Archibald Garrod & the Black Urine Disease

Kristin Gabel & Douglas Allchin

Overview

This case study focuses on Archibald Garrod and his identification of several "inborn errors of metabolism," which linked Mendelian factors to physiological chemistry at the turn of the 20th century. Nobel Prize winner George Beadle later heralded Garrod's work as an early expression of the "one gene, one enzyme" concept. The case begins with Garrod's analysis of patients with alkaptonuria, whose urine turns black. This was the first human trait recognized as exhibiting Mendelian inheritance patterns. Yet Garrod himself remained aloof from the debates that followed the rediscovery of Mendel's work, and his subsequent work was largely unappreciated by geneticists for several decades. Major nature of science elements include:

- the nature of unknowns (and the border between the known and the unknown)
- evidence and alternative explanations
- theoretical contexts in interpreting evidence
- professional responsibilities of scientists
- the role of persuasion
- conceptual change

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The Many Colors of Urine

It is London, 1892 [01].

In June, *The Strand Magazine* publishes the twelfth in a popular series of stories about a fictional detective, Sherlock Holmes [02], who strives for social justice.

A grand new bridge with two towers [03] is being built across the Thames River.

Not far away, on the north side of the river is St. Bartholomew's Hospital [04], the oldest hospital in Britain and one that provides free care for many patients.

Here, Dr. Archibald Garrod [05] is treating a patient with involuntary movements of the arms and feet, a nervous disorder known as chorea. But Garrod notes with equal interest that the patient's urine is red [06]! Why? Unlike many other physicians, Garrod has a background in chemistry. So for him, the red color of the urine is like a chemical indicator. He wonders whether the chemical difference might be important, and help someone interpret what causes the disease.

Garrod also has some interest in research [07]. He comes from a scholarly family. His father worked on gout and rheumatoid arthritis, which Garrod himself now studies too. "The scientific spirit," he wrote in 1890, "does not rest content with applying that which is already known, but is a restless spirit, ever pressing forward towards the regions of the unknown. ... It acts as a check, as well as a stimulus, sifting the value of the evidence, and rejecting that which is worthless, and restraining too eager flights of the imagination and too hasty conclusions." So, the case of the red urine gives him a chance to apply his chemistry knowledge to a human disease.

> **THINK (1)**: Given this clue, how would you approach interpreting or treating this disease?

Garrod identifies the red pigment as hematophorphyrin. But he finds that it is unrelated to the chorea. Healthy people can have this chemical in their urine, or it can be caused by a sedative.

But Garrod begins to think further about urine color as an indicator [08] of disease. Meanwhile, through collecting butterflies he meets a young chemist at a nearby hospital who shares his interest in nature. They decide to collaborate. Together they begin to study other pigments in the urine [09], normal or otherwise.

In 1897, Garrod encounters a patient whose urine turns a remarkable *black* when it is exposed to the air or alkaline solutions [10]. Yet no adverse effects are observed. This conspicuous but apparently harmless disease is already well known, first described in 1822. It is called alkaptonuria or, informally, "black urine disease." But the cause is still unknown.

> **THINK (2):** Describe how interpreting the case of the red urine might help in investigating the cause of alkaptonuria.

Chemical Anomalies and their Cause

Other scientists have worked on alkaptonuria. In 1891, Woklow and Baumann concluded that the black pigment was due to homogentisic acid, which is closely related to tyrosine, an amino acid normally found in the body. They suggested that the chemical breakdown of tyrosine was disrupted, creating the homogentisic acid [11]. Garrod confirms that alkaptonuria patients

have homogentisic acid in their urine. Again, he sees a clear chemical basis for the disease.

But why? Earlier, in 1859, Carl Bodecker thought it was caused by patients with diabetes. By the 1870s, germ theory emerged and so many diseases were considered in terms of possible microorganisms. Woklow and Baumann suggested that microbes in the intestines were responsible for the chemical anomaly. Garrod, however, has encountered individuals who have had alkaptonuria for their entire lives. He doubts it can be caused by an infection. He believes instead that alkaptonuria is congenital. That is, one may think of the unusual condition as a chemical “malformation” or ”derangement” of the body. He calls it “an alternative course of metabolism” (Garrod 1901, pp. 1, 10).

> **THINK (3)**: What evidence would help support or rule out the Woklow and Baumann hypothesis on microorganisms? What evidence would help Garrod show that alkaptonuria is due instead to a congenital chemical anomaly? If possible, identify other alternative explanations for alkaptonuria that should be considered.

Garrod investigates other known cases of the disease. The disease is not common, but when it is found, it is often shared with other members of the family [12]. Garrod notes, in particular, a trend among siblings. This is not entirely surprising. Many diseases seem to show familial patterns. Much earlier in his career, Garrod studied the frequency of rheumatoid arthritis in various families, concluding that the disease seemed influenced by inheritance. He presents his observations and his speculation on the black urine disease in 1899.

