The American Journal of Human Genetics

Vol. 10 No. 2 *June 1958*

Sir Archibald Garrod's "Inborn Errors of Metabolism"

II. Alkaptonuria

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"Of inborn errors of metabolism, alkaptonuria is that of which we know most, and from the study of which most has been learnt." *Alkaptonuria*, A. E. Garrod, 1908.

INTRODUCTION

THE LINES ABOVE, quoted from the opening of Garrod's Croonian Lecture on Alkaptonuria, are as true today as when written, though this fact cannot be easily confirmed by casual reading of the available texts. The usual source presents only a restricted view of what has been a rich and many-faceted problem, and one which in turn is still enriching our understanding of biological processes. This disease was the prototype of the inborn errors of metabolism. It was the first hereditary human disease whose mode of transmission was known, and through its use the first intermediary metabolic pathway was elucidated. The papers marking the milestones in its study constitute an interdisciplinary education in clinical chemistry (Bödeker, 1861), pathology (Virchow, 1866), organic chemistry (Wołkow and Baumann, 1891), internal medicine (Osler, 1904), biochemistry (Neubauer, 1928), statistical genetics (Hogben, Worrall & Zieve, 1932), plus recent work too near to characterize, and above all a conceptual scheme on a grand scale which cannot be otherwise characterized (Garrod, 1902). It is intended here to trace the history of our knowledge of alkaptonuria and critically to evaluate the advances that have been made in its study in the fifty years since Garrod's lectures introduced it as the type example of a new class of disease.

Alkaptonuria consists of the life-long excretion in the urine, after the first few days of life, of the strongly reducing substance, homogentisic acid. This is a normal intermediary metabolite formed in the body from tyrosine. It accumulates because the enzyme reaction normally oxidizing it is missing. Homogentisic acid can be spontaneously oxidized in alkaline medium to a brown or black polymer. This polymer causes the most famous signs of the disease: old urine is darkened and wetted clothes are stained. Something like this same black polymer also accumulates slowly in the body in certain mesenchymal tissues, producing by middle life the blackening of

Received February 10, 1958.

* This laboratory is supported by U. S. Public Health Service Grant A567 and by U. S. Atomic Energy Commission Contract No. AT (30-1)-901 with the New England Deaconess Hospital.

cartilages and related tissues called ochronosis. This coloration can often be observed through the skin, but when seen in the dissected tissues is a most arresting sight. At least some of the dyed tissues degenerate prematurely. Arthritis, the only result of this degeneration now generally recognized, is almost inevitable in this disease after middle age and it may be incapacitating.

The vast majority of the cases of alkaptonuria are adequately accounted for on the basis of a single autosomal recessive gene hypothesis. A few pedigrees showing direct transmission of alkaptonuria from one generation to the next have given rise to the suggestion that there may also be a very, very rare, dominant form. The evidence for the existence of a dominant form is weak, and it is more likely that all cases conform to the recessive mode of inheritance.

I. HISTORY

Sir Archibald Garrod was, among other things, a scholar with a beautiful and lucid style which makes him one of the most quotable of medical writers. His version of the history of alkaptonuria deserves to be given in his own words, and especially so since it has been the source of the introductory paragraphs of so many papers about this disease: "Until the early years of the nineteenth century no distinction was drawn in medical writings between urines which were black when passed and such as darkened on exposure to air, but it is difficult to suggest any other diagnosis than that of alkaptonuria for some cases referred to in works of the sixteenth and seventeenth centuries, such as that mentioned by G. A. Scribonius (in 1584) of a schoolboy who, although he enjoyed good health, continuously excreted black urine, and that cited by Schenck (in 1609) of a monk who exhibited a similar peculiarity and stated that he had done so all his life. The most interesting record of this kind is to be found in the work of Zacutus Lusitanus, published in 1649. The patient was a boy who passed black urine and who, at the age of fourteen years, was submitted to a drastic course of treatment which had for its aim the subduing of the fiery heat of his viscera, which was supposed to bring about the condition in question by charring and blackening his bile. Among the measures prescribed were bleedings, purgation, baths, a cold and watery diet, and drugs galore. None of these had any obvious effect, and eventually the patient, who tired of the futile and superfluous therapy, resolved to let things take their natural course. None of the predicted evils ensued, he married, begat a large family, and lived a long and healthy life, always passing urine black as ink." (Garrod, 1908).

It would appear that undue emphasis has been placed upon the darkening of alkaptonuric urine, since many know of it only in this way. Black urine, of course, is one of the more striking of the manifestations of this whole group of diseases "which advertise their presence in some conspicuous way, either by some strikingly unusual appearance of surface tissues or of excreta, by the excretion of some substance which responds to a test habitually applied in the routine of clinical work, or by giving rise to obvious morbid symptoms." (Garrod, 1909, p. 16). But the urine blackens slowly by itself, and must be looked at a day later with seeing eyes to note the change, while its response "to a test habitually applied in the routine of clinical work" is much more regularly observed by the busy practicioner. This test, one for the reducing action of

urine to detect diabetes, is responsible for most of the diagnoses of alkaptonuria. It is also the reason why most of the early patients, and about half of those seen even today, were considered for a shorter or longer time to be diabetics.

The First Patient: The confusion of diabetes and alkaptonuria was to be expected when Bödeker (1859, 1861) described the first alkaptonuric patient seen in modern times as also having glycosuria. We can now distinguish glucose by the specific tests for it. Alkaptonuric urine can be differentiated by its negative results with bismuth reduction, fermentation, phenylhydrazine precipitation and optical rotation, and identified by its blackening of undeveloped photographic film (Fishberg, 1942). Yet when Bödeker first met this problem he fared better than many of his successors. The urine reduced Fehling's solution as if it contained glucose, but it did not reduce bismuth hydroxide (e. g., the Nylander reaction), a test still useful in distinguishing homogentisic acid from glucose. From the result of these tests and others, he concluded that the reduction was not caused by sugar. Yet the bias that reducing urine meant diabetes was strong. By greatly concentrating the urine, he detected some fermentation with yeast, and concluded that sugar was present "above the usual small quantities—certainly not above 1 per cent." From his amazement that the patient continued well, without polyuria or other diabetic symptoms, it is clear that he thought he was dealing with an atypical form of diabetes.

What had first caught Bödeker's attention was the color change of the urine as he made it alkaline for the test for sugar. This observation was made in the course of a routine examination of urine from a forty-four year old man, hospitalized without relief, it should be noted, from his incapacitating arthritic pain in the lumbar spine. "Sobald ich Behufs der Prüfung auf Zucker den Harn zuerst nur mit etwas Aetznatron kalt mischte, sah ich, sie die blass röthlichgelbe Flüssigkeit sich von oben herab auffallend braun verdunkelte. . . . " As the urine darkened *from the surface*, "it took up somewhat more than its own volume of oxygen gas," and this gave the substance its name. "I call it for this reason 'Alkapton' (admittedly a somewhat barbarous combination from the Greek participle of *κάπτειν*, to suck up greedily, and the Arabic *al kali*), after its outstanding behaviour toward oxygen in alkaline solution." It should be mentioned here that the word, alkaptonuria, is not less of a barbarism when the "k," which is at least half Arabic in origin, is changed to "c" as is frequently done in the English-speaking world.

Bödeker isolated by lead precipitation and ether extraction, methods which became classical, a sugar-free but nitrogen-contaminated, crystalline sample of his "alkapton." Before he could obtain more for determination of its structure, the patient left the hospital. Bödeker's "fervent hope" that the man would again seek hospitalization was after two years still "sadly unfulfilled," although he then received "a couple of ounces of urine," enough to repeat the qualitative tests and establish the condition as a chronic one. Not for thirty years was another of these rare patients to come into the hands of a biochemist equally as competent as Bödeker. Although he did not find the structure of the "alkapton," he had recognized that its uptake of oxygen in alkaline solution with the formation of dark brown or black pigment was similar to the behavior of hydroxyphenols like pyrogallol and quinone.

The Search for "Alkapton": The subsequent guesses about the chemical nature of

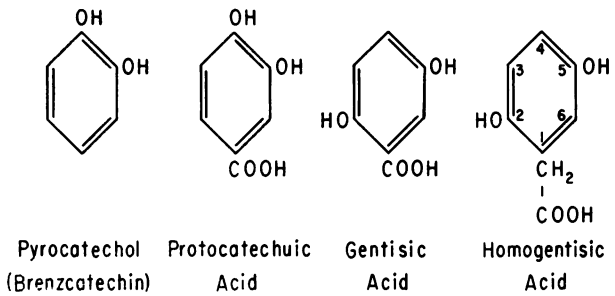

FIG. 1. Structural formulae suggested for the "alkapton", and of the metabolite of salicylic acid, gentisic acid, from which homogentisic acid derived its name.

the compound kept closely to the hydroxyphenols with which Bödeker compared it, but the names given it were limited only by the number of cases seen before 1891, when the structure was at last established. Not until 1875 was alkaptonuria again reported, and then two cases were found. Ebstein and Muller (1875) assumed that the substance excreted was pyrocatechol, simply on the basis of its color with ferric chloride. For this reason their patient was excluded from the series Garrod later collected, although the urine tests were typical of alkaptonuria. Fleischer (1875) then obligingly isolated a small amount of pyrocatechol (Fig. 1) from the urine of Fürbringer's (1875) patient, and though he also isolated similar amounts from the urines of several non-alkaptonurics, the German workers (including Bödeker—see note in Fürbringer, 1875), were in temporary agreement that alkaptonuria was "brenzcatechinuria."

