CASE REPORT

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Contribution of Microscopic Plant Anatomy to Postmortem Bone Dating

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ABSTRACT: The authors describe a microscopical method of studying plant anatomy for estimating the age of vegetal organs which can help to date bones. This procedure was not based on counting the number of annual rings, as usually, but on demonstrating a completely primary structure without development of secondary formations; this material was considered as a part of a young root system whose development would correspond to about one year. The usefulness and limits of this procedure are discussed.

KEYWORDS: forensic science, forensic anthropology, plant, vegetal, root, annual rings, forensic medicine, bone, postmortem interval

Some extremely difficult problems are raised in forensic anthropology by postmortem bone dating. In the light of the current legislation in France, a question asked by magistrates is whether human remains date from more or less than ten years. Other than in certain special cases, legal action is no longer pursued more than 10 years after death. The dating methods used in archaeology are usually of no use in forensic anthropology.

We describe a method of utilization of plant anatomy examined by microscope for estimating the age of vegetal organs which can help to date bones. The usefulness and limits of this procedure are discussed.

Methods

Some human remains were given to us for forensic anthropological analysis and individual examination. This examination took place in two stages: Reconstructive identification, then comparative identification.

Anthropological Study

Skull measurements have been recorded, wherever it was possible. Radiographs showed that there were no projectiles nor fragments in the bones. In the right fronto-parietal region, a depressed fracture, in the shape of the weapon that caused it, was seen with an oval part measuring 16.4 by 15 mm which continued as an elongated fracture, also in the shape of the weapon, measuring 26.6 by 4.3 mm, and ending as a point at the temporal line of the frontal bone (Fig. 1). The above-mentioned fracture was seen on skull autopsy with three bone fragments pushed inwards. The right half of the encephalic cavity was completely filled with plant roots. The left half contained a soft putrefied substance which showed itself on sectioning to be brain tissue (Fig. 2).

The usual postcranial measurements were taken wherever possible and the indices calculated. An important unusual feature was noted on the right tibia, where there was 15° rotation of the tibial plateau to the right, with respect to the diaphysis. The right tibial plateau was incomplete. It is impossible to say if the individual had one leg shorter than the other because the femurs were incomplete, but an orthopedic insole was present in one of the shoes discovered near the bones.

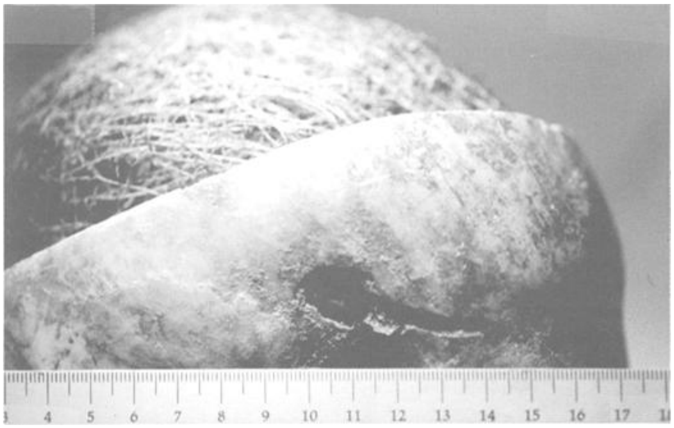

FIG. 1—*Depressed fracture in the right fronto-parietal region.*

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FIG. 2—*Putrefied brain and plant roots.*

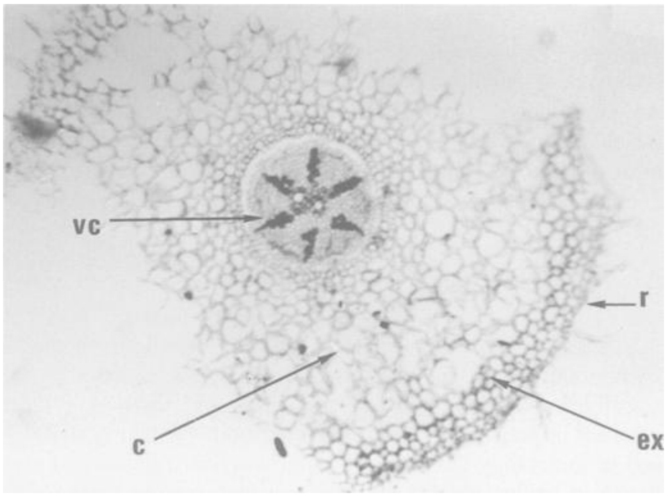

FIG. 3—*Transverse section of the vegetal sample (×50). r, rhizodermis; ex, exodermis; c, cortex; vc, vascular cylinder.*

