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> ONE MONTH, ONE PROTEIN <

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liquid yellow

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When the opportunity to write a piece on urine arose, I thought "wonderful, here's something we can all relate to". I had no idea, however, where it was going to lead me: from Hippocrates, uroscopy and the tradition of Hebridean waulking to alchemy, quacks and The Pisse Prophet, a 17th century satire. Very early on, physicians took a keen interest in what each one of us exudes at least twice a day. Gradually, urine became a sort of medical manual *per se* in which the overall state of health of its host could simply be read – so much so that physicians began to feel that it was unnecessary to even meet their patients. Still today, urine tests help doctors form their diagnoses, but they certainly do not exclude carrying out other tests or talking to their patients. Urine has many tales to tell – depending on its colour, its scent, its molecular composition. In healthy individuals, it is usually a shade of pale yellow owing to the yellow pigment found in it: urobilin. Urobilin is just another component of the total waste product that forms urine and whose presence depends on an enzyme of bacterial origin: biliverdin reductase.

"Stephen peeing"

Lila Copeland (1912-?)

Urine, and its excretion, is one of the many ways humans – like so many other animals – have of ridding their bodies of chemicals and molecules that are uninvited or no longer welcome. It is, in a way, a by-product of an organism's overall metabolism, besides being one of the most conspicuous. Unsurprisingly, urine is mainly water while the rest, barely 10%, is composed of inorganic salts, a lot of urea – which is full of nitrogen – as well as myriads of other organic compounds and ammonium salts, with a sprinkling of protein, hormones, metabolites and toxins. It really all depends on what you have ingested and what is going on inside you.

Humans have always had this way of putting to good use many things which may seem basic to us today – such as using yeast in the process of brewing beer or making bread. In this way, our ancestors made use of urine in several inventive ways. It soon became a fertiliser, where the nitrogen infiltrates the soil and is pumped back into the life cycle. It was used to make gunpowder, which sounds utterly incongruous. How? Left to rot on straw, the water evaporated, leaving in its wake crystals of potassium nitrate (saltpetre crystals) which form the greater part of what is needed to make gunpowder – and fireworks for that matter. In preindustrial times, urine-derived ammonia, whose smell we know so well, was used as a cleaning fluid and a mordant for textile dyes. In the Hebrides, woven wool was soaked in urine, though preferably an infant's.

Physicians, too, were swift to realise that urine could perhaps reveal what was going on inside us.

In fact, urine marks the very beginnings of laboratory medicine which is thought to have emerged with the Sumerians about 6000 years ago – although it is the Greek physician Hippocrates who is credited with being the founder of uroscopy, i.e. the inspection of urine. As the centuries rolled on, uroscopy gradually became the most acclaimed diagnostic tool – to the extent of flirting with the absurd, perhaps even proving to be dangerous. An accurate diagnosis depended on the shape of the vessels in which urine was collected. Some physicians believed that different parts of the vessel echoed different parts of the human body. Charts emerged describing up to twenty types of urine related to different bodily ailments. Some physicians even thought it redundant to talk to a patient. By the 17^{th} century, there were so many 'pisse-prophets' and 'pissemongers' that the British physician Thomas Brian published The Pisse Prophet, a satirical text which ridiculed the ongoing practice of uroscopy. The science subsequently lost its aura. In came urinalysis, as we know it today.

Urine is a filtrate of the blood, processed by our kidneys. Its colour depends on the presence of urobilin, a yellow pigment that was discovered over a century ago. However, until recently, scientists did not know how this pigment was actually synthesized. Urobilin occurs following the normal ageing and destruction of red blood cells. Red blood cells carry oxygen around our bodies thanks to chemical compounds known as haems that are an integral part of haemoglobin. Urobilin is a direct product of haem degradation. In the blood, it exists in the form of bilirubin. Bilirubin is then reduced to urobilinogen which oxidises spontaneously to give urobilin – the yellow pigment.

Are enzymes responsible for these changes? Yes. One in particular: biliverdin reductase, or BilR. BilR belongs to the Old Yellow Enzyme (OYE) family so named in the 1930s. Why old? Because a second "new" yellow enzyme was found shortly after the first. Why yellow? Because the enzyme's cofactor is a yellow pigment: flavin mononucleotide (FMN). Bilirubin reductases have motifs (HGDR motifs) which are exclusive to them. These particular reductases are thought to be the key step in haem degradation, deciding which haem by-products are reabsorbed and which are excreted. BilR acts on bilirubin by reducing multiple carbon-carbon double bonds. Surprisingly, the enzyme is expressed not by us but by microorganisms that belong to our microbiome, namely *Clostridia* of the phylum *Firmicutes*. In fact, this particular bacterium is itself solely responsible for the reduction of bilirubin to urobilinogen. Hence: for the colour of our pee.

All in all, BilR activity, or haem excretion, is an essential function of the healthy human gut. Then, surely, its presence or its absence is indicative of certain diseases, or at least of a body's homeostasis. Indeed, infants who suffer from jaundice shortly after birth seem to lack BilR. This may be because their microbiome is not yet fully developed. Levels of BilR in patients suffering from inflammatory bowel disease (IBD) are also low which demonstrates that the enzyme is essential for the balance of important metabolites. The level of BilR in urine is thus indicative of a patient's state of health and can help to pin down certain diseases. BilR, however, is also present in bacteria found in water and in soil, suggesting that the enzyme breaks down bilirubin – or metabolites similar to it – in environments other than human gut. A notion which would have surely inspired a uroscopist or two.

Cross-references to UniProt

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