Sir Archibald Garrod's "Inborn Errors of Metabolism"

I. Cystinuria

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"It is an old experience that Nature often grants us through her errors unexpected insights into her secrets, which are otherwise a closed domain." *Über Cystinurie.* A. Loewy and C. Neuberg, 1904 (54).

INTRODUCTION

THE OCCURRENCE in certain individuals of renal or bladder stones composed of pure cystine has attracted attention since the early nineteenth century. The specific chemical nature of the anomaly, and its frequent familial incidence, guaranteed its interest to different investigators, just as the dramatic symptoms of renal colic often seen with the condition compelled the interest of physicians. It has a further claim on our interest as one of the bases of a great theoretical advance. It was one of the four diseases singled out by Sir Archibald E. Garrod in 1908 as the "inborn errors of metabolism." This concept grew out of considerations of all facets of the disease, both scientific and clinical, and the same integrated consideration has been attempted in this history of the study of this disease written fifty years later.

Cystinuria is an hereditary anomaly of renal function, with defective tubular reabsorption of cystine, lysine, arginine and ornithine. These amino acids, two of them essential to the body, are excreted in the urine in abnormal amounts throughout life. Only the excretion of the least soluble one, cystine, was recognized until recent times, and both the name and the clinical importance of the condition is entirely referable to this one amino acid. The only clinical consequence appears to be the frequent formation of urinary calculi composed of almost pure cystine. Most individuals homozygous for the cystinuria gene sooner or later form such stones, and the stones tend to recur. The sequelae may well lead to eventual renal insufficiency and death. Some of the individuals heterozygous for the cystinuria gene also excrete more than the normal amount of cystine, which can be detected by chemical tests such as the cyanide-nitroprusside reaction, but the amount here is not great and these individuals only very rarely form cystine stones. The incidence of chemical cystinuria is nevertheless much greater than the incidence of stone-forming cystinuria, since heterozygotes are vastly more common than homozygotes, and not all homozygotes have stones at a given time.

The separate identification of heterozygotes and homozygotes is essential for extending our knowledge of this disease, and this requires more than the qualitative

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recognition of cystine excretion. A quantitative determination of cystine is also needed for the proper treatment of those subject to clinically important complications. It is now reasonable to believe that cases diagnosed as homozygotes before calculus formation has occurred can be successfully managed so that all complications will be prevented. However, this successful treatment depends upon the full understanding of the disease mechanism.

The possibilities of treatment and of further research can best be appreciated in the perspective of the studies that have been done in the past. Not only are there many nearly forgotten things that need not be rediscovered, but there are also errors commonly accepted as fact. The clinical literature, in particular, has perpetuated a number of beliefs about cystinuria that were long ago shown to be false. The history of the investigations of cystinuria also provides an unusual opportunity to trace the laborious development of human understanding about a disease. Particularly is this so, since the history can now be written from the vantage point of a reasonably complete understanding. Our knowledge grew by the successive solution of limited problems: first, the clinical definition of the disease; the chemical nature of cystine and its role in metabolism; then the relation of heredity to metabolic events; and finally the specific biochemistry of renal physiology. But this stepwise progress was not intentional on the part of the successive investigators. In each generation the investigators were concerned with the integration of the whole problem, were plagued by what they did not yet understand, and were often uncertain even about what was definitely established. These human frailties and errors are not without interest and instructional value, but the epic quality of the investigations of cystinuria is most apparent in relation to the different concepts of mechanism which, one after the other, flowered and faded until one, for the present at least, has provided an adequate basis of understanding for this disease and related ones.

I. EARLY STUDIES

The opportunity to investigate this relatively rare disease occurred only sporadically and was not always given to those best prepared to advance our knowledge. Even more importantly, the cystine with which such studies began was provided by the patients themselves, upon whom the supply of this essential material was dependent for nearly 100 years (in 1904 (54) and even in 1936 (52) cystine metabolism was tested by giving to the individual cystine from a stone which he had formed). For these reasons Niemann could well bewail in 1876 that "our knowledge of cystinuria has indeed developed unusually slowly and by small degrees" (64). Through these limitations of clinical and chemical materials the knowldege of cystinuria was slowly lifted by its bootstraps.