Then cases began to be found abroad. From Dublin, Smith and Armstrong (1882) reported a three year old girl with alkaptonuria. They noted a simple fact, that the "alkapton" could not be extracted into ether unless it was first acidified. This showed that it had an acid group, and they guessed that it was protocatechuic acid. Brune (1886, 1887) proved that the material isolated from the urine of the first American case was not protocatechuric acid. He also stated that all the contemporaneous workers were dealing with the same substance—one which was of no pathological importance since it did not indicate diabetes! The purest compound, with nearly the correct melting point, was isolated by Marshall (1887) from urine of a patient he did not see, one whose "diabetes" prevented his buying life insurance. Marshall called this substance "glycosuric acid" and strangely enough made no mention of its darkening reaction. This patient was actually the same as Brune's (see King, 1915), and was later described by Osler (1904). The patient was the younger of the two brothers in whom ochronosis was first diagnosed clinically, as well as the somewhat overdiagnosed first American case.

Up to this time there had been a remarkable unanimity, despite the several names used, about the properties of the "alkapton" and, most importantly, about the fact that only one substance caused the phenomena observed in the affected urines. This was changed by Kirk in Britain. A charitable assessment of his studies should emphasize that he established the familial incidence of alkaptonuria. Three younger boys in a family had alkaptonuria, while an older boy and the parents were normal.

This important observation was reinforced by Kirk's parenthetic remark (1886) that Smith had written him to say that the three year old Dublin patient now had a sibling, also alkaptonuric. Perhaps mention should also be made of Kirk's belief that the "alkapton" was a disinfectant. He believed its presence in these children accounted for the mildness of their illnesses during a typhoid epidemic when many people on their street were dying (Kirk, 1889a). He could not explain the fact that the youngest child nevertheless died of whooping cough.

By his own admission, chemical matters were beyond Kirk (1889a). He isolated fractions from urine containing the "alkapton" and named them by their colors: "urrhodinic acid" (1886), "uroxanthic acid" (1888) and "uroleucic acid" (1889b). Huppert of Prague may be really to blame for taking seriously this example of phenomenology. He was in search of the latest facts for a new textbook on urine, and though he realized (correspondence in Kirk, 1889a) that all of Kirk's fractions were impure "uroleucic acid," he assumed that at least it was real and different from the "alkapton." Eventually he published a probable structure, based on analyses of Kirk's impure samples (Huppert, 1897), as 2,5-dihydroxyphenyllactic acid. Not until years later was this error corrected, when Garrod saw that the excretion of two substances instead of one would weaken the case for the single metabolic block he envisaged, and when no other patient had been found to excrete more than the one substance homogentisic acid. Garrod then reexamined Kirk's patient. This youth excreted only homogentisic acid (Garrod, 1902). Still later, after Kirk's death, Garrod examined the samples of "uroleucic acid" and found nothing but impure homogentisic acid (Garrod, 1909).

"The New Era": These early experiments were authoritatively reviewed in 1891 by Wolkow and Baumann. They did this with a fine sense of history, for as they said in the introduction to their paper, having now "prepared the alkapton in pure form, determined its structure, shown how it is related to metabolism, and found some of the conditions for its creation . . . the alkapton problem moves into a new era [neues Stadium]." Much of this classical paper is indeed relevant to the next phase of alkaptonuria investigation, the metabolic studies, where it will be discussed. For the moment, it is enough that they furnished elaborate chemical proof that the substance isolated was not one of the sixteen known acids with the same empirical formula, and identified it as a compound unknown till then, 2,5-dihydroxyphenylacetic acid. They named it homogentisic acid, since it was the next higher homologue of the familiar salicylic acid metabolite, gentisic acid. They also carefully outlined the great similarities of the urine from their patient to the urines already described. They left no doubt that where verifiable properties of the isolated substances had been recorded, namely for Marshall's "glycosuric acid" and Brune's isolated compound, these substances were also homogentisic acid. As for Kirk's "uroleucic acid," their first deep red extract, the subsequent yellow fraction, and the final white material seemed very similar, but in the end they proved that such a substance as Kirk had described was entirely absent from the urine they studied. This otherwise excellent work also contained a forceful statement that homogentisic acid was formed by the putrefaction of protein in the gut and not by the tissue metabolism.

An Inborn Error of Metabolism: The chronology of discoveries about alkaptonuria

—the chemical identification of homogentisic acid in 1891—the pathological identification of ochronosis with alkaptonuria in 1902—the clinical recognition of the accompanying arthritis in 1907—the realization about the same time that it was a metabolic disease—would seem to have set the stage for Garrod's grand synthesis in 1908 of the clinical signs with heredity and with step-wise metabolic aberrations. Nothing would be farther from the truth. The concept of alkaptonuria as an inborn error of metabolism was developed almost independently of the above discoveries, and it was formed by 1902 (Garrod, 1902).

There can be no doubt that alkaptonuria was the prototype of the inborn errors of metabolism. It was the first of these diseases that Garrod studied, and the one to which he personally made important contributions. It is also the disease which first fitted and which most closely fits, then and now, the definition he gave for the group. His publications on the subject permit us to follow the genesis of his concept.

Garrod began his studies with the description of five new cases of alkaptonuria (making a total of twenty-eight), almost certainly more than any one person had seen up to that time (Garrod, 1899). His cases confirmed the fact, first reported by Kirk, that this very rare disease might be met in several members of one family. His subsequent contributions were curiously off the main paths of the then current investigations of alkaptonuria. He seemed almost indifferent to much of the reported work. He assembled new information, and verified or disproved earlier statements, but only of a sort which interested him. He was from his first paper a man with an idea.

The sort of information which interested him and was reported in his second paper (Garrod, 1901) concerned the duration, the metabolic nature and the familial incidence of alkaptonuria. The staining tendency of the urine of a new-born child, whose older sibling had already been diagnosed as an alkaptonuric, was described from birth, diaper by diaper. There was slight staining of the diaper thirty-eight hours after birth, deep staining of one after fifty-two hours, and staining of all subsequent ones. Garrod associated the beginning of the alkaptonuria with the child's first feeding. The lag of a day or two before stained napkins were noted, also supported by the history of several additional cases, might now be ascribed to the time necessary for maturation of the phenylalanine and tyrosine oxidizing systems in the liver of a new-born child (Kretchmer *et al.*, 1956; Kenney *et al.*, 1958). Although patients with intermittent alkaptonuria had been described, Garrod was not convinced they occurred (Garrod, 1902), and by the above observations he had demonstrated that the condition was congenital in at least some cases.

Garrod doubted the theory almost forced on the world by Baumann that alkaptonuria resulted from a specific form of infection of the alimentary canal. To check this, he measured the urinary homogentisic acid of his four year old patient at intervals after feeding. He found that the maximal excretion occurred four to seven hours after a meal, coincident with the peak excretion of nitrogen, and not earlier as might have been expected if homogentisic were formed in the gut before the absorption of tyrosine. From this he concluded that tyrosine from the diet must be absorbed and then converted by the body's metabolism to homogentisic acid. "The facts lend support to the view that alkaptonuria is what may be described as a 'freak' of metab-

olism, a chemical abnormality more or less analogous to structural malformations." This is a much more pregnant statement than an earlier one by Kirk (1886), that the anomaly "must result from a profound perversion or arrest of metabolism."

Two new observations about the familial incidence of alkaptonuria reinforced the earliest statement of the concept of inherited biochemical abnormalities. "There is, as yet, no known instance of its transmission from one generation to another. . . ." And later in the same paper, "I am able to bring forward evidence which seems to point, in no uncertain manner, to a very special liability of alkaptonuria to occur in the children of first cousins." The latter information concerned four sibships, all from first cousin marriages, "including no less than eleven alkaptonuric members, or more than a quarter of the recorded examples of the condition" (Garrod, 1901).

The words "freak" and "sport" were used to describe the appearance of alkaptonuria among sibs whose parents were unaffected. It was the language of the day for such phenomena. Darwin had used the same terms for the differences within a species on which selection might act to produce evolutionary changes. Understanding of these matters had not yet gone beyond that possessed by an observant husbandryman, but Garrod had marshalled the important facts of heredity, metabolism and disease. The spark that would fuse them together was then in press: the rediscovery of Mendel's work.

The year 1902 saw the emergence of modern ideas of hereditary disease in a surprisingly mature form. William Bateson published "Mendel's Principles of Heredity" (Bateson, 1902), which contained an explanation of why the mating of first cousins would enable a rare recessive condition to show itself. In an obscure footnote, Bateson actually said that a rare recessive factor would explain the observed incidence of alkaptonuria (Bateson & Saunders, 1902). In the same year Garrod made use of this information and more that he had gathered to publish a précis of the inborn errors of metabolism (Garrod, 1902). The intellectual climate of the times can be gauged from some of its words: "The question of the liability of children of consanguineous marriages to exhibit certain abnormalities or to develop certain diseases has been much discussed, but seldom in a strictly scientific spirit. Those who have written on the subject have too often aimed at demonstrating the deleterious results of such unions on the one hand, or their harmlessness on the other, questions which do not here concern us at all. There is no reason to suppose that mere consanguinity of parents can originate such a condition as alkaptonuria in their offspring, and we must rather seek an explanation in some peculiarity of the parents, which may remain latent for generations, but which has the best chance of asserting itself in the offspring of the union of two members of a family in which it is transmitted" (Garrod, 1902).