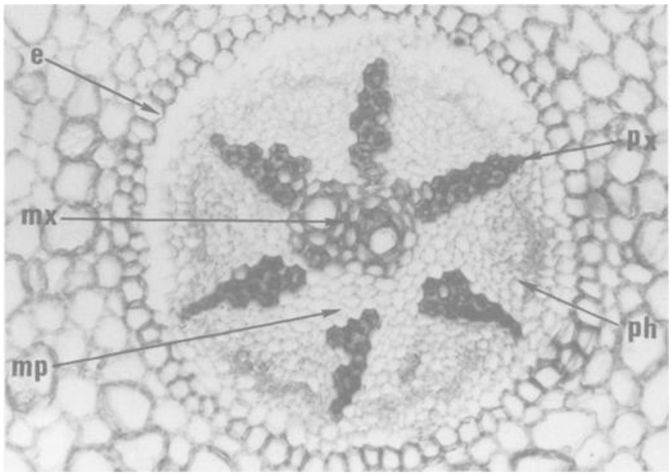

FIG. 4—*Detail of vascular cylinder (×200). e, endodermis; ph, phloem and px, protoxylem (exarch vascular poles); conducting bundles in alterning arrangement; mx, metaxylem; mp, medullary parenchyma.*

In summary, the bones were from a man between 1.69 and 1.71 m tall, who was middle-aged, probably around 40 years old. There were some discordant features regarding his age: Examination of the spinal column did not show any osteoarthritis, however, there were other features that were more in favor of an old person: The appearance of the ribs was that of someone aged between 40 and 45 years old; the gonial angle was wide obtuse; many teeth were missing with filling in by the alveolar bone; severe periodontal disease and calculus deposit were noted; a mild dental overbite was observed; there was partial synostosis of the palatine and cranial sutures which was in favor of an older person; Lamendin's technique also suggested an older person. In view of all the features present, we conclude that it was a middle-age person probably between 35 and 45 years old.

There was a depressed skull fracture: The severity of the injury could easily have been the cause of death and was probably the result of a blow with an heavy blunt instrument.

There were no other soft tissues to be found on the other pieces of bone examined. Very surprisingly, part of the brain was still present in the skull, although it was completely putrefied. In view of forensic examination of the remains, data in the literature and our own experience, we felt that death had probably occurred one or two years previously. The finding of roots in the skull led us to date the concerned plant using microscopic anatomy techniques.

Identification of the Plant

An anatomical examination of the sample gave the following characteristics (Figs. 3 and 4) (1–4): (i) Confirmation that the vegetal organ is a root: Presence of a rhizodermis with absorbing hairs and a multilayered exodermis (suberic protective zone); alternating arrangement of sieve conducting bundles (xylem and phloem) with exarch vascular poles (protoxylem) and centripetal differentiation (actinostelic model); (ii) only a primary structure without secondary formations accruing from a cambial zone (no identifiable meristematic cells) and persistent areas of medullary parenchyma: These characters are representative of a young root system whose development would correspond to about one year; (iii) the plant is a species belonging to the Dicotyledon Angiosperms and more exactly to the Ranunculaceae family: Small central cylinder, simple endodermis (thick-walled cells), limited number (six) of vascular bundles. Probably, it is an individual of *Ranunculus ficaria L.*, a perennial herbaceous plant, corresponding to geophyte type, common in forest and scrubs of the whole French territory (5,6).

Comparative Identification

The anthropological features and the information gained from an enquiry pointed to the identity. This person had apparently been hospitalized some years previously for a minor cerebral injury. The clinical X-rays were therefore seized by a court order, allowing comparative identification to be made. Comparison of X-rays with the same views revealed a number of identification criteria, of which a comparison of the frontal sinuses would appear to be the most obvious and simplest. A comparative dental expert opinion drew the same conclusion and allowed the individual's identity to be confirmed.

Discussion

The use of animal and plant material in forensic medicine has been described in the literature. Medico-legal entomology can contribute considerably in certain cases and there is a recent increase in interest for this technique, both for dating death (7) and in toxicology (8,9). In the same way, crustaceans and algae found on bodies which have been in water, may be used.

In forensic medicine, vegetation has been used to identify criminals, by comparison of a sample taken from the scene of the crime and one found on the suspect. It is, however, more difficult to recognize plant when it is dry than when it is wet (10).