Cystine, and cystine stones, were recognized before the patients with cystinuria. Wollaston, who had thirteen years earlier described five different types of urinary calculi, added a sixth in a report to the Royal Society of London in 1810, "On Cystic Oxide, a New Species of Urinary Calculus" (86). Two examples of bladder stones had come into his hands, one from a collection at Guy's Hospital ("No. 46—according to the present arrangements—which, it is to be hoped, will not be altered") and

one from a physician. "It had been taken from his brother when he was five years old." Wollaston had considered the substance to be an oxide because of its tendency to unite with both acids and alkalies. From its occurrence in the bladder, he gave it the name "*cystic oxide*, which will serve to distinguish it from other calculi; and as this is unlike any other term at present employed in chemistry, it is to be hoped that it will not be thought to require any alteration."

But the name became a focus in the first debates about the new disease. The next few cases also had bladder stones, perhaps not simply because "Was mann weiss, sieht mann," as Goethe observed about that time. Stones do develop in the bladder "remarkably often" in cystinuria (70), especially in young people. It was assumed that cystine stones were therefore formed by the bladder, until Marcet in 1818 found three patients (two at autopsy) with renal stones of cystine. He suggested that *nephritic oxide* was a better name. "Venables suggested *nephrine*, but Civiale objected to this term as unphysiologic. Instead he suggested *scorodosmine*, alluding to the odor of garlic given off by the substance when heated before the blowpipe" (70). Chemistry had its say through Berzelius, who observed that whatever its structure, the substance was not an oxide. He suggested the name *cystin*. "Of this new name, which has since been universally adopted, Civiale wrote, in 1838, that although it corrected an error of chemistry it perpetuated an error of physiology, for cystine is excreted by the kidneys and does not have its origin in the bladder" (Garrod, Lecture III (32)).

The renal origin of cystine was established when Prout in 1820, and Strohmeyer in 1824 (cited in 70), recognized the same hexagonal platelets of cystine in urinary sediment, which Wollaston had formed from dissolved stones. The identification of these crystals through a microscope has remained until recent times the primary means of diagnosis of cystinuria. The collection and weighing of these crystals was first done, according to Renander (70), in 1855 by Toel, who found a cystine excretion of 1.33 to 1.50 grams per day in a cystinuric. This procedure also became the basic method for the study of cystine metabolism until it was displaced after 1900 by Folin's urinary sulfur fractionations.

II. CONTRIBUTION OF NIEMANN

In the study of the literature of a relatively rare disease, where every "Case Report" is "With a Review of the Literature," the superficial and the fragmentary bury the rare work of scholarship. Also, errors tend to be perpetuated by plagiarism. Two works on cystinuria stand out among less than a dozen authoritative reviews, one in 1876 by Niemann, and one, almost never quoted, by the Swedish radiologist, Renander, published in 1941. References not otherwise identified here can be found in the latter work, which cites correctly and fully, for the most part, the literature on cystinuria to that time. This literature was first given form by Niemann.

The slowly accumulating information from sporadic cases of cystinuria was harvested and winnowed in 1876, when the writing of Niemann's medical Inaugural Dissertation at Göttingen happily coincided with the referral of a case of cystinuria to his professor (Ebstein). From what began as a case report by a young medical man willing to undertake some chemical investigations on his patient grew the

first definition of this obscure medical condition. Stimulated by the fact that the latest review, published two years earlier, had listed only fourteen cases of cystinuria, Niemann tabulated in chronological order and discussed, in addition to his own patient, fifty-two cases which he found in the medical literature. He consulted the original references for most cases. Subsequent reviews and research followed the pattern he set in this first authoritative discussion of the disease.