Since his last paper on the subject, Garrod had corresponded with many who had investigated alkaptonuria to obtain information they had neglected to publish about the families of these patients. The results were still fragmentary, but "more cannot be learned until new cases are described." For contemporary readers the clarity of the presentation must have suffered, because Garrod sought to do much more than support the recessive hereditary nature of the condition. This paper on "The Incidence of Alkaptonuria: A Study in Chemical Individuality," which Hogben *et al.*,

(1932) called "a landmark in the history of human genetics," is also the key reference for the development of the concept of inborn errors of metabolism.

Garrod (1902) first recorded the results of his reexamination of Kirk's patient, who now excreted only homogentisic acid and no "uroleucic acid." It is not reading too much into this introduction to assume that he checked the finding because he felt the excretion of a single compound, instead of two or more, was the more likely result of a single metabolic block of the sort he had in mind. Next he reviewed his earlier conclusions that alkaptonuria "is not a manifestation of a disease, but is rather of the nature of an alternative course of metabolism, harmless and usually congenital and lifelong" (though as a course of metabolism it was "somewhat inferior to the ordinary plan . . . ," with "a certain slight waste of potential energy."). Next followed a table giving the amounts of homogentisic acid (2.6 to 5.9 g.) excreted per twenty-four hours by nine different patients. None was found in the urine of normal individuals, so the excretion represented a qualitative trait, not simply a quantitative difference. Garrod had learned about the sibships of nineteen patients. These nineteen plus their twenty-nine sibs made up nine families. In one family, that of the two brothers described by Osler, a son also had alkaptonuria, but in all other families only the members of one generation were affected. In ten families about which information on the relationship of the parents was available, six (containing twelve alkaptonurics among thirty-six sibs) were the offspring of first cousin marriages. The parents of four sibships (containing seven alkaptonurics among more than fifteen sibs) were not known to be related. Garrod contrasted this incidence of 60 per cent consanguineous marriages in alkaptonuric families with the estimate by Darwin that less than three per cent of the marriages in England were consanguineous. He concluded that a very rare "latent peculiarity" in the parents asserted itself under these conditions. He was quick to state that this did not occur in most consanguineous marriages, else there would be upwards of 50,000 alkaptonurics in London alone, in place of the mere six he had located by searches at two active hospitals. At the end of his paper Garrod quickly sketched the salient characteristics of a "sport" such as alkaptonuria: a conspicuous and specific chemical deviation occurring among brothers and sisters, often the product of first cousin marriages. Albinism, and possibly cystinuria, might also represent similar conditions, and this would make the first more believable. If so, a new class of diseases would be known.

In the Croonian Lectures, and his first edition of them, Garrod (1908, 1909) noted some evidence in the larger sibships of a 3:1 Mendelian segregation, but he did not further develop the study of the heredity of alkaptonuria. He had become occupied with albinism, cystinuria and a fourth condition, pentosuria, as additional examples which bolstered the validity of his concept. Most of his discussion of alkaptonuria dealt with the biochemical aspects which were then very imperfectly known. In effect, he left those parts of the problem, like the fact of inheritance which was moderately well-established, and turned to work on those aspects which were still without form. Though in his lectures his discussion of the biochemical problems of homogentisic acid excretion was not particularly well-founded on the most recent work, his contribution was of permanent value. He intuitively imposed the correct form on the incomplete and contradictory data available at a time when it was not

really known that metabolism occurred in discrete enzyme-catalyzed steps, when homogentisic acid was widely believed to arise from putrefaction in the gut, and when other evidence identified it as an abnormal compound formed only by alkaptonuric patients. Subsequent work has fully borne out his view of compartmentalized metabolism, each step of which was under hereditary control. The initial statement of this view was the guide to the understanding of alkaptonuria, the basis of his concept of the inborn errors of metabolism, and represented the birth of biochemical genetics: "The conception of metabolism in block is giving place to that of metabolism in compartments. The view is daily gaining ground that each successive step in the building up and breaking down, not merely of proteins, carbohydrates, and fats in general, but even of individual fractions of proteins and of individual sugars, is the work of special enzymes set apart for each particular purpose.

"It may well be that the intermediate products formed at the several stages have only momentary existence as such, being subjected to further change almost as soon as they are formed; and that the course of metabolism along any particular path should be pictured as in continuous movement rather than as series of distinct steps. If any one step in the process fail the intermediate product in being at the point of arrest will escape further change, just as when the film of a biograph is brought to a standstill the moving figures are left foot in air. All that is known of the course of catabolism tends to show that in such circumstances the intermediate product in being is wont to be excreted as such, rather than that it is further dealt with along abnormal lines. Indeed, it is an arguable question whether, under abnormal conditions, the metabolic processes are ever thrown out of their ordinary lines into entirely fresh paths, with the result that products are formed which have no place in the normal body chemistry" (Garrod, 1908).

II. METABOLISM

Enough was earlier said of Wolkow and Baumann's (1891) work to identify it as the second milestone in the study of alkaptonuria, to be compared only with Bödeker's (1859) discovery and Garrod's conceptual synthesis (1902). This single paper contained not only a discerning assessment of the virtues and defects of earlier work, and the chemical isolation and identification of the "alkapton" as homogentisic acid, but it also laid the groundwork for the studies that were to occupy biochemistry in this area for the next sixty years. It also contained a very strong statement about the nature of the disease which seems ludicrous today.

As soon as the aromatic ring structure of homogentisic acid was certain, its origin was deduced. Baumann, who had much experience in the study of the origin of excreted compounds, stated flatly that only plants and not animals could make aromatic compounds out of substances without benzene rings. The aromatic compounds such as homogentisic acid that were excreted by animals could come only from the protein in the body or in the diet. There were regularly only two (then) known aromatic substances in protein, tyrosine and phenylalanine. Wolkow and Baumann had in the laboratory sufficiently large samples for metabolic studies only of one of these substances, tyrosine. They developed a method for the quantitative assay of homogentisic acid in urine by silver titration, and fed their patient the tyrosine. The results

TABLE I. ORIGIN OF HOMOGENTISIC ACID FROM TYROSINE OF DIETARY PROTEIN (WOLKOW & BAUMANN, 1891)

Regimen	Homogentisic Acid Excreted (g/day)	
	Total	Extra
Hospital diet (av. 14 days)	4.6	—
Hospital diet + 10 g. Tyrosine	11.5	6.9
Hospital diet + 11.5 g. Tyrosine	14.2	9.4
Meat diet (av. 17 days)	6.4	—
Meat diet + 12.5 g. Tyrosine	15.8	9.4

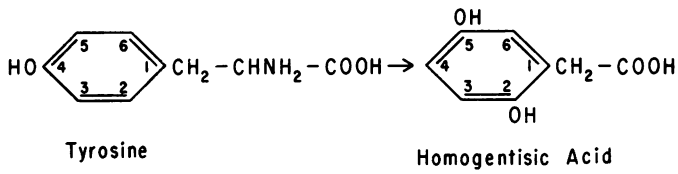

FIG. 2. The over-all conversion of tyrosine to homogentisic acid, deemed impossible for mammals in 1891.

showed that the amount of homogentisic acid excreted by their patient was increased with a high protein diet and greatly increased when the tyrosine was fed (table I).

Man or Microorganism? Baumann's chemical knowledge misled him only when he attempted to deduce the mechanism by which the precursor tyrosine was converted to homogentisic acid. The conversion involved the unheard of change of a 4-hydroxyphenyl compound to a 2,5-dihydroxyphenyl compound; i.e., the removal of one hydroxyl group and the appearance of two new ones (Fig. 2).

"Man hat niemals das Verschwinden einer Phenolhydroxylgruppe durch Reduction in den Organen des Thierkörpers beobachtet." In fact, Baumann made an eloquent appeal that if such a conversion occurred in the tissues *which was not already known to chemistry*, the certainty built up by the study of the metabolism of hundreds of compounds over the past decade would be lost. Metabolism might then do anything and to investigate the metabolism of any substance would be useless. He concluded with the observation that, although some excreted substances were intermediate products of metabolism, "Das ist aber bei der Homogentisinsaure . . . ganz und gar nicht der Fall." Having denied the ability for this conversion to man, he had no recourse but to attribute it to the little animals, the bacteria in the gut: "Auf Grund obiger Darlegungen sind wir zu dem Schlusse gelangt, dass die Bildung der Homogentisinsäure aus dem Tyrosin nicht curch eine an sich unerklarbare abnorme Function des Stoffwechsels in den Geweben bedingt, sondern als eine Wirkung einer besonderen Art von Mikroorganismen anzusehen sei." Kluyver and Zijp (1951) greatly enjoyed this quotation while pointing out that in the intervening sixty years no one had been able to find microorganisms that produced homogentisic acid. They, and Utkin (1950), recorded the only known examples, the conversion of tyrosine and phenylacetic acid to homogentisic acid by *Aspergillus niger*.

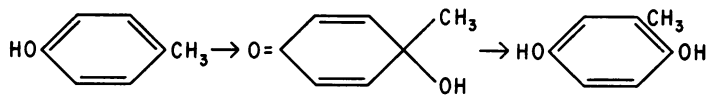

FIG. 3. The chemical reaction of side-chain (CH_3) migration, through an intermediary quinol This reaction served as the prototype of homogentisic acid formation.

The proof offered for this strongly held theory, that the bacteria in the intestine formed homogentisic acid, came from a single attempt at intestinal disinfection. The patient was given 6 g. of salol (phenylsalicylate) daily for three days while the diet was kept constant. The amount of homogentisic acid excreted daily was unchanged, except on the third day when 60 per cent of the usual amount was excreted. Contrary to their usual standards of work, this crucial experiment was not repeated because the patient left the clinic. But the theory was indirectly disproved by a still more important experiment whose meaning was not then fully appreciated. A small dog metabolized nearly all of a dose of 4.5 g. of homogentisic acid. When it was later shown in a similar experiment that a normal man could also metabolize large doses of homogentisic acid (Embden, 1893), it should have been clear that the metabolism was abnormal in the alkaptonuric patient.