Garrod expands his collection of data. He writes to other physicians in the United States, Italy, and Germany, inquiring whether they have treated families with this condition. “Of 32 known examples, which were presumably congenital,” he finds, “no less than 19 have occurred in seven families” (Garrod, 1902, p. 3).

But Garrod [13] wants more direct evidence. He knows, in particular, a family with several children who have alkaptonuria. The mother is having another child. Garrod instructs the nurses at the hospital to watch the “nappies” used to wrap the newborn child. At 52 hours, they observe black stains in the fabric. With this confirmation, Garrod now suspects that the disease probably passes from the unaffected parents to the child. He publishes a second note on alkaptonuria in 1901.

Other physicians have read Garrod’s note in the medical journal and send information on their own. Rarely does either parent have the disease themselves. But the two parents are often cousins, or otherwise related by blood. Garrod receives one pedigree from Germany, which includes three different marriages of cousins. Garrod begins to tabulate the rate of alkaptonuria in cases of consanguinity, or intermarriage of relatives [14].

> **THINK (4):** In what ways does this new data about consanguinity provide significant information beyond the table of siblings in the same family?

Intersecting with Mendelian Genetics

During the same period that Garrod is working on the patterns of alkaptonuria, biologists experience some exciting new ideas. In 1900 Hugo de Vries (in the Netherlands) and Carl Correns (in Germany) each publish findings on remarkable patterns of heredity. They find specific ratios of offspring, echoing the earlier work of an Austrian monk, Gregor Mendel [15a,b,c]. Their work highlights the importance of discrete hereditary factors for particular observable traits. The factors form pairs that segregate and recombine in each generation. Thus, some traits may not be observable but reappear (in reunited pairs) in future generations.

In England, William Bateson, who has been studying evolution and the nature of variation, dramatically shifts his attention to the new Mendelism and becomes an enthusiastic advocate of the new principles of heredity.

In fact, Bateson does research on inheritance of the traits in pea plants that Mendel studied. He discovers that Mendel's round versus wrinkled seeds differ in starch content and the size of starch granules in the seed cells [16]. The observable traits seem to have a biochemical and cytological basis.

Just who encounters whose work first is unclear (historically), but Garrod and Bateson [17a,b] begin to correspond. The two men share ideas and become friends. Bateson, for his part, describes Mendel's work to Garrod. Can the Mendelian patterns explain the frequency of alkaptonuria in his families? Equally important, Bateson notices the pedigree patterns of alkaptonuria as plausibly matching Mendel's principles whereby hidden traits recombine. In a meeting of the Evolution Committee of the Royal Society in 1901, Bateson boldly presents alkaptonuria as an example of Mendelian inheritance — the first such trait attributed to humans.

> **THINK (5):** Explain how Bateson might interpret the evidence of siblings and consanguineous families with alkaptonuria as fitting Mendelian patterns. What kinds of proportions, or ratios, would he expect among offspring?

Bateson comments:

> Now there may be other accounts possible, but we note that the mating of first cousins gives exactly the conditions most likely to enable a rare, and usually recessive, character to show itself. If the bearers of such a gamete mate with individuals not bearing it the character will hardly ever be seen; but first cousins will frequently be the bearers of similar gametes, which may in such unions meet each other and thus lead to the manifestation of the peculiar recessive characters in the zygote. (quoted in Garrod, 1902, p. 9)

Meanwhile, Garrod likewise notes the significance of Mendelism for medicine, in possibly explaining certain human diseases through hereditary patterns. In 1902, Garrod writes a third paper: "The incidence of alkaptonuria: a study in chemical individuality." Here he explains alkaptonuria as an inborn chemical error that seems to be a Mendelian recessive trait: sometimes unexpressed, but re-emerging especially in consanguineous families. He presents other conditions — albinism and cystinuria (described more fully below) — that seem to follow a similar pattern.

> **THINK (6)**: As Bateson, how might you plan to capitalize on Garrod's findings? As Garrod, how might you plan to capitalize on Bateson's work on Mendelian genetics?

Soon Bateson, along with colleague Reginald Punnett, become embroiled in a debate with those that feel that Mendel did not adequately explain inheritance [18a-e]. These critics focus on the patterns of traits at the population level, where traits are not categorized into dichotomous types, but seem continuous. They measure the variation, which follows statistical distributions. It does not exhibit discrete ratios. In this view, inheritance involves blending of traits from both parents, with diminishing influence traced back mathematically through the generations. For this group of scientists, the Biometricians, Mendelism seems too simplistic. The Mendelians, on the other hand, emphasize individual crossing experiments as fundamental models for understanding inheritance. Powerful personalities on both sides amplify the debate.