Diagnostic Problems: Nearly all of the cases in Niemann's collection had been diagnosed on the basis of urinary stones of cystine, although several had been recognized by cystine crystalluria. He provided careful drawings and crystallographic studies of these transparent, shiny, hexagonal leaflets seen with a microscope in the sediment of urine which had been allowed to stand for a few hours. Although he called the recently described nitroprusside reaction, which gave a violet color with cystine, an "elegant" test, the identification of cystine rested primarily upon Wollaston's criteria: the solubility of the crystals in ammonia and the insolubility in acetic acid, and, when strong HCl was added to the dry crystals under the microscope, the rapid growth of stellate clusters of delicate prisms consisting of the water soluble cystine hydrochloride. Chemical analysis depended largely upon the "sulfur reaction," the formation of a precipitate of lead sulfide when urine or a cystine solution were heated with KOH in the presence of lead acetate. He recognized that cystine could be present in the urine, as indicated by the lead sulfide precipitate, in abnormal amounts without the presence of stone or crystals. The brother of his patient had such abnormal amounts of dissolved cystine. However, Niemann did not make a definite diagnosis of cystinuria on this basis.

By his criteria, a probable diagnosis of cystinuria, suggested by a stone or crystals, was to be confirmed by the identification of cystine through the strong sulfur reaction given by the solid material. Additional confirmatory evidence which he mentioned was the smell of hydrogen sulfide, occurring when the urine was allowed to stand for many days, and the development of a particularly foul smell in the urine in hot weather or when admixed with rotting urine. The latter was no doubt due to the cadaverine and putrescine formed by bacterial action on the other amino acids now known to be present in these urines in abnormal amounts. Since the methods for detecting cystine depended upon supersaturated solutions from which it would precipitate, it is not surprising that he was cautious about making or excluding the diagnosis of cystinuria. He noted that cystine crystals were sometimes absent from the urine of individuals with a known cystine stone; that cystine crystalluria was sometimes present without stone; and in several cases, that stones of other chemical constitution were found in known cystinurics. The latter are now known to develop with the aid of the urinary infections set up by the original cystine stones. With the diagnostic precautions used by Niemann, it is evident that patients with cystinuria might be missed, but few who were not cystinurics would be diagnosed as such.

Patterns of Occurrence: Both males and females were affected with cystinuria, the males predominating. Thirty-eight males and fourteen females, with one patient of unstated sex, made up Niemann's collection of fifty-three patients. A similar predominance of males, though slightly less marked, has characterized all subse-

quent collections of cases (47, 50, 59). Niemann attributed this difference to a natural reluctance of women to see a doctor, and to the greater ease with which small stones were passed through the female urethra, with the result that fewer women than men were forced to seek medical help. Whether or not this simple explanation gives the correct reason, women were once upon a time more reluctant than men to go to a doctor. Now this reluctance is vanishing, and with it is disappearing the sex difference in incidence of cystine calculi. No significant sex difference occurred in a group of cases diagnosed by chemical examination (40).

Niemann believed that cystinuria might well be present from birth. Although it has not even yet been observed in a new-born child, the youngest patient seen at that time was two years old. Since then a cystine stone has been passed spontaneously by a child of nine months (Case VI (62)). The ages of the patients were known to Niemann in thirty-six cases, which revealed that there were similar frequencies of stone formation in the successive decades of life (Age < 10 yrs., 5; 10–20 yrs., 6; 20–30 yrs., 12; 30–40 yrs., 7; 40–50 yrs., 6), with no stones then reported to have developed after age 50. Renander tactfully mentioned that Niemann overlooked two cases, aged 61 and 73 (70). Kretschmer found a similar distribution with age in 107 cases, 73 per cent of which developed by age 40 (47). In one patient stone formation occurred at age 42, and recurred at age 81 (81). The oldest case reported was a woman of 87 (57). Because no particular age was singled out, and since actual diagnosis of stone was often preceded by many years of symptoms suggestive of urinary lithiasis, Niemann believed that the underlying cystinuria was lifelong, with stone development occurring sooner or later in most individuals with this condition.