Despite the effective work of Baumann's student, Embden, who disproved his master's theory in several ways, Baumann's putrefaction theory of the origin of abnormal metabolites was not weakened until the scientific reason for its proposal was removed. An alternative to the metabolic formation of homogentisic acid was needed only because that type of a chemical transformation was unknown. Meyer (1901) suggested that the side chain instead of the hydroxyl group could migrate on the ring. Soon after, Bamberger (1903) demonstrated the oxidation of *p*-cresol with side chain migration to form toluhydroquinone (Fig. 3). It was seen that this chemical reaction provided an analogy for the reaction of tyrosine to homogentisic acid in metabolism (Friedmann, 1908) and Baumann's theory collapsed.

The many observations on alkaptonuria made in the two decades at the turn of the century could then be ascribed to metabolic processes, but the study was immediately hung on a new dilemma: Did homogentisic acid represent an accumulation of a metabolite before a blocked step of normal metabolism, or was it an abnormal compound formed by an abnormal series of reactions? Whether normal or abnormal, the series of reactions leading to homogentisic acid was determined. Substances that were precursors of homogentisic acid could be distinguished by their causing an increased excretion of homogentisic acid when fed to alkaptonurics. Protein and phenylalanine, as well as tyrosine and homogentisic acid itself, increased the excretion of homogentisic acid in alkaptonuric patients. So did a number of postulated intermediates, while a much larger number of possible intermediates did not increase homogentisic acid excretion, and they were excluded from the projected pathway. This exciting period was later reviewed in detail by one of the main participants (Neubauer, 1928), and Fig. 4, taken from this review, shows the pathway finally deduced. All of the compounds shown, except 2,5-dihydroxyphenylalanine, had been

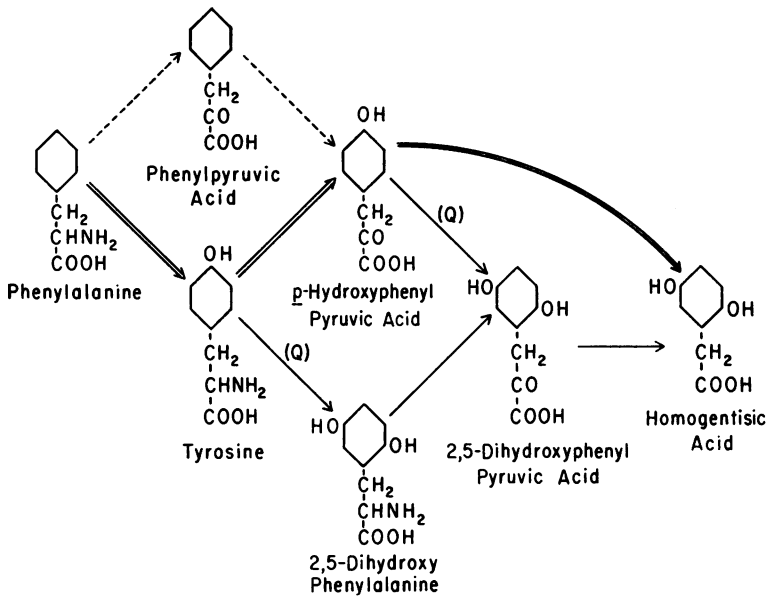

FIG. 4. Possible pathways of the metabolism of phenylalanine to homogentisic acid, deduced from feeding experiments with alkaptonuric patients (Neubauer, 1928). Neubauer indicated the less likely paths with dotted lines. The reactions involving the hypothetical quinol intermediate are marked "Q". The doubled arrows have been added to show the actual pathway (see Fig. 5).

tested. Later Neuberger *et al.*, (1947) synthesized 2,5-dihydroxyphenylalanine and proved that it too was converted by the alkaptonuric patient to homogentisic acid. Neubauer was aware that his method of testing would include compounds which, though not on the direct pathway from phenylalanine to homogentisic acid, would feed freely into the pathway. The final decision about which of the possible routes deduced represented the direct pathway actually used had to await the step by step analysis of the various enzyme reactions in the liver (Knox, 1955).

In the meantime, the objection gained momentum that the first metabolic pathway of some complexity to be elucidated was an abnormal one. To this crucial question Garrod had given a succinct answer: "It appears to me that at present the evidence in favour of the theory of an intermediate product far outweighs that which can be brought against it. Perhaps the most serious objection which can be raised to the view that homogentisic acid is an abnormal product, peculiar to alkaptonurics, is that such a view involves the assumption that the alkaptonuric, who alone has the power of forming homogentisic acid, is also exceptional in having no power of destroying it when formed" (Garrod, 1909). This answer would carry more weight today than it did then, when sequential metabolic reactions were almost unknown and what is now called biochemical genetics was on trial. Dakin, who was sceptical that a normal metabolic pathway had been elucidated, devised an ingenious test of the whole scheme. The over-all reaction was thought to depend upon the intermediate formation of hypothetical quinol, through which a 4-hydroxyphenyl could be converted to a 2,5-dihydroxyphenyl compound (see Fig. 3). A compound blocked in the para

position so it could not form a quinol, like *p*-methyl- or *p*-methoxyphenylalanine, should not be oxidized by a normal individual, if the postulated scheme were indeed the normal metabolic pathway. But when such compounds were given to normal animals, or perfused through liver, or even when administered to an alkaptonuric subject, they were said to be completely oxidized (Dakin, 1911). The conclusion appeared inescapable that the metabolism of these compounds occurred by another route, perhaps the truly normal route and one which did not involve either a quinol or homogentisic acid.

The results of Dakin, confirmed by similar studies (Fromherz & Hermanns, 1914), created a paradox unresolved until recently. Such was the appeal of Garrod's thesis, including the belief in a blocked normal metabolism, that investigations along this line persisted until the metabolic pathway of phenylalanine through homogentisic acid was finally established as the normal and major route. Only recently were the experiments of Dakin repeated. Ichihara (1957) and Pirrung, Gottesman and Crandall (1957) then found that the compounds were not metabolized. It must be inferred that the methods used by the earlier workers could not detect the unchanged compounds that they administered.

There remained some other evidence, which has not yet been explained, against the now accepted normal pathway of phenylalanine metabolism. One example out of many will be given. A three year old boy with alkaptonuria, when fed a diet sufficiently low in carbohydrate to cause ketosis, ceased to excrete homogentisic acid (Katsch, 1918). It appeared that either the blocked reaction began to function in acidosis or the "abnormal" route through homogentisic acid was depressed by ketosis relative to another, perhaps the normal, metabolic route. It was suggested that ketosis decreased the ability of a normal individual to metabolize homogentisic acid (Katsch, 1920). This pointed to the existence of an alternative metabolic route for tyrosine. The careful experiments of Katsch were not confirmed in two adults (Lieb & Lanyar, 1930; Diaz, Mendosa & Rodriguez, 1939). However, the results of Katsch, the examples of intermittent alkaptonuria, and the failures of normal individuals fully to metabolize homogentisic acid "under certain poorly understood circumstances" (see Galdston, Steele and Dobriner, 1952, references 31–49, for additional examples) cannot be overlooked, even if they are not accepted as evidence against Garrod's concept.

The problem may be solved eventually by an understanding of the regulation of metabolism. The metabolic reactions of the body are not simply present and working at full speed in the normal individual, or absent in the individual with a condition like alkaptonuria. Each reaction is modulated to fit the momentary demands of the body and the environment. The enzyme for the first of the reactions in the metabolism of tyrosine is now known to increase ten-fold in amount in a few hours after tyrosine administration, or after hydrocortisone treatment (Lin & Knox, 1957a). These instances of metabolic adaptation (Knox, Auerbach & Lin, 1956) are a sort of temporary somatic variation superimposed on the genetically determined metabolic plan. Adaptive variations in the other enzymes may occur to alter alkaptonuria under certain conditions.

Katsch was also involved in a premature and erroneous proof of Garrod's thesis,

the supposed absence of an enzyme which oxidized homogentisic acid. The absence from alkaptonuric serum of such an enzyme, and its presence in normal individuals was reported by Gross (1914). Katsch and Stern (1926) said it was not an enzyme that was absent in alkaptonuria, but an inhibitor that was present, although the functional result was the same. Any difference between normal and alkaptonuric urine was promptly denied by Lanyar and Lieb (1929), who showed that poor pH control produced these results, but this first "proof" of a missing reaction in an hereditary disease was already widely publicized. The disproof has not yet caught up with the "proof" cited in many standard texts.

Experimental Alkaptonuria: Support for Garrod's thesis of a blocked normal metabolism gradually evolved from numerous instances of homogentisic acid excretion by normal animals under somewhat abnormal conditions (see Knox, 1955, for references). The premium associated with the identification of homogentisic acid in urine led Abderhalden to the heroic extremity of administering 50 g. of tyrosine to an assistant. A trace of homogentisic acid was excreted. When the assistant refused to repeat the test for confirmation, Abderhalden himself took 150 g. between 9 a.m. and noon. He did not excrete any homogentisic acid (Abderhalden, 1912). Excretion of homogentisic acid was observed in some rats who survived toxic doses of phenylalanine plus ascorbic acid and in rats on certain protein or amino acid deficient diets. The most physiological experiment was the eventual development of alkaptonuria in rats fed phenylalanine for at least three weeks (Papageorge & Lewis, 1938; Lin & Knox, 1957b). Contrary to the widespread impression, homogentisic acid excretion was found only once in scorbutic guinea pigs fed tyrosine (Sealock & Silberstein, 1940) and is not a regular finding in scurvy. Ascorbic acid is not directly concerned with homogentisate oxidation (Knox, 1955). The most ingenious example of experimental alkaptonuria was that produced in guinea pigs by dosage with α,α-dipyridyl, an inhibitor of the iron-containing homogentisic acid oxidase (Suda, Takeda, Sujishi & Tanaka, 1951).

