Micro-estimation of Porphyrins in Bones, Teeth and Shells

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Porphyrins are known to be deposited in bones and teeth of men and animals with congenital porphyria (Borst & Königsdörffer, 1929; Maacke, 1931; Rimington, 1936), as well as in the shells of several species of molluscs (Comfort, 1949*a, b*), and the porphyrins found in these structures are known to be uroporphyrins. During the study of Danish strains of pigs and cattle with congenital porphyria (Jørgensen & With, 1955) it was found desirable to arrive at a method of analysis giving an easy and accurate classification of the severity of the porphyria of the individual animal, and quantitative determination of the porphyrin in the teeth of the living animal and in the skeleton after necropsy was found to meet these requirements.

Earlier investigators studying porphyrins in hard structures (Rimington, 1936; Comfort, 1950) employed relatively large amounts of material and rather laborious procedures including defatting of the bones. The method described below requires only about 50 mg. of material or even less and is extremely simple; it does not include defatting.

EXPERIMENTAL METHODS

The method is based on the finding that porphyrin from hard structures is easily and quantitatively extracted, without previous defatting, with dil. HCl if the structures are finely minced to a powder with a saw or file before the extraction. The concentration of 0.5*N*-HCl was preferred because Sveinsson, Rimington & Barnes (1949) and Rimington & Sveinsson (1950) used this strength of acid for spectrophotometric estimation of uroporphyrins. If file dust from bones, teeth or shells containing porphyrins is inspected in Wood's light, a beautiful red fluorescence is seen, and after addition of 0.5*N*-HCl (about 5 ml./50 mg. solid) and energetic shaking for about 1 min., the fluorescence is seen to have passed from the powder into the solution; if the powder is centrifuged down it shows no more fluorescence.

The minute teeth from newborn pigs with congenital porphyria are often very dark-brown and sometimes almost black; and this colour cannot be due to porphyrin alone because such black teeth do not always fluoresce in Wood's light. If the teeth are treated with 2–3 ml. of 0.5*N*-HCl after appropriate mincing, the porphyrin goes into solution while the black non-porphyrin pigment remains undissolved and can be removed by centrifuging. In several cases black teeth which did not show fluorescence on direct inspection yielded HCl extracts with marked red fluorescence.

If concentrated hydrochloric acid, giving complete dissolution of the bony structures, was employed, and dilution

to 0.5*N* performed before the reading, the results were lower than after extraction with 0.5*N*-HCl. Most of the porphyrin was extracted in 1 hr., but if the extraction was prolonged to 24 hr. the results were somewhat higher.

Analytical procedure. First the material is inspected in the light from a Wood's lamp (e.g. an Osira mercury discharge lamp with Wood's glass as used by Rimington (1943) or an Osram analytical bulb, type HQV 300, 75 w as employed in the present studies) to choose the parts from which material is to be removed for analysis, and these parts are marked. Then powdered material is produced with a saw or file from the marked parts. Small objects like teeth from newborn pigs are crushed in a mortar. Then about 50 mg. of material are weighed into a centrifuge tube and 5 ml. of 0.5*N*-HCl added, the tube is corked and shaken vigorously for about 1 min. After standing for 1 hr. the tube is centrifuged to remove the powder suspended in the solution, and the reading is performed in a spectrophotometer at 380 $m\mu$., 430 $m\mu$., and at the Soret maximum (405 $m\mu$. for uroporphyrin); in each case the maximum is determined by measuring at 403, 404, 405, 406 and 407 $m\mu$., and the highest extinction measured is employed. From the reading, the volume of the solution and the weight of the material extracted the porphyrin concentration is calculated by means of the following equations (Rimington & Sveinsson, 1950): $d = 2E_{\text{max.}} - (E_{380} + E_{430})$; $d^{\text{corr.}} = d/1.844$; $d_{1\text{cm.}}^{\text{corr.}} = 0.65$ corresponds to 1 $\mu\text{g.}$ of uroporphyrin/ml. of solution in the cell ($E_{1\text{cm.}}^1$ at the Soret maximum in 0.5*N*-HCl for uroporphyrin I is 6517). The measurements were performed with a blank consisting of 0.5*N*-HCl.

RESULTS

Table 1 presents analyses carried out on different bones from the same pig with three different extraction methods; each of the bones used was filed until sufficient powder was produced to give six portions of about 50 mg. each. Only the

Table 1. Duplicate analyses of pig bones by three methods

Method ...	I		
	0.5 <i>N</i> -HCl, 1 hr.	0.5 <i>N</i> -HCl, 24 hr.	25% HCl to complete dissolution. Dilution to 0.5 <i>N</i> before reading
	Porphyrin ($\mu\text{g.}$ /g. bone)		
Fibula	106	132	57
	103	126	95
Tibia	106	111	72
	104	118	78
Femur	131	156	113
	166	163	108

Table 2. *Porphyrin concentration at different locations (designated I and II) of the same bone and in different bones of the same animal*

Bone	Costa		Mandibula		Femur		Ulna		Ileum	Pubis
	I	II	I	II	I	II	I	II	I	I
	(µg./g. of bone)									
Pig I	7.7	4.4	10.3	6.0	11.0	11.5	—	—	4.2	0
Pig II	7.3	9.9	8.7	9.2	—	—	11.3	12.0	5.2	0
Bull	—	—	94	33	—	—	—	—	—	—

cortical substance of the diaphyses was employed. Apparently the porphyrin is partially destroyed by the strong hydrochloric acid and the extraction is not quite completed in 1 hr. with dilute acid.

Variations of the porphyrin concentrations from one part of the skeleton to another is illustrated by Table 2. The distribution of the porphyrin is not even and significant variations within the same bone may occur. This is most easily demonstrated on direct inspection of the structure in Wood's light. Some variation from one long bone to the other in the same animal is already seen in Table 1, but this point is more clearly illustrated by Table 2.

The pigs I and II were full sisters. The figure 33 from the bull's lower jaw was from the spongy portion of the condyl process, and 94 from the cortical bone of the mandibular body.

Teeth from newborn pigs with porphyria contained from a fraction of 1 µg./g. up to 165 µg./g. and teeth from cattle with porphyria (at present only four studied) 25–240 µg./g.

Bones from an ox which died as long ago as 1920 and had since been stored in formalin as a museum specimen at the pathological museum of the Royal Veterinary School, Copenhagen, showed 82 µg./g.

In a shell from the Persian pearl-oyster (*Pinctada vulgaris*) the whole shell showed 87, its dark parts 136, and its light parts 73.5 µg./g.

SUGGESTIONS

If one wants to subject porphyrin extracted by means of hydrochloric acid from bony structures to closer qualitative analysis, the easiest method is to dilute the HCl to 0.1N and adsorb on talc; McSwiney, Nicholas & Prunty (1950) state that the most convenient pH for talc adsorption is 3.4, but neutralization of the extracts from the bony structures to this pH gave precipitation of the porphyrins, and the adsorption of the porphyrins on the talc was found to be nearly complete at pH about 1. The calcium salts may be removed

from the talc by washing with dil. HCl and distilled water, and the porphyrins can be eluted with acetone-HCl, or with methanolic HCl as esters.

SUMMARY

1. An easy method of micro-determination of porphyrins in bony structures is described. The material is powdered with a saw or file and the powder extracted with 0.5N hydrochloric acid without previous defatting. The porphyrins are determined by spectrophotometric measurement in the extract after centrifuging.

2. Some results of the use of this method are given.

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