In forensic anthropology, plants have rarely been used to date bones. Kerley (11) noted the potential of the association of roots with forensic bodies for determining the relative time since death. Vanezis et al. (12) described various possibilities. The first one (root injury) is studying the development of a root after it has been damaged, in particular, roots which have been damaged when a grave is dug to bury a body. It is in fact possible to count the number of annual rings that have been formed since the injury. If the cambium (the meristematic zone) between the bark and the wood, where the rings are formed, is destroyed or damaged, no secondary xylem (wood) is formed by the damaged area in the years following the damage. Although the traumatized area may heal over laterally, a permanent lesion remains. The number of rings can therefore be counted since the injury, and this can also be compared with a section made through an adjoining part of the root which has not been damaged. The second possibility (root growth), is a study of the rate of root growth. In the case described by Vanezis et al., a small root had grown in, and then out of the waistcoat of a cadaver, through the shoulders. It was possible to date the event by counting the number of annual rings in the root (dendrochronology). Roots in fact first grow longitudinally to look for nutrients and water, this being a primary structure, and then grow radially producing annual rings. The third possibility (branch growth), is a study of the annual longitudinal growth rather than the radial growth. The annual longitudinal extension can be estimated; therefore, the size and approximate spatial position of a branch can be estimated for each time period in the past. Warren (13,14) and Stewart (15) studied the rate of root penetration as an estimator of absolute time since death, concluding that caution should have been exercised, because there is no standard rate of root growth, even for roots of the same plant. Denne (16) described a case of dating from a birch branch. A skeleton had been found hanging from a branch. The rope had formed a groove in the bark and microscopy showed severe disruption. Dating was possible by counting the annual rings in this scar. In another case, the same author studied the structure of a root found on the side of a tomb. He assumed that the structure could have been altered by changes in the soil or by an increase in the amount of nutrients in it from decomposition of the body. He found a noticeable difference in the size of the vessels and so he attempted to date the events by counting the growth rings. The vessels from inside the grave were considerably broader that those from outside. In fact, the churned soil of a grave or the decomposition of a body may increase subsequent growth of plants; comparison between disturbed and undisturbed plants of the same species is necessary to determine the year of change. Willey and Heilman (17) draw attention about the fact that, to be used, plant parts must grow through the clothing, other personal effects, or bone (penetration rule), or be affected by soil disturbance or body decomposition.

Various biological limitations were underlined (18). Our knowledge of the seasonal production of wood by the roots of each tree is insufficient to be able to date the time of death from root growth rings with complete confidence. It is possible to over or underestimate when counting annual rings, especially due to false rings (i.e., extrarings formed during a single calendar year), or incomplete rings (i.e., rings that form following irregular growth around the circumference and result in oval or other distorsions from a circular cross section (18,19). Ring definition depends, in part, on the orientation of the root (growing horizontally, obliquely, or vertically to the ground surface) (17). Also the cambium has an irregular growth pattern as a result of environmental factors (light, drought, flooding, fires, parasites, or toxins) and because of interspecies and intraspecies variability. Some variations are, however, easy to recognize and interpret, for example, when the cell size varies systematically with the branch topography in the tree. We also found some biological limitations in our case. For example, our calculations took into account that the plant was from the Ranunculaceae family. Some members of this family, among which precisely Ranunculus ficaria, do not produce secondary structures whatever their age. At any rate, in our case, the young structure of the plant was confirmed by the total absence of any cambium (meristematic zone) and by the medullary which was not completely invaded by metaxylem.

The main limitation is in fact medico-legal. Even if the age of plant found growing on or inside the bones is estimated accurately, this does not necessarily mean that the human remains have been there for the same length of time. Decomposition must first reach a certain stage before roots can penetrate areas of skin loss on the cadaver. The bones may be secondarily colonized by vegetation which is already well developed. Alternatively, the plant development may be more recent than the date of disposal of the body, especially if the body has been moved. We have to be careful because roots may have been replaced over the body during burial, and surface burials may have been covered with living plants to obscure the remains (17).

In our case, the anatomic characters of the vegetal sample show that it is a young plant with roots corresponding to a development stage of about one year. We can, therefore, confirm that the body had been in this place for at least one year. We cannot, however, say if the plant developed secondarily, when the body had already been there some time. It could, therefore, have been there for more than a year. The enquiry in this particular case later led us to the disappearance of this individual that proved to be a year and a half before the discovery of the bones.

Conclusions

We used a microscopic dating method of plants which is not based on counting annual rings. The use of plant material is not very widespread in forensic medicine nor forensic anthropology. It must be stressed that plant growth is used to estimate minimal time since death. These data are, however, of interest in forensic anthropology, then all vegetation close to a grave or bones should be preserved as well as any control samples of the same plant found close by it. Sometimes, a botanist's help may be required at the time of exhumation, even before any digging has begun. Despite the number of biological and medico-legal limitations outlined above, forensic botany may prove to be of valuable help in medico-legal anthropology or thanatology.

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