Spontaneous alkaptonuria has not been observed in any animals but man. There is a report of one rabbit which excreted a urine which darkened on contact with air and gave some qualitative tests for homogentisic acid. But homogentisic acid was not identified, and the animal died without offspring (Lewis, 1926).

Separate Enzymic Steps: Modern experimental approaches provided firm evidence for the normal metabolism of phenylalanine through homogentisic acid. Isotopic labelling experiments revealed that a rearrangement of carbons occurred in the course of the oxidation of phenylalanine to acetoacetic acid (Schepartz & Gurin, 1949). This rearrangement was like that which would be expected if a side-chain migration had occurred to convert a 4-hydroxyphenyl to a 2,5-dihydroxyphenyl compound. Homogentisic acid was not accepted as the normal intermediate in this metabolic path, however, until the step-wise series of enzyme reactions, including that forming homogentisic acid and that oxidizing it to known metabolites, were demonstrated *in vitro* in extracts of normal liver (Knox & LeMay-Knox, 1951; Knox, 1955). The completeness of our present knowledge of this pathway is illustrated by Fig. 5, every step of which is catalyzed by a known isolated enzyme.

The reactions of Fig. 5 also give a surprising answer to the pivotal chemical

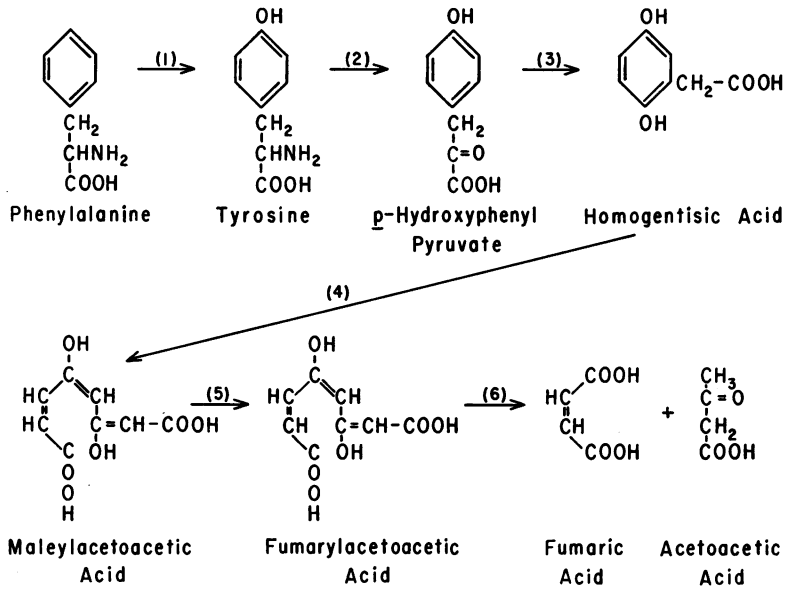

FIG. 5. The individual enzyme reactions of phenylalanine and tyrosine metabolism. The final products shown, fumaric and acetoacetic acids, enter the tricarboxylic acid cycle. Reaction (4) is the one inactive in alkaptonuria. The enzymes catalyzing each reaction are: (1) phenylalanine hydroxylase; (2) tyrosine transaminase; (3) *p*-hydroxyphenylpyruvate oxidase; (4) homogentisate oxidase; (5) maleylacetoacetate *cis-trans* isomerase; and (6) fumarylacetoacetate hydrolase (Knox, 1955).

question in the study of alkaptonuria. The intermediate quinol whose postulated existence was the basis of the metabolic studies does not exist unless as a transitory form on the enxyme surface.

Our present understanding leaves only a very small unexplained residue from all the observations made over two generations on this metabolic system. Three loose ends have persisted. These are the experiments, like those of Katsch, which suggested an altered or diminished activity of tyrosine oxidation in ketosis, and other poorly defined physiological states. It is known that ketosis leads to excretion of *p*-hydroxyphenylpyruvate, the precursor of homogentisic acid (Takeda *et al.*, 1952). The explanation may reside in the regulatory activities of metabolic adaptations. Second, it was observed that gentisic acid, formed from salicylic acid, was not further metabolized by the alkaptonuric patient as it was by normal individuals. Homogentisate oxidase, the enzyme missing in alkaptonuria, does not oxidize gentisic acid (Knox & Edwards, 1955), so the disturbed gentisate metabolism (based on non-specific chemical determinations of two very similar compounds) would indicate that there was another unsuspected abnormality of metabolism in alkaptonuria. The unified concept of the disease introduced by Garrod and now supported by all other evidence would then be incomplete. Third, the basic assumption about the nature of alkaptonuria, that the enzyme normally oxidizing homogentisic acid in liver and kidney is inactive in alkaptonuria, remained confirmed by direct investigation on the tissue of such

patients. As this is written, La Du, Zannoni, Laster and Seegmuller (1958) report that such an investigation was made. All of the other enzymes of tyrosine metabolism (see Fig. 5) were present in a biopsy sample of liver from an alkaptonuric subject, but that the homogentisate oxidase was inactive. There remain other complex and unexplained aspects of alkaptonuria, such as the pathogenesis of the pigmentation and the arthritis, which will be discussed with these clinical signs.

III. HEREDITY

Hogben, Worrall and Zieve (1932) correctly referred to Garrod's paper (1902) instead of his lectures (1908) or book (1909) as the primary study of the inheritance of alkaptonuria.

Single Recessive Factor: In the paper already described (Garrod, 1902), it was established that alkaptonuria was 1) a congenital and familial condition; 2) manifested in siblings whose parents were unaffected (with one exception); and 3) associated with an excess of consanguineous marriages among the parents. From these facts Garrod proposed that alkaptonuria was determined by a single recessive Mendelian factor, although he did not call it exactly that. By 1909 he knew of two pedigrees in which direct transmission occurred, those of Osler (1904) and of Orsi (1889). He intrepreted these as examples of the mating of a homozygous recessive with a heterozygote:

"When a recessive individual mates with an apparent dominant, who produces gametes of both kinds, a larger proportion of the offspring will be recessives, and we should expect that recessive children of a recessive parent, but whose other parent is apparently normal, will occasionally be met. Of such direct transmission of alkaptonuria from parent to child, the other parent not being alkaptonuric, two examples are known."

Fromherz included in his review (1908) the pedigree of the family he had observed with an "intermittently" alkaptonuric mother and three of twelve children alkaptonuric. Garrod had not included this pedigree as an example of direct transmission because of uncertainty about the diagnosis of the mother (Garrod, 1909). Fromherz collected fifty-eight cases at that time which confirmed the familial tendency of the disease, and incidently confirmed Garrod's statement that more could not be learned about the heredity of alkaptonuria until new cases were reported with adequate family studies. However, forty-five of the fifty-eight cases had been seen in the past fifteen years, so the prospect for more data was improving.

Toenniessen (1922) repeated a type of analysis used by Garrod (1909) to avoid the difficulties caused by lack of knowledge of the number of individuals in affected families and the failure to identify the proband for use in Weinberg's method. He used only the recently described, large families, with sufficient siblings "to give a good approximation of the statistical proportions of Mendel." His two large families plus the one of Fromherz (none of the parents were related) had a total of twenty-three normal and eight alkaptonuric siblings, nearly a 3:1 ratio. Adding to this those sibships numbering four or more from Fromherz' review, there was a total of thirty-five normal sibs and thirteen alkaptonurics, again nearly 3:1. This result confirmed Garrod's earlier evidence for the Mendelian segregation of the character. Toenniessen

also reprinted a pedigree from Umber and Burger (1913), one that Hogben *et al.*, later credited to Toenniessen, "which is the only pedigree besides that cited by Garrod showing direct inheritance." The pedigree will be described later. The 1:1 ratio of normal to alkaptonuric individuals in the second generation (four of each) with one affected parent would be expected with a dominant character as Hogben later interpreted it, but this ratio was actually cited by Toenniessen as proof of its recessivity, arising "through the cross RR x DR, and then in F_1 the results must be 1:1, so this pedigree gives the theoretical number."

The most complete and scholarly study of the inheritance of alkaptonuria is still that of Hogben *et al.* (1932). The number of recorded cases had grown to 120 by 1923 (Garrod, 1923) and then in 1932 to 151 cases included in the study by Hogben *et al.* Forty-two were isolated cases without reference to familial incidence or parental consanguinity. In forty-five fraternities with such information more than half had more than one affected sib. If the isolated cases were included, there was still more than one affected sib in nearly one-third of the fraternities. The familial nature of alkaptonuria was thus evident from an incidence of a very rare disease among sibs that was "vastly higher than could be accounted for by pure chance." The observed number of cases in thirty-seven fraternities of sizes one to fourteen where complete information was available was compared with the expected number on a recessive hypothesis (corrected for ascertainment only through affected individuals). There was no significant difference between the observed number of 66 and the expected number of 61.9, and the incidence therefore fit the hypothesis of a recessive gene. The large number of affected sibships with normal parents, and of normal sibships with one affected parent, confirmed the recessive character. This was further borne out by the fact that 42 per cent of the sixty-three patients whose parents' relationship was ascertained were the offspring of consanguineous matings. From this "highest incidence of consanguinity" that had been found, appropriately in the "rarest disease studied," they calculated the incidence of alkaptonuria to be between one in a million and one in ten million in the population. The validity of this evidence for a simple recessive inheritance in the majority of families with alkaptonuria does not come into question when another mode of transmission is considered for a small number of additional cases.