Niemann was led to the latter conclusion, that most cystinurics ultimately formed stones, because persons with only cystine crystalluria were even rarer than those with stones. The incidence of stones in an unbiased series of cases, i.e. one selected chemically and not on the basis of clinical symptoms of stones, was recently estimated to be well above 50 per cent of homozygous cystinurics (22). Niemann mentioned the possibility that abnormal amounts of cystine in the urine detectable by chemical methods might be more common than generally thought. This H. B. Lewis demonstrated fifty years later to be true (50). It was impossible to make an estimate of the absolute frequency in the population of cystinuria characterized by cystine stone or crystalluria, and Nieman pleaded for the study of urine samples from large numbers of individuals to supply this data. The relative rarity of the condition was clearly attested by the number of cystine stones among all urinary calculi found in clinical practice or in museum collections. He cited the finding of three cystine stones among 649 urinary stones from the Hunter Museum, and the experience of three clinics treating urinary calculi with reported incidences of cystine stones in 1 in 300 patients, 6 in 1100 female patients and 4 in 105 male patients. This incidence of cystine stones, making up approximately 1 per cent of all urinary calculi, has been generally confirmed by all subsequent surveys (63, 78), of which Mörner's is the most complete (58).

Niemann mentioned that observers had been impressed with the familial disposition of cystinuria. The fourth and fifth cases, described by Marcet in 1818, were

two brothers about thirty years old. Four other instances in which two siblings were affected had been reported, and in one of these instances, two sisters with cystine stones, the mother had also excreted abnormal amounts of cystine but did not have stones or crystalluria. It was quite unclear to Niemann at this time, before the rediscovery of Mendel's work, why a number of these cases showed this striking familial tendency, and yet surveys of the families of other patients failed to reveal any additional cases. Of course, most of the individuals studied in such surveys belonged to earlier or later generations than the propositus, and affected cases of a recessive condition would not usually be expected except among siblings. It is interesting to note that the brother of Niemann's patient, who might well have had the same genotype, excreted abnormal amounts of cystine by chemical test, while the mother and maternal uncle of the patient were normal in this regard. A passage in Niemann's paper states that despite the care with which a case was studied ". . . in unserer Kenntniss über die *genetischen* Beziehungen der Cystinurie nicht weiter gebracht." This curious anticipation of a word soon to be used in another sense must refer simply to the failure to learn more about the origin or pathogenesis (genetischen Beziehungen) of the disease, since nothing was then known about the genetics of this or any other condition.

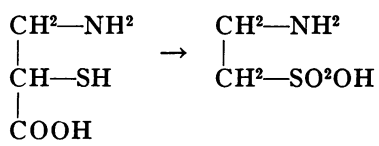
Possible Causes: His large collection of cases put Niemann on firm ground to evaluate the alleged "constitutional" or environmental causes of the disease, which were usually based on coincidental findings from single causes. Since five patients were less than ten years old, he dismissed all possibilities of an association with an occupation. Six patients were of high social standing, and for this reason he dismissed the suggestive influences of poor housing and coarse nutrition on the development of the disease. He admitted that "scrofula, chlorosis, and anemia" were sometimes associated with cystinuria, but considered them incidental to, or results of, the complications of cystinuria, rather than predisposing causes. The cases of the two v. Planta brothers reported by Civiale confirmed his view that one need not be sickly to have cystinuria: they were men "of the strongest nature, without any apparent constitutional disturbances, and in the most fortunate of circumstances, yet they had the most marked degree of cystinuria." An alleged association with rheumatism had been based on a theory that rheumatism was caused by cystine crystals in nerves and blood vessels, and in chronic cases the crystals appeared also in the urine. This he mentioned merely "as a curiosity," since most cystinurics were without a trace of rheumatism. Yet statements that there may be this same association could be found in medical writings more than fifty years later (50)! Niemann exhibited a similar restraint about incidental findings in the study of his own case. This eighteen year old student had an intention tremor of the upper extremities. Niemann did not wish to attribute this finding to the coexistent cystinuria, but suggested that it might be a consequence of the fact that "the boy drank more beer than his parents knew about." Niemann was in a position to estimate accurately the intake from his measurements of the daily urinary output.

