

Questioned Gastric Content: Analysis of Human STR Polymorphisms and Scanning Electron Microscopy Study

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Introduction

It is known that, in order to estimate the time of death, the gastric content and its state of digestion are indecisive (1, 2). Although Rose (3) considered a reasoned opinion possible, as long as time, quantity and character of the last meal eaten were known, and the many factors that can affect gastric emptying were taken into consideration, more recent authors believe that the variables involved are too many to express a valid opinion; nevertheless, the identification of food found in the stomach, when the composition of the last meal ingested is known, can provide useful data to estimate the time of death (4) or even to identify the author of a crime (5).

If the visual recognition of ingested food in the early stages of digestion is relatively easy, with the passage of time food undergoes modifications that make the immediate identification difficult, if not impossible. In these cases it is possible to obtain useful elements with laboratory techniques that also include the observation with the stereomicroscope, the optical microscope and the scanning electron microscope (6-9).

We presented a homicide case in which the gastric content was the most debated topic. The study at our laboratory has allowed some useful considerations about the identification of the last food consumed and the time of death.

Case history

In April 2011, the corpse of a young woman, who disappeared two days earlier, was found in a forest in Ripe di Civitella del Tronto (Province of Teramo, Italy). The conclusions of the first medicolegal consultants were widely disputed, so the Judge for the Preliminary Investigation of the Court of Teramo asked our laboratory to review the case.

One of the most debated topics was the gastric content of the death woman which was given great importance for the evaluation of the time of death. The first medicolegal consultants had concluded that the last meal consisted of a glass of milk and perhaps a piece of *piadina*¹ and that the death had occurred within two hours from the ingestion. Heated arguments with experts defense derived from the interpretation of the smear examination of the stomach contents and the uncertainty of the available data, since the description of the gastric contents, always defined mush, had changed as regard to the quantity² and the colour³ in three different autopsy reports.

For these reasons, we decided to acquire the collected gastric contents.

Gastric content re-examination

The evidence came frozen at our laboratory. Even before defrosting, we observed the modest amount and the beige-brown colour. After thawing, the gastric contents appeared frankly liquid. Given the circumstances, to make sure that the collected material belonged actually to the young woman, three samples of 100, 300 and 400 μ l were taken for the study of DNA polymorphisms. The remaining gastric contents, about 20 cc, was poured into the Petri dish for a better observation, which allowed to identify, immersed and in part outcropping, some frustules of variable color (Fig. 1a,b). Observing with the stereomicroscope, the liquid surface appeared formed by minute droplets of fat. With the aid of a sterile pipette tip, the frustules were taken and placed on a microscope slide. In this manner we also appreciated the solid consistency and the colour, varying from beige to light brown. Some had a "pellicle" appearance (Fig. 1c).

Analysis of human STR polymorphisms

DNA extraction was performed with "NucleoMag[®] 96 PCR" kit (Macherey Nagel), a magnetic beads-based technique, and KingFisher mL (ThermoFisher). A pre-lysis was carried out with buffer FLB (Macherey Nagel) and protease K. DNA was eluted in 60 μ l of buffer MB6. DNA quantitation was performed with 7300 RealTime PCR System and "Quantifiler[™] Human DNA Quantification kit" (Applied Biosystems) (Table 1).

Only the sample containing the greater amount of DNA was subjected to amplification by the use of "Investigator[®] ESSplex Plus" kit (Qiagen); amplified products were genotyped using capillary electrophoresis on an ABI PRISM 310 Genetic Analyser and data were processed using GeneScan[®] Analysis Software and Genotyper[®] Software Version 3.7 (Applied Biosystems).

The genetic profile obtained from the gastric contents was compared with the genetic profile of the victim. A positive outcome was obtained for all analyzed markers. The RMP of the genotype shared by the gastric contents and the victim was calculated using the allele frequencies concerning the Italian population (6.2×10^{-18}).

Study with a scanning electron microscope

In order to clarify the nature of the frustules⁵, we proceeded to study with a LEO 1430VP scanning electron microscope (LEO Electron Microscopy Ltd, Cambridge, UK) with a Link ISIS 300 dispersive X-ray analyzer (EDX) equipped with the Cameo program for X-ray imaging color (Oxford Instruments, High Wycombe, UK). The microscope slide was placed directly into the microscope chamber and studied in variable pressure, mode of operation that allows the observation without alteration of the sample. The examination was conducted on each frustule, individually numbered for clarity purposes.