The unequal sex ratio of patients with alkaptonuria did not find an explanation in the hereditary mode of transmission. A higher proportion of males than females had been noted by both Garrod and Fromherz. Garrod had compared it to the similarly unequal sex ratio observed in cystinuria. Hogben *et al.*, found one hundred males and forty-six females among the recorded alkaptonurics of known sex. They discarded the hypotheses of sex-linked inheritance or lower penetrance in females because of the nearly correct Mendelian ratios observed, and they also found no clinical evidence that the condition might be semi-lethal in females. They showed how at least part of the disproportion could be ascribed to the fact that males were often the probands in affected sibships. Their conclusion was similar to that suggested by Niemann (1876) for the preponderance of males with cystinuria: that sociological factors accounted for the apparent disproportion. These included reticence about micturition in women and the more frequent subjection of males to medical and life insurance examinations.

This suggestion also implies that many cases are not diagnosed until the reducing action of the urine is found. Hogben *et al.*, noted that there were actually more females than males reported among infants, where the sociological factors favoring diagnosis of males were less important.

Direct Transmission: Five separate pedigrees showing direct transmission of alkaptonuria had not been included in the above study. These were separately discussed, and though exceptions could be taken to several of them, that of Pieter (1925), in particular, was considered to *compel* the recognition of a dominant form of alkaptonuria. The major position taken by Hogben *et al.*, in this discussion was that the direct transmission of such an extremely rare disease by the mating of a homozygous recessive individual with one who was heterozygous, as was suggested by Garrod and Fromherz, would be very, very unlikely to occur unless the mating was consanguineous. The observed pedigrees did not have evidence *for* the expected degree of consanguinity, and it was therefore assumed that at least some of them represented a dominant form of alkaptonuria. This controversial conclusion of Hogben *et al.*, is worth detailed discussion and reevaluatiom.

New information on the incidence of alkaptonuria, and new pedigrees of families showing direct transmission of alkaptonuria must be considered in this reevaluation. There is reason to believe that the incidence of alkaptonuria in the population may be one or two orders of magnitude less rare than Hogben *et al.*, estimated. Cases of alkaptonuria have continued to be recorded with increasing frequency until only those with unusual features are now regularly published. Twelve primary cases were diagnosed at a single clinic over a period of twenty-six years (Martin *et al*, 1955). Most importantly, the incidence of alkaptonuria in Northern Ireland, determined by complete ascertainment, has been estimated by Prof. A. C. Stevenson to be three to five per million individuals (unpublished). Not only is the possibility of matings between individuals homozygous and heterozygous for the alkaptonuria gene therefore much greater than Hogben *et al.*, assumed, but some subsequent studies have been made of the same pedigrees, and of some new ones, which showed direct transmission of alkaptonuria. These studies somewhat alter the evidence for the existence of a dominantly inherited form of the disease.

Families with Directly Transmitted Alkaptonuria: Personal communications from the several authors to Garrod were cited by Hogben *et al.*, as an additional source of information about the pedigrees of alkaptonuria. However, comparison of the data in the original reports with that used by Hogben *et al.*, showed that no significant new information was added. The pedigrees must therefore be evaluated on the data available in the literature. They are reproduced in Hogben *et al.* (1932) and some are brought up to date in Milch (1955).

Orsi (1889): This fragmentary report of "familial pirocatechinuria" contained no information except that the mother and her son and daughter were affected. There is little doubt about the correct diagnosis from the tests reported, despite the name given the condition. "Pirocatechinuria" is the Italian version of "brenzcatechinuria," which was used by Ebstein and Muller.

Osler (1904) (and others): These two brothers, originally described by Marshall and Futcher, were the ones on whom Osler made the first clinical diagnoses of ochro-

nosis. Their parents were unrelated, and the older brother (Futcher, 1898) had two sons (know to Osler), one of whom was alkaptonuric. No statement was ever made about the possible relationship of the older brother and his wife in the critical mating, that which produced the alkaptonuric son. However, another brother (there were actually seven sibs in the first affected generation) is now known to have married a first cousin (Milch, 1955), so the possibility of consanguinity in the critical mating is not precluded. A subsequent investigation of this pedigree revealed no addition cases of alkaptonuria besides the son in the second affected generation (Milch, 1955). The affected son in the second generation was one of five siblings. The others were normal. Depending on the son's identity, there were either three third generation offspring or six third generation and eight fourth generation offspring, all of whom were normal. The occurrence of one consanguineous marriage in the generation which directly transmitted alkaptonuria, its appearance in only one of five offspring in the second generation, and its failure to appear in the third and possibly the fourth generations, are new facts to be considered in determining the mode of inheritance operating in this pedigree.

Fromherz (1908): This family was omitted by Garrod and by Hogben *et al.*, because of the uncertainty of the diagnosis of the mother. In this large family of twelve children, two were alkaptonuric by actual test, and from the mother's report, one more was alkaptonuric, one not alkaptonuric and one uncertain. The parents were unrelated, and not knowingly related to other alkaptonurics, although the mother came from the same locality as some previously described alkaptonurics. The father was normal. The mother gave a history of having often noticed that her urine was dark or quickly became dark on standing. Fromherz regularly left flasks at the house for each member of the family and collected them several days later. Two were regularly positive—those from the two alkaptonuric children. On one day three were positive—the third was from the mother. On repeated testing, no further positive sample was obtained from her. Though Fromherz could find no evidence, motive or admission of a mixup, he could not take the finding as free from exception until it could be repeated with the mother isolated in the hospital, or with the younger alkaptonuric child, who slept with the mother, out of the house. He suggested, however, that the mother might represent a case of "periodic alkaptonuria." Herein lies whatever importance the pedigree has, since other instances of intermittent alkaptonuria have been descrived, though not recently, and the documentation of them did not convince Garrod that they existed (Garrod, 1902). Yet the possibility remains that an "incompletely recessive" form might exist, which in the heterozygous form would occasionally give rise to detectable amounts of homogentisic acid excretion. Against this is the fact that the single positive sample from the mother in Fromherz' pedigree contained the usual amount of homogentisic acid, "too much to have been introduced only by contamination."

Umber and Burger (1913): This pedigree, though attributed by Hogben *et al.*, to Toenniessen (1922), was actually observed by Umber and Burger (1913). The high incidence of arthritis observed in the affected members, and Toenniessen's use of it to support the hypothesis of recessive inheritance alkaptonuria have been described. One alkaptonuric in an otherwise normal fraternity of six married a normal,

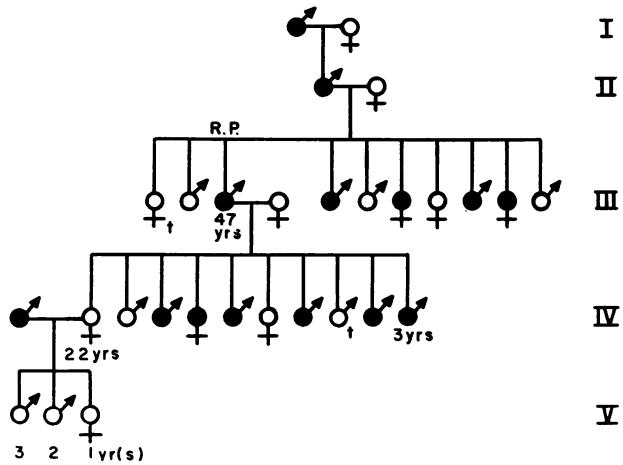

FIG. 6. Pedigree showing direct transmission of alkaptonuria, from Pieter (1925). The diagnosis of R.P., the propositus, is certain. A footnote said that alkaptonuria was *not* transmitted by any members of the third generation except R.P. (see text). ●, affected; ○, unaffected; †, stillbirth.

unrelated woman (both came from Holstein). There were eight offspring, four of them alkaptonurics. It should be mentioned that one of the four alkaptonurics in the second affected generation had two sons, both normal, and one of the latter had a son who was also normal. No new information about this family is known.

Pieter (1925): This was the remarkable pedigree which "compelled" Hogben *et al.*, to consider a dominant form of inheritance for alkaptonuria in certain instances. It is reproduced in Fig. 6. The original report, which described an agricultural family of mixed Spanish and Indian blood on the island of Santo Domingo, is of considerable interest in itself. The diagnosis of the proband is certainly correct. He was the forty-seven year old father (R. P., Fig. 6) of ten children, who was first diagnosed during hospitalization for removal of a bladder stone because his urine stained the bed sheets. Elaborate chemical tests, including the development of exposed photographic film in a solution of the urine (plus alkali, potassium bromide and sulfite) to give "a faint image after three hours," left no doubt that he excreted homogentisic acid. This use of photographic film anticipated the very useful test (1942) of Fishberg:

"Pour jeter un grain d'humour dans tous les ennuis que je lui ai causés, j'ai proposé à ce malade de s'improviser photographe. Il pourrait ainsi, lui dis-je, tenir boutique avec un minimum de frais généraux, car sa dépense en révélateurs pour plaques et papiers se trouverait réduite de beaucoup. En effet, comme on l'a vu, ses urines réduisent les sels d'argent. Cela tient sans doute à la nature chimique de l'acide homogentisique. . . . Son alcaptone serait donc un acide hydroquinone-acétique, et tout le mond connaît l'emploi de l'hydroquinone dans l'art de Daguerre."