The most scathing remarks were reserved for the then current hypothesis that Marowsky had developed in 1868 about cystinuria. This hypothesis about the disease mechanism emerged after the observation of a single case of cystinuria, in a patient

who also had chronic acholia and liver disease (56). Taurine, excreted in the bile as taurocholic acid, and cystine were both known to contain sulfur. Marowsky suggested that cystinuria was caused by liver disease. He believed that in his jaundiced case of cystinuria the usual taurine-secreting activity of the liver was suppressed, and replaced by "vicarious secretion of the sulfur-containing taurine through the kidney in the form of cystine."

Niemann said of Marowsky, "I will only remark that the cystine excretion in his case was not continuous, while the acholia and disturbed liver function were continuous. One may demand at least a continuous 'vicarious activity' of the kidney if this view is to be represented as plausible." It seemed unlikely to Niemann that cystinuria could be the result of underlying liver disease, with conversion of taurine to urinary cystine, without the appearance in the urine of other parts of the bile acids. To this point he adds the observations that neither taurine nor cystine could be found in the urine of other patients with jaundice. Lewis later cited analyses showing no deficiency of taurocholic acid in the bile of cystinurics (50).

Niemann nevertheless revamped Marowsky's hypothesis into the form later to be known as an "arrest of metabolism," a concept from which Garrod developed his fertile idea of the "inborn errors of metabolism." Instead of taurine being converted to cystine, as Marowsky implied, the formulas of the substances, as then known, indicated to Niemann that taurine was the "natural oxidation product of cystine":



Cystin Taurin

(According to Niemann, 1876)

"Should one reason from these pure chemical considerations to the situation in the human organism, one would think that in certain cases, from unknown causes, cystine was not oxidized to taurine and therefore appeared in the urine." Niemann had to point out, however, that plausible as this scheme was from a theoretical standpoint, the current knowledge of animal metabolism provided no support for it. He believed that wider investigations of other separate substances in the urine should be undertaken. His own measurements disclosed a low sulfate excretion in cystinuria. Such investigations, in time, would reveal the other amino acids present in abnormal amounts in cystinuria. Niemann failed to add the now obvious suggestion that a failure of cystine oxidation would cause the low urinary sulfate, but the pathway of cystine oxidation through taurine to sulfate was not yet known.

Treatment: No treatment was used on Niemann's case: "All means attempted up to now have proved useless." He thought treatment would continue to be unsuccessful until based on an understanding of the origin of cystine in the organism. On this subject he was ironic: "In the beginning, when cystine was known only to exist in bladder stones, it was believed to be formed by the bladder, and when cystine

was later found in the kidney, that was believed to be the site of formation; and Scherer's discovery of cystine, though only once and in the liver of an alcoholic, suggested the liver as the possible place of origin of the cystine." He reviewed earlier attempts at treatment, which included seltzer water to increase the urine volume and minimize precipitation. He suggested, apparently for the first time, that alkalinization of the urine might dissolve the cystine (Magendie is credited with the same suggestion, plus restriction of protein, in 1828 (70). But on one occasion his patient excreted an alkaline urine containing cystine crystals. This observation, plus the fear of precipitating phosphate with alkali and so enlarging the stones already present, kept him from trying alkali therapy. Only surgical treatment was to be offered when necessary, and that sometimes tardily by modern standards, so it was fortunate that many of the patients spontaneously passed their stones. In the group of those who were not candidates for the surgery of the day he included "a woman of 50 years who passed 13 cystine stones through a fistulous opening between the symphysis and the navel."

