are found, which belong to the economically important Lom and Sofia coal basins, respectively (9). It is possible though these coal deposits to be hydrologically linked to the endemic area or undocumented (non economic) lignite deposits to exist in the BEN area. Moreover, the area where the Bulgarian BEN endemic sites are located is geologically defined by Pliocene sediments corresponding to the Southern margins of the Tertiary Dacian Basin. In other words, Vratza region emerged as a BEN endemic area in paleoenvironmental conditions similar to those encountered in the Romanian Drobeta Turnu Severin endemic region. Hence, wet, swampy lands characterizing the margins of the Tertiary Balkan interior shallow seas are a common denominator for the sites where the current BEN endemic regions are described.

Why is the disease geographically restricted?

Similar Tertiary sediments and Pliocene coals as those described above are found in other countries of the Balkan Peninsula or elsewhere, from Slovenia and Hungary in the north, to Greece, Albania and Turkey in the south, where no BEN cases have been described so far. Then, why has BEN such a limited geographical distribution? According to the hypothesis involving the Pliocene lignite derived compounds in BEN etiology, two factors responsible for this limitation can be in play: 1. the geochemical composition of the Pliocene coal associated to the BEN areas; 2. the local hydrogeological environment in the affected areas. We will briefly discuss below the two factors.

1. If compounds leached from the Pliocene lignite are related to BEN etiology than it is likely that the quality of the lignites is a significant factor in making those lignites etiopathogenic or not. In this regard, geochemical analyses revealed a higher degree of alteration of lignites sampled from Bosnia, from an area free of BEN and from Greece, compared to the lignites from the BEN endemic regions. The later ones also proved to be very rich in organic functionalities containing methoxyl, phenolic, and O-bonded aliphatic hydrocarbon groups (10). We have also shown in this work the particular composition of the Pliocene lignite methanol extracts compared to that of other coals, from nonendemic regions.

A specific organic composition of the Pliocene lignites, resulting from some particular coal forming and sedimentation conditions in the Tertiary swamps, would be a major factor in restricting disease distribution. In other words, coal geochemistry has to be unique, distinct from that of the Pliocene coals occurring in other regions, either in the same countries where BEN occurs or worldwide. For instance, in Romania, Pliocene lignites were identified in many places along the southern Carpathians, in the Carpathian foredeep (corresponding to the northern border of the Dacian Basin) but BEN is restricted only to the area corresponding to the westernmost margins of the Dacian Basin. It is likely that some specific coalification processes have occurred in these places during different stages of the Neogene, starting from the late Miocene and continuing up to the early Pleistocene. Recent studies have shown that the Oltenian lignites (sampled from depositional layers found around or underlying the endemic villages) have a peculiar organic composition. What individualizes these lignites from other lignites of Romania or other nonendemic areas seems to be the huminite fraction, rich in phenolic and other aromatic structures, probably originally derived from lignin. It is possible this huminite fraction contains certain nephrotoxic and/or carcinogenic molecules not found in the organic composition of other, "non-BEN causing", coals. Leached into the groundwater, these molecules could lead to BEN in a susceptible population group.

The major coal forming plants growing in the Balkan Peninsula Tertiary Basins belonged to several distinct phytocenoses, growing in specific paleoenvironments: the reed marsh, the *Nyssa-Taxodium* swamp, the Myricaceae-Cyrillaceae moor and the *Sequoia* moor (11,8). Although all these plants contributed to the formation of the coal deposits, by far the most frequently involved was *Glyptostrobus europeus,* which formed large forest swamps during Tertiary. Although *G. europeus* was widespread in the Dacian Basin (and very likely in the other coal forming basins of the Balkan Peninsula), some species, like *Sequoia* were restricted in distribution only to certain areas (for instance, to the western part of the Dacian Basin in Romania), probably due to some limiting paleogeographic circumstances (8). Such species could have contributed to a particular organic composition of the Pliocene lignite connected to the endemic villages. In a similar way, a specific ratio in the coal generating plants could also have been responsible for a distinct composition of the BEN-inducing coal and not encountered in other coals.

2. BEN endemic villages are located in alluvial valleys, and for most of the villages the only water supply is ground water from shallow wells dug in each yard or on the streets. We found that the depth to water in most of the open shallow wells and springs used for drinking water in the endemic villages is between 0 to 15 meters. Groundwater circulating through the Pliocene lignites and organic shales in the rocks underlying and adjacent to alluvial valleys would leach the toxic organic compounds from the rocks, and transport them to the shallow wells/springs. Even the unconsolidated alluvium in the vicinity of endemic villages may contain weathered particles of shale and lignite from adjacent hills, and these particles also contribute soluble toxic compounds to the groundwater. The occurrence of endemic and non-endemic villages within a few kilometers of each other is probably a result of variations in concentrations of toxic organic compounds in well water. This variation can be influenced by: a) proximity to formations containing the toxic compounds, b) differences in concentrations of toxic compounds in the sediments, c) differences in flux of groundwater through the sediments, and d) differences in dilution of toxic compounds due to rainfall and permeability of the soils, especially in the vicinity of water wells. The relatively low concentrations of toxic organic compounds found in the drinking waters in endemic villages could explain the long period of exposure leading to BEN.