Unfortunately Pieter was less solicitous and informative about other members of the family. He did not give the evidence of other diagnoses, whether by actual tests or by history or hearsay, nor any mention of relatedness of the spouses. He gave only those details which appear in the pedigree (Fig. 6), except for an illuminating footnote apparently missed by Hogben *et al.* In this he says of the proband's siblings,

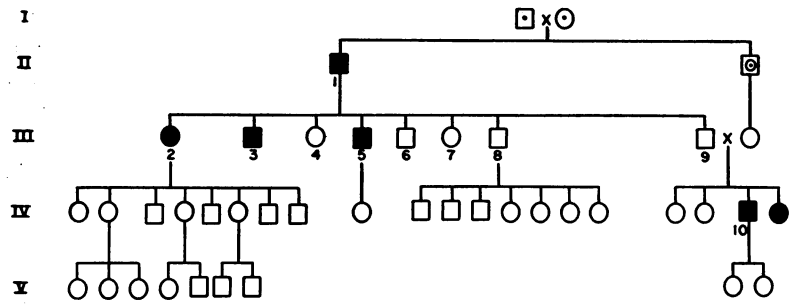

FIG. 7. Pedigree of alkaptonuria in Family 3 (Milch, 1955). Possibly this family was related to that of Pieter (Fig. 6). ■, affected male; ○, unaffected female; ⊡, status unknown.

four of whom were alkaptonurics: "Aucun autre sujet de la III[e] lignée (tous mariés et avec enfants) n'a produit des alcaptonuriques." The presumed dominance of the disease in this pedigree was manifested only in the descendants of *one out of the five affected individuals*! An alternative explanation of the apparently direct transmission by one of five siblings would be a certain degree of consanguinity in the marriages of agricultural people on an island. Some evidence that inbreeding may have occurred can be found in a report of a possibly related pedigree (Family 3, Milch, 1955).

Milch (1955, Family 3): Fig. 7 shows the pedigree from Milch (1955) of a family which was "established with fair certainty" to be "descendents of the family earlier reported by Pieter. . . . Preliminary data tend to suggest that the 'original' male parent (II-1, Fig. 7) is in fact probably one of the males in Pieter's fourth generation." This particular relationship is difficult to believe, in view of the chronology. Since there is no reason to think that Pieter's report was long delayed between his observations and the publication, R. P. was nearly forty-seven years old in 1925, and the ages of his oldest and youngest children ("Pieter's fourth generation") were given as twenty-two years and three years. It is difficult to believe that even the oldest of these children would be a great grandfather, with seven greatgrandchildren, twenty-six years later when the pedigree was first described (Milch & Milch, 1951).

Whether or not the pedigree of Fig. 7 is connected with that of Pieter's (Fig. 6), it does illustrate the remarkable tendency, seen in some of the other instances of direct transmission, for the condition not to reappear in the third and fourth generations. It also proves that alkaptonuria genes were present in Santo Domingo in some concentration. Another, apparently unrelated, patient from Santo Domingo has also been described (Galdston *et al.*, 1952, Case III). If the pedigree of Milch is connected with that of Pieter's, it shows that consanguinity was not unknown in the family, since an unaffected member of the second affected generation (III-9) "married his (unaffected) first cousin." Two of their four offspring were alkaptonurics, strongly suggesting that at least here the usual recessive mode of inheritance was operating in this family said to compel belief in a dominant form. Dr. Milch now has reason to believe that his "original male" (II-1) may also have been married to a second or third cousin (personal communication).

Milch (1955) (Family 2): This family was described in part in several clinical studies before Milch (1955) restudied the family to correct discrepancies. Alkapto-

nuria occurred in four of nine children, the offspring of two normal parents who were first cousins. Alkaptonuria occurred in three out of four sibs in the second affected generation, but solely among the offspring of one of these alkaptonurics from the first affected generation who married his first cousin, and not among the five offspring of two other alkaptonuric siblings who married non-relatives. In the third generation, the three children of one alkaptonuric married to a non-relative were normal. Another alkaptonuric, also apparently married to a non-relative, had two children. One was normal and the status of the other "remains somewhat nebulous." He was thought to have alkaptonuria, but was never tested. Except for this "nebulous" case, the pattern of transmission in this pedigree conforms exactly to that expected of a rare recessive trait.

Other Pedigrees Showing Direct Transmission: Fifteen alkaptonuric individuals were found among a kindred of three hundred traced for seven generations (Hall, Hawkins and Child, 1950). The pattern of inheritance was evidently that of a simple recessive character, and this was supported by the occurrence of extensive inbreeding in the first five generations, and the presence in the area of about three hundred additional members of the kindred who were not studied. Klein (1953) stated that: "On rearranging the pedigree, the dominant transmission with three affected sibships of non-consanguineous parents, one of whom was likewise affected, becomes more evident." This is misleading. There were two, not three, instances of direct transmission, and in one of these the affected parent was known to have married his first cousin. The relationship of the wife to the affected parent in the second instance of direct transmission was unknown, since he lived in the early days of the settlement of the county. There were a total of only three affected sibships whose parents were not known with certainty to have been related. This uncertainty cannot be cited as evidence that they were not related, however, especially in view of the extensive inbreeding elsewhere in the kindred, the presence of an equal number of unstudied relatives in the immediate area, and testimony that at least one of these couples were related. Again it is observed in this pedigree that direct transmission stopped with the second generation. Wherever the pedigree is complete it illustrates recessive inheritance.

Cases 3 and 5 of Martin, Underdahl, Mathieson and Pugh (1955) each gave a history that their mothers had alkaptonuria. The sister of one of them had "funny" urine. No further details of the families were given, and as they stand the pedigrees are less informative than that of Orsi.

A pedigree at present unpublished (Khachadurian & Abu-Feisal) shows a total of seven alkaptonurics in four successive generations of a village family in Lebanon, an instance of direct transmission comparable only to that recorded by Pieter. However, investigation proved that not only were all affected individuals in each generation the offspring of consanguineous marriages, but that the population of the whole village was in some way related and many of them were familiar enough with the phenomenon of alkaptonuria to state positively who had it and who did not.

Dominance by Default: Ten pedigrees showing direct transmission of alkaptonuria are now known. So little is known about that of Orsi and the two described by Martin *et al.*, that they can only be enumerated. That of Fromherz is still unproved. That of

Milch (Family 2) and the large kindred described by Hall *et al.*, show typically recessive inheritance, with the instances of direct transmission accounted for by RR x RD crosses between first cousins, as was originally suggested by Garrod for the Osler pedigree. Of the remaining four pedigrees, on which a decision must be based, one is new (Milch, No. 3), there is new information about Osler's and Pieter's and only that of Umber remains as it was when considered by Hogben *et al.* Even in the latter pedigree the absence of alkaptonuria in the two sons and the grandson of one of the alkaptonurics, which Hogben *et al.*, suggested might be a chance occurrence, assumes a new importance in view of the almost uniform failure of directly transmitted alkaptonuria to appear later than in the second generation. It was not transmitted to three (or six) offspring of the third generation in the Osler pedigree, to nine in the third generation of the Milch No. 3 family, or to four fraternities each with an affected parent in the Pieter pedigree. The two instances with three or more generations affected depend upon the presence of the disease in the father and grandfather of Pieter's forty-seven year old proband, and in the "nebulous" but possible alkaptonuric in the third affected generation of Milch's family 2. In neither of these instances is there even evidence that the diagnosis was correct, and the latter family shows an otherwise typically recessive inheritance. In a recessively transmitted condition the failure to reappear in a third generation could be expected.

Inbreeding occurred at least in the family, when it was not known to have occurred in the critical mating, in the Osler and Milch # 3 pedigrees (and possibly by relationship with the latter, in the Pieter pedigree).

To the high probability of some inbreeding in the families showing direct transmission of alkaptonuria and failure of the disease to appear with certainty after the second generation, must be added the newer estimates of the incidence of the disease. On the basis of an incidence of five alkaptonurics per million in the population, about one in every 200 people would be heterozygous for the gene. A small but definite number of cases showing direct transmission of this recessive anomaly could therefore be expected to occur in each generation solely on the basis of chance. All of the ten pedigrees showing direct transmission of alkaptonuria are therefore most probably instances of recessive inheritance, none compel belief in a hypothesis of dominant inheritance, and the choice of a dominant over a recessive inheritance must be based on information which is lacking in each pedigree instead of on positive facts.

The overwhelming evidence that the great majority of cases inherit alkaptonuria through a single autosomal recessive gene is also supported by a wealth of biochemical evidence for a single inactive enzyme reaction as the basic cause of all the disease manifestations. This evidence should also be considered in favor of a simple hereditary transmission. The same kind of a biochemical lesion could also be caused by an allele with more serious effects, like the "incompletely recessive" variant of the cystinuria gene. But is is unlikely that a more complex action involving several genes would have such a precise biochemical effect. It should also be noted that the cases showing direct transmission of alkaptonuria do not differ biochemically in any known way from the great majority of cases. The success of Garrod's concepts about alkaptonuria is primarily due to his insistence on maintaining the simplest possible hypotheses for both the heredity and the metabolic abnormality. An hypothesis recently pro-

posed of an "incompletely penetrant dominant gene which co-exists with at least one other pair of modifying gene factors" (Milch, 1955), could, of course, be fitted to cover all cases. Such an hypothesis is undesirable, however, and for the great majority of cases, unnecessary.