The study has confirmed primarily the presence of abundant starch grains of varying size (Fig. 2); we observed that all the frustules were characterized by a structure of "spongy" appearance, with the cavity of polyhedral shape, typical of the plant parenchyma (Fig. 3). Some frustules, then, those of darker colour, with a "pellicle" appearance, showed a surface structure characterized by relatively large thin-walled cells (Fig. 4).

Considered the solid consistency, the variable colour from beige to light brown, the "pellicle" appearance of some frustules and the microscopic structure, a few foods evoked from these characteristics were investigated with the scanning electron microscope, especially hazelnut, walnut and almond (Fig. 5). The comparison has permitted to conclude that the frustules were minute fragments of almond: characteristic of this food is in fact the surface layer of large thin-walled cells (10).

Discussion and conclusions

In the presented case the use of a scanning electron microscope has permitted to hypothesize concretely the last foods ingested by the victim.

Much was debated on this topic not only owing to the different descriptions of gastric contents already reported, but also because of the uncertainty of the testimonial evidence. The husband of the victim had reported that his wife, the day of disappearance, at approximately 1:00 P.M., had just taken a glass of cold milk (but actually he had not seen her eat), while the victim's mother declared that her daughter, around 1:30 P.M., had reported by telephone that "she had eaten a *piadina*", without further details. Always the victim's mother added that she had "never seen her dining with a glass of cold milk and nothing else. Sometimes she had dined with milk and cereals".

The first medicolegal consultants, based on the examination of the smear and testimonial evidence, concluded that the starch granules and droplets of lipid material, which they attributed to milk, were referable to a meal made up of a glass of milk and a piece of *piadina* taken between 1:00 and 1:30 P.M. of the day of the disappearance, assuming that death had probably occurred within two hours from the ingestion of food. These conclusions were contested by defense experts and considered arbitrary on the sole basis of the examination of the smear.

The re-examination of gastric contents has enabled us to observe primarily that it was a modest amount of liquid containing some frustules, that is to say a gastric content in an advanced digestion. Cleared the field of the identifying doubts by analysis of DNA polymorphisms, in addition to confirming the presence of a large number of starch granules, the use of the scanning electron microscope has allowed us to identify minute fragments of almond.

The available data suggested, therefore, that the last meal eaten by the victim included foods containing starch, fat and almonds. From the deeds, it was result that the victim had started a weight control diet in the days before her disappearance. Moreover, in addition to the already mentioned testimonial evidence, also those of inspection in the home of the victim were available. We was observed that in a kitchen cabinet there was a packet of muesli containing cereal flakes (food rich in starch) and almonds. All these elements together allow concretely to assume that the last meal ingested by the woman was made up of milk and cereal.

Similarly, considered that the muesli, containing plenty of fiber, slows gastric emptying (11, 12) and that the gastric content was in advanced digestion, it was plausible to assume that between the last meal and the death about three hours had passed instead of the two hypothesized by the first medicolegal consultants.