Conclusion and future prospects

The correspondence between the Tertiary margins of the major basins located in the Balkan Peninsula and the BEN sites is intriguing and could offer, along with the similar correspondence of the Pliocene lignite deposits and BEN foci, a resolution to the old mystery of the etiology of Balkan nephropathy. The search for the toxic molecule(s) is underway through geochemical investigations of water and coal samples collected from the endemic villages, and if the suspect molecules will be found, animal experiments will follow in order to prove their nephrotoxicity and/or carcinogenicity. The nature of this compound(s) is unknown, as hundreds or thousands of organic molecules can be found in coal composition, many of them with potential toxicity. Due to the local climatic and geological conditions in the Balkan Peninsula, the coalification process of the peat has been incomplete, leaving partially decayed compounds, aromatic and nonaromatic, starting from the originating compounds of the dead plants. Such compounds are, for instance, the terpenoids, considered biomarkers of incomplete degradation of fossil fuels, like coal and oil (12,13,14). These compounds are also sometimes found in association with aromatic hydrocarbons. Also, certain of these molecules or their derivatives can have mutagenic effects (e.g., terpineol) (15), or could be nephrotoxicants, and we have found similar compounds in the methanol extracts of the endemic area Pliocene lignite but not in the other "non-endemic" coal samples. Detecting similar or identical compounds in the water collected from the endemic areas will bring a solid connection between the etiology of Balkan endemic nephropathy and the geological background of the endemic villages, dominated by the presence of Tertiary lignites.

Acknowledgments. Part of this work was supported by an internal US Geological Survey grant to William H. Orem and by funding from the Ministry of Health, Romania. We are grateful to Dr. Dan Popescu from the Institute of Public Health Timisoara, Romania, for kindly providing us a car for the field trip, and to the villagers from the endemic area for the many helpful discussions.

References

1. Plestina R: Some features of Balkan endemic nephropathy. Food Chem. Toxicol 1992; 30: 177-181.
2. Ceovic S, Hrabar A, Saric M: Epidemiology of Balkan Endemic Nephropathy. Food Chem. Toxicol 1992; 30: 183-188.
3. Tanchev Y, Dorrossiev D: The first clinical description of Balkan endemic nephropathy (1956) and its validity 35 years later. IARC Sci. Pub 1991; 115: 21-28.
4. Feder GL, Radovanovic Z, Finkelman RB: Relationship between weathered coal deposits and the etiology of Balkan endemic nephropathy. Kidney Int 1991; 40 (Suppl. 34): S9-S11.
5. Jasko S: Lignitbildung im Pliozaen in Suedost-Europa; Lignite formation in the Pliocene of southeastern Europe(In German). Braunkohle 1973; 25: 67-71.
6. Ghenea C: The Pliocene-Pleistocene boundary in Romania, in Van Couvering JA (ed): The Pleistocene Boundary and the Beginning of the Quaternary. London, Cambridge University Press, 1997, pp.216-221.
7. Ticleanu N, Diaconita D: The main coal facies and lithotypes of the Pliocene coal basin, Oltenia, Romania. In: European coal geology and technology (R.A.Gayer and J. Pesek, eds.) Geological Society Special Publications 125, London, 1997, pp.131-139.
8. Ticleanu N: Vegetal associations generating the Neogene coals of Romania. (In Romanian). Dari de Seama ale Sedintelor 1984; 70-71: 219-233.
9. Siskov GD: Bulgarian low rank coals: geology and petrology. In: European coal geology and technology (R.A.Gayer, and J. Pesek, eds.) Geological Society Special Publications 125, London, 1977, pp.141-148.
10. Orem WH, Feder GL, Finkelman RB: A possible link between Balkan Endemic Nephropathy and the leaching of toxic organic compounds from Pliocene lignites by groundwater. Int J Coal Geol 1999; 40: 237-252.
11. Taylor GH, Teichmuller M, Davis A, Diessel CFK, Littke R, Robert P: Organic Petrology. Gebruder Borntraeger, Berlin, 1998.
12. Naihuang J, Jieming S, Jian Z: Terpenoid compounds in an immature source rock. Acta Sediment. Sinica 1987; 5: 125-131.
13. Venkatesan MI: Terpenoid hydrocarbons in Hula peat; structure and origins. Geochimica Cosmochimica Acta 1986; 50: 1133-1139.
14. Zumgerge JE: Terpenoid biomarker distributions in low maturity crude oils. Org Geochem 1987; 11: 479-496.
15. Gomes-Carneiro MR, Felzenszwalb I, Paumgartten FJ: Mutagenicity testing (+/-)-camphor, 1,8-cineole, citral, citronellal, (-)-menthol and terpineol with the Salmonella/microsome assay. Mutation Res 1998; 416: 129-136.