Niemann's success in defining cystinuria, achieved largely by the use of logic to divest the known cases of their adventitious trappings, can be measured by comparing his conclusions with the definition of cystinuria written in 1955 by Dent and Senior (22): "It is a condition presumed to be present from birth and characterized by the excretion in the urine of large quantities of cystine (in the adult, about 1 g. in 24 hours), lysine, arginine and ornithine. The condition is often present in the patient's siblings and may result in the formation of stones composed almost entirely of cystine. Apart from the possibility of kidney damage and the other complications resulting from stone formation, the patients enjoy good health and are clinically indistinguishable from normal." Niemann's definition agrees with this modern one in every detail, with the exception only of the abnormal amounts of lysine, arginine and ornithine in the urine of these patients.

III. STATISTICS AND CLINICAL CONFUSION

Niemann's review altered the character of the clinical reports on cystinuria which appeared later. Little could be added of clinical importance, except validation of his definition of the disease, until additional understanding was available. Clinical interests were contented by the accumulation of statistics. Ebstein, Niemann's professor, did him the honor of republishing his count eight years later and adding ten cases. One of the best of the early clinical articles was that of Simon (74), who reprinted Niemann's chronological list of fifty-two reported cases and brought it up to 1900, making a total of 103 cases, plus four of his own and one from the Norwegian literature added to the paper when in proof. One of his cases was a Negro, the only one reported to have cystinuria. In general, Simon repeated Niemann's arguments about the nature of the disease. Though without direct reference to them, the cases collected by Niemann and Simon were repeated in the collection made by Kretschmer in 1916 (47). This also contained 107 cases, though more had been reported in the interim since Simon's paper. In fact, three more reviews, of 114 cases in 1904 (Wasserthal), 153 in 1907 (v. Hoffmann) and 164 in 1912 (Link) had been published before Kretschmer's article. In the ten-year period between

1921 and 1932 Lewis counted seventy-one cases reported (50). Morrison listed seventy-five cases studied radiographically between 1920 and 1940 (62). Sum-Schick counted 180 by 1929 (78). Renander's estimate that published cases by 1940 "only slightly exceeds 200" was conservative.

Until 1932 case finding depended solely on the identification of cystine stones or crystals in urines "of patients presenting symptoms of renal colic or calculi of the genito-urinary tract. Cystinuria uncomplicated by calculus formation has been detected almost entirely by examination of the urine of relatives of patients who have been compelled to seek surgical aid for the removal of calculi" (47). But the number of cases seen depended on the interest in the disease. For example, the first case was seen in Sweden in 1870 and the second only in 1901. Only six were seen from 1901 to 1920, and these were reported by Mörner. In the five years following this, galvanized by Mörner's continued interest, sixteen additional cases were observed, four of whom did not have stones (59). These latter were discovered in surveys of the families of known cases and were diagnosed by the presence of cystine crystalluria. The diagnostic criteria had not changed. The stones might resemble triple phosphate stones, as mentioned by Wollaston, and even be admixed with mineral deposits; if not investigated more thoroughly they would not be properly identified. There were consequently serious limitations to the estimates of the absolute incidence of cystinuria. But the surveys of large numbers of urine samples for cystine crystalluria pleaded for by Niemann had uncovered one case in 15,000 (74), one case in 20,000 (Primavera, cited in (34)) and four cases in approximately 35,000 urine specimens (75). Since the crystalluria was known to be intermittent in closely observed patients, the true incidence of cystinuria was undoubtedly higher, but it was not imagined how much higher chemical methods would show it to be. For some time cystinuria was regarded as a rare condition, fortunately since Garrod used rarity as a criterion for an inborn error of metabolism.

Stone formation is indeed a rare occurrence. Something like a complete ascertainment of the incidence