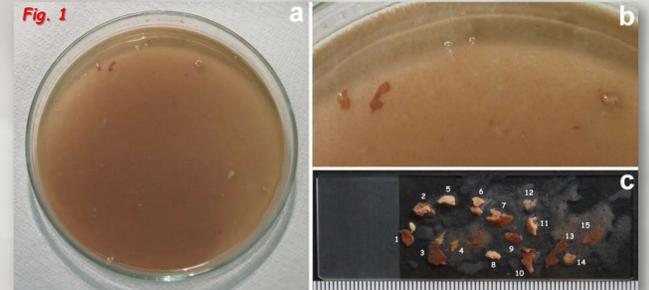
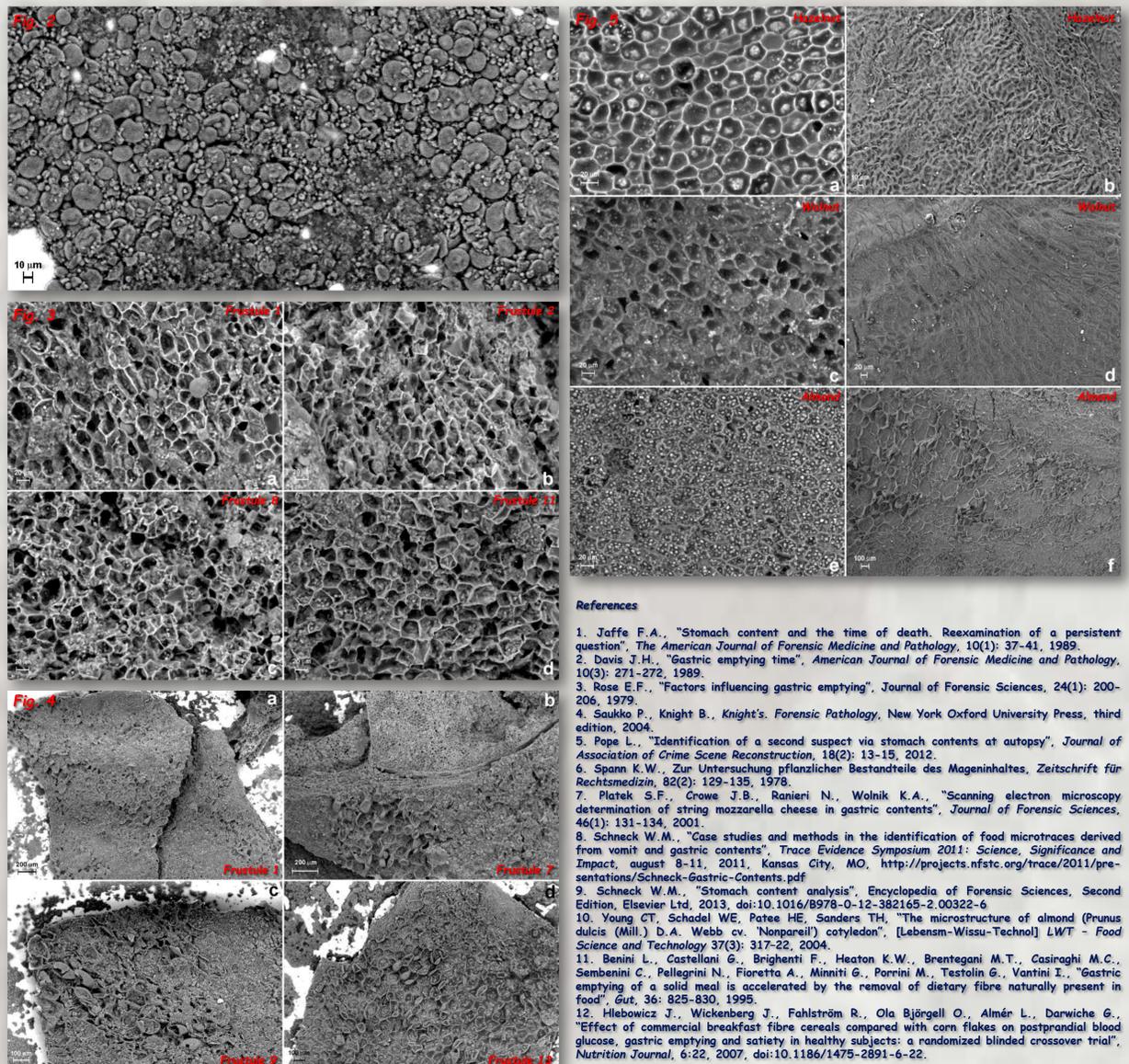


Table 1

SAMPLE	DNA CONCENTRATION (ng/ μ l)
Gastric content 100 μ l	0.659
Gastric content 300 μ l	0.258
Gastric content 400 μ l	0.217
Blood of the victim 100 μ l	13.946



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¹ For the food identification had been prepared a smear, which was thus described: "presence of numerous elements with typical 'Maltese cross', indicative of starch granules. There are also lipid vacuoles of milky substance, identifiable with fats of milk origin (milk, cheese); presence of numerous prokaryotic formations (bacteria) to type lactic acid bacteria".

² In first report are not described, quantified in 200cc in the second report and 140 cc in the third report.

³ In first report are described white-greenish, greenish in the second report and brownish in the third report.

⁴ We also took a sample of 100 μ l of blood of the victim for comparison.

⁵ For all frustules the maximum size was measured, that can only be approximate because of the minute fragments. It is nearly 4 mm for the fragment 1 (the smaller side measure nearly 2 mm); nearly 4 mm for the fragment 2; nearly 5 mm for the fragment 3 (the smaller side measure nearly 3 mm); nearly 3 mm for the fragment 4; nearly 4 mm for the fragment 5; the fragment 6 is composed to two frustules of nearly 2.5 mm; the fragment 7 measure nearly 6 mm; the fragment 8 nearly 3.5 mm; the fragment 9 nearly 4 mm; the fragment 10 nearly 6 mm; the fragments 11 and 12 nearly 3 mm; the fragment 13 nearly 7 mm; the fragment 14 nearly 2 mm; the fragment 15 nearly 5 mm.