IV. PATHOGENESIS OF CLINICAL SIGNS

The diagnosis of alkaptonuria is usually said to rest upon a classical triad of darkening urine, pigmentation of cartilages and arthritis. In actual fact, the diagnosis more often rests upon the correct interpretation of an atypical test for sugar in the urine, and only in later life are the pigmentation and arthritis at all prominent. The reducing action of the urine, caused by something which turned dark in alkali, was the only clinical sign of the disease known until the turn of the century, and it is even now sometimes repeated that the condition is completely benign.

Pigmentation: Not until a decade after the work of Wolkow and Baumann in 1891, by which time over forty patients had been reported, was any mention made of the second most characteristic sign of alkaptonuria—the pigmentation of the tissues. The black tissues had been observed, however, though not during life and not knowingly in association with alkaptonuria. Virchow himself reported the first case as a pathological curiosity. Autopsy of a sixty-seven year old man who died with generalized anasarca had disclosed, "at the first cutting of the thorax," that the cartilages were coal black, a condition to which Virchow gave the descriptive term "ochronosis" (Virchow, 1866). Almost nothing is known of the patient's clinical history, but this patient, like that of Bödeker, also had "arthritis deformans," especially of the knees. Virchow likened the black deposits and osteophytes in the joints to the tophi of gout. Cartilages and tendon insertions in the bones all over the body were stained black to light grey. Under the microscope the tissue clearly showed intercellular distribution of brown or yellow (ochre) pigment, from which the name was derived. Since the origin of the pigment or the factors directing it to its specific location in the mesenchymal tissues remain unknown, it is interesting to note that even the arterial intima, and especially the sclerotic aortic plaques in Virchow's case, were strongly pigmented. The colored parts of the cardiovascular system, like the colored cartilages, had degenerated to some extent.

The second case of ochronosis was reported twenty-five years after that of Virchow (it was presented in a "Festschrift" in 1891 honoring Virchow's seventieth birthday). Others soon followed, and the total of six were carefully reviewed by Albrecht (1902), when he added the seventh. These were pathological studies and very few clinical details were given, but in two of the cases a history of long-standing melanuria was reported. There had also been at least two autopsies on known alkaptonurics by that time (Osler, 1904), but they had included no mention of ochronosis (the ages of these patients were twenty-nine and forty-three years). It remained for Albrecht (1902) to demonstrate the connection between alkaptonuria and ochronosis. His patient had grey-blue ears "like dilated veins," and his urine turned dark on standing. He died with miliary tuberculosis soon after hospital admission, and when section revealed ochronotic cartilages, an heroic attempt was made to isolate homogentisic acid from the 20 ml. of clear, yellow urine found in the bladder (Zdarek, 1902). This

effort failed, but enough was learned of the properties of the reducing material present to recognize the case as one of alkaptonuria. This association of a clinical anomaly with a pathological curiosity was subjected to an ill-founded attack (Langstein, 1904) by a protege of Hansemann (1892), who had seen the two reported cases of ochronosis with melanuria. But confirmation of Albrecht's thesis by Sir William Osler (1904) silenced all further opposition, although Pick's invention of "exogenous ochronosis" caused by prolonged exposure to phenol had overtones suggesting it was also a subtle attack on Albrecht's thesis (Pick, 1906).

Osler, whose interest in this disease had undoubtedly been stimulated by Garrod's correspondence with him (Garrod, 1902), confirmed Albrecht's suggestion by reporting that two brothers who had been described earlier as alkaptonurics (Marshall, 1887; Futcher, 1898), could be recognized during life to have ochronosis of their sclerae, ears and across their noses: "There is no question that these are cases of ochronosis in long standing alkaptonuria and they support Albrecht's suggestion that the pigmentation of the cartilaginous tissues is associated with the remarkable disturbance of metabolism which we have heretofore only recognized by the changes in the urine. The condition is thus brought within the range of the clinical physician. *Fortunately it is not of much moment, so far as we know, and in the recorded cases there have been no symptoms directly due to the alkaptonuria*" (Osler, 1904). At least one of Osler's patients, from his own description, had disabling arthritis.

The pigmentation, as such, is not a really prominent sign during life, and is a minor cosmetic affliction. It accumulates slowly and has rarely been mentioned in patients less than forty years of age, though it can be seen earlier in the eye (Smith, 1942). In time, the pigment collection can become very dark. That located in the sclerae at the rectus insertions was once mistaken for a melanosarcoma and the eye enucleated (Skinsnes, 1948). When alkaptonurics have had urinary stones for other reasons, some of these stones were black (e.g. Martin, 1955, Cases 3 and 6). Like other melanin pigments of the body this one is a high polymer that can be dissolved in alkali and precipitated with acid—along with the proteins to which it is bound. It has been alleged that it is different from the usual melanins, since the oxidation product of chemically pure homogentisate has an absorption spectrum (Milch *et al.*, 1957) different from that of hair or choroidal melanin (Stein, 1955). But this assumes an identity between the pigment formed by autoxidation of pure homogentisic acid and that accumulating in the body tissues which is entirely inferential. Such autoxidation may occur in the tissues, since a few cases with similar pigmentation allegedly due to chronic phenol exposure are known (Pick, 1906). The deepest interest in the pigmentation concerns its possible causal relation to the degeneration of the cartilages and of the other tissues where it is concentrated.

Arthritis: It was casually mentioned in the original reports that Bödeker's first patient with alkaptonuria had "neuralgia" of the lower lumbas spine for two years, unrelieved after three months' hospitalization; that Virchow's first case with ochronosis had "arthritis deformans" (the report contains a colored drawing of the knee joint); that one of Osler's patients had osteoarthritis and Heberden's nodes; and that several other of the earliest patients had chronic polyarthritis (e.g. Embden, 1893). But unless such an associated condition is both unusual and almost invariably pres-

ent, it is often not immediately related to the primary clinical syndrome. The arthritis of alkaptonuria is not unusual, except for a particular pattern of intervertebral disc calcifications seen in advanced cases. It resembles the common types and affects the spine and large joints, though differently in each individual. It is usually like osteoarthritis (degeneration of the articular surfaces), but it may be much more active and assume the nature of rheumatoid arthritis (acute inflammation with later ankylosis). The older part of the normal population commonly complains of a similar process in its milder forms, calling it lumbago, sciatica, rheumatics, stiffness, etc. The arthritis associated with alkaptonuria also develops late, like the pigmentation, patients were seen as children, long before any arthritic changes occurred. Thus the arthritis associated with alkaptonuria is neither an unusual complaint nor is it invariably present in the patients seen, and consequently it was not recognized as part of the disease till about 1907.

The possible association of ochronosis with other pathological changes was considered, however, even before ochronosis was recognized to be part of the picture of alkaptonuria. Heile (1900) suggested that ochronosis was part of a "gouty-rheumatic diathesis," because the reported cases had either joint or cardiovascular pathology, or both. Albrecht fully discusssed the problem: of the seven known cases of ochronosis, three had marked arthritic changes, and all had some disease of the heart or aorta. Yet he was forced to conclude that this was a chance association: "We see heart failure and senile or arthritic joint changes of different degrees occurring together so often without ochronotic pigmentation, that we cannot accept Heile's almost unsupported suggestion." But one of the next cases of alkaptonuria to be reported (Gross & Allard, 1907) had such far advanced arthritis, with ankylosis of both upper and lower limbs, that he was nearly helpless. In Umber's (1913) family containing five alkaptonurics, all the affected members had arthritis. Umber was a specialist in arthritis, and his insistence that it was part of the disease was heeded. Of forty-six patients for whom Hogben *et al.*, (1932) gave some clinical details, twenty-four had some form of arthritis. We now know that in time arthritis develops almost invariably in all alkaptonurics. The consequences are often severe and painful, and may lead to a completely bed-ridden existence in later life. Pomerantz *et al.* (1941) have given one of several radiological descriptions of the lesions. Osler's statement that the disease "is not of much moment" or Garrod's strong belief in its benignity (so that he listed this as one of the cardinal though non-essential attributes of the "inborn errors"), simply point up the difficulty for even practiced observers to recognize clinically each of the pleomorphic consequences of an hereditary disease.

In view of the difficulty of identifying all facets of an hereditary disease, the records of these patients should be continuously scrutinized for unrecognized, late developing complications and for complications of less than invariable incidence. A cursory survey of the published cases of alkaptonuria reveals, for example, that the cardiovascular system, whose elements are known to be pigmented, often is the site of pathological changes, just as Heile indicated. Whether this process is more common, or is accelerated, in alkaptonuria can only determined by careful analysis.

The unknown pathogenesis of alkaptonuria remains a challenge. The explanation of the intermediate mechanisms is needed to connect a discrete gene abnormality

to a precisely located enzyme inactivity, then in time to pigmentation of cartilage and to arthritis. This challenge was provocatively phrased by Thannhauser (1929): "The arthritis of alkaptonuria is particularly instructive for general pathology, because we have here an endogenously produced metabolite, whose chemical structure is completely known, giving rise to a deforming joint disease. It is to take but one step to suggest that in the more common arthritis deformans of unknown etiology there is another endogenous metabolite acting like homogentisic acid and producing the joint changes." The knowledge of how a single hereditary abnormality ultimately produces its predictable arthritis in the rare alkaptonuric may be usefully applied to a very much larger number of patients with more complex and less well understood disorders.

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