The Mendelian debate goes on for the next decade. Mendelians use Garrod's case as

an example of human recessive inheritance. But Garrod [19] himself does not take part in the debate. Garrod feels that the “genes” and “genetics” are too vague to debate. Still, Bateson and Punnett point to his work as an example.

> **THINK (7):** Is Garrod justified in avoiding the debate or should he take an active role in it? Does he have a professional responsibility to participate? What is the consequence of participating or not participating?

In 1904, St. Bartholomew’s Hospital opens an out-patient department for the medical diseases of children [20]. Garrod helps initiate the program and is co-director for the next six years.

Chemical Individuality

Garrod’s 1901 paper marks an important step in a gradual change in focus. He shifts from the cause of the disease itself to the uniqueness of the physiology of each person. He emphasizes how persons vary chemically. He develops an image of individuality on a *biochemical* level. In a new book in 1909, he characterizes the specific deficits of reactions in the cell as “inborn errors of metabolism.” In each case a particular enzyme for catalyzing one reaction seems to be missing. The normal cell biochemistry is disrupted. He now describes, in addition to alkaptonuria, several other examples to illustrate his general idea.

The first case is albinism [21]. Albinos, Garrod notes, lack pigments in the melanin group, resulting in striking white hair, pale untinted skin, and pink eyes. The hereditary nature of albinism is easily observed and has already been studied by Bateson and other early geneticists such as William Castle, Karl Pearson, and Charles Davenport. There are interesting exceptions to complete albinism, such as individuals who gain pigment in childhood or as adults, and those whose pigmentation is patchwork, or piebald. Overall, the evidence points to not just a lack of pigment, but the “lack of a specific intra-cellular enzyme” that *produces* the pigment from tyrosine or other precursor molecules (Garrod 1923, p. 34). The cells of albinos have an identifiable biochemical nature, which can be inborn and inherited.

Another example is cystinuria. Rare individuals produce urine which can deposit hexagonal crystals of cystine [22]. Sometimes, they form stones inside the bladder, which can be life-threatening. Cystine is a component of proteins, so it is a part of the body’s normal chemistry. But ordinarily it is not excreted. The sulfur it contains is usually found in the urine, but in other chemical forms. Garrod reasons that in cystinurics cystine escapes destruction and thus is excreted in abnormally high amounts. Here, the absence of a metabolic step directly reflects normal biochemical function, which must also be inherited.

A fourth case is individuals excreting large amounts of 5-carbon sugars, or pentoses [23]. Only about 50 cases have been documented historically around the world. But they appear conspicuously in families, especially of Jewish heritage. Not much is known about the specific biochemistry. But again, an uncommon chemical in the urine reflects something distinctive about the person’s biochemistry that can be linked to heredity.

For all of these he focuses on the differences in the chemical makeup of the individual. “It would seem that there is a chemical basis for those departures from type which are styled mutations, and I for one believe that the liabilities of certain individuals to, or their immunity from, certain maladies — what may be called their diatheses — have chemical origins” (Garrod, 1928).

Garrod acknowledges that all these conditions are extremely rare. Nonetheless, they seem to indicate something fundamental about all individuals. The visible traits, like albinism, have a biochemical basis which determines someone’s unique biological character. “The most probable cause is the congenital lack of some particular enzyme, in the absence of which a step is missed, and some normal metabolic change fails to be brought about” (1923, p.16). Each

successive step in the build up and break down of cellular molecules seems “the work of special enzymes set apart for each particular purpose” (1909, p. 6) [24]. Moreover, these visible cases indicate a larger group of “other such abnormalities which do not so advertise their presence” (1923, p. 12). Thus, biochemical differences would seem to underlie other hereditary diseases of metabolism, such as diabetes, gout and obesity. They would also be equally responsible for other externally visible but very individual non-disease traits, such as eye and hair color, blood types, personal dietary reactions, and responses to certain drugs. So the study of a few diseases potentially opens an understanding of how the body works more generally.

> **THINK (8)**: Garrod emphasized biochemical individuality, with virtually no discussion of genetics. What do his findings imply in terms of the nature of genetic traits or genes?

In 1923 Garrod [25] reprints and expands his 1909 monograph. He adds two new cases of inborn errors of metabolism: hematoprophyria congenita and congenital steatorrhea.

Eight years later, in 1931, Garrod publishes an expanded version, *The Inborn Factors in Disease*, which brings together all his work and gives specific examples of biochemical individuality to disease. He presents further evidence to relate individuality to diathesis, or an inborn predisposition to disease. Physicians are largely uninterested because he has focused on rare diseases. Also, he offers no remedies. Geneticists hardly give it notice, because there is so little attention to heredity, and the study of genetics now focuses on chromosomes and other topics. They continue to study genes in guinea pigs, fruit flies, maize, and other crops. Garrod’s work thus remains obscure.

> **THINK (9)**: What might you do to increase the chances of a more positive or active reception to the ideas?

Epilogue

By 1958, however, the context for interpreting Garrod’s work has changed dramatically. George Beadle [26a] refers to it in accepting the Nobel Prize in Medicine. Beadle, collaborating with Edward Tatum, had studied mutations in a simple bread mold [26b], concluding that each mutation inactivated a specific enzyme. Thus, they firmly linked individual genes with biochemical reactions at the cellular level. The Nobel award acknowledged “their discovery that genes act by regulating definite chemical events.” The principle has since become known popularly as “one gene, one enzyme.” Beadle reflected:

> In this long, roundabout way, first in *Drosophila* and then in *Neurospora*, we had rediscovered what Garrod had seen so clearly so many years before. By now we knew of his work and were aware that we had added little if anything new in principle. ... We were able to demonstrate that what Garrod had shown for a few genes and a few chemical reactions in man was true for many genes and many reactions in *Neurospora.* [27a-d]

> Biochemists ..., especially medical biochemists, knew of Garrod’s inborn errors of metabolism and no doubt appreciated them in the biochemical sense and as diseases; but the biological world was inadequately prepared to appreciate fully the significance of his investigations and his thinking. Geneticists, it should be said, tended to be preoccupied mainly with the mechanisms by which genetic material is transmitted from one generation to the next.

> Regardless of when it was first written down on paper, or in what form, I myself am convinced that the one gene-one enzyme concept was the product of gradual evolution beginning with Garrod ... (pp. 10, 11, 12)

Beadle's tribute has since received mixed responses. Some credit Garrod for his great insight and a discovery that was "ahead of its time." Others note that Garrod refrained from talking about genes (discussing Mendelian "factors" instead), that he had not identified any specific enzymes or full reaction pathways, and that he did not stress the connection between his few cases and the widely applicable principles of genetics or cell metabolism.

> **THINK (10)**: What would it mean to say that Garrod's work was "ahead of its time"? How should one interpret the similarity between Garrod's early work and Beadle and Tatum's later prize-winning work?

Coincidentally, also in 1958 — 22 years after Garrod's death — the biochemical reason for alkaptonuria was identified. Bert N. La Du and colleagues identified the deficient enzyme as homogentisic acid oxidase [28a], that catalyzes the oxidation of homogentisic acid to maleyl acetoacetic acid, a step in the breakdown of tyrosine. In 1995, the gene for alkaptonuria was identified on Chromosome 3 [28b].

In recent years, health care has developed a growing focus on personalized medicine, based on the concept that each person's genetic make-up, and hence their biochemistry, is unique. A highly individual set of enzymes will govern distinctive and variable responses to drugs. The hope is that by profiling an individual's genome in detail, one will be able to select the most appropriate drugs and other treatments. Of course, this modern vision is rooted in the same principle that guided Garrod's understanding of chemical individuality over a century earlier.

THINK (Review): What does the case of Archibald Garrod and the black urine disease [30a,b] indicate about the following aspects of how science works:

- the nature of unknowns (and the border between the known and the unknown) (THINK 1, 2)
- evidence and alternative explanations (THINK 3, 4)
- theoretical contexts in interpreting evidence (THINK 5, 6, 8, 9, 10)
- professional responsibilities of scientists (THINK 7)
- the role of persuasion (THINK 9)
- conceptual change (THINK 10)

Extension Activity

Select a human genetic trait and investigate its chemical and molecular basis. Prepare a small poster, along with a 1-minute summary to share orally with the class.
The primary resource is OMIM (Online Mendelian Inheritance in Man):

http://www.ncbi.nlm.nih.gov/omim

Possible traits to investigate:

- blue-skinned Fugates of Kentucky (methemoglobinemia, OMIM #250800)
- hairy face, or "werewolf syndrome" (hypertrichosis, Ambras type; OMIM #145701)
- muscular dystrophy (OMIM #310200)

- dwarfism (achondroplasia; OMIM #100800)
- pseudohermaphroditism (5-alpha reductase deficiency; OMIM #264600)
- early aging (progeria, OMIM #176670)
- extra fingers and toes (polydactyly; OMIM #174200 and #174700)
- “Elephant man” David Merrick (Proteus syndrome, OMIM #176920)
- scaly skin (ichthyosis; OMIM #242300)
- “fish-odor syndrome” (trimethylaminuria, or TMAU; OMIM #602079)
- short sleeper (OMIM #612975)
- kinky hair in Menkes disease (OMIM #309400)
- reversed internal body organs (situs invertus; OMIM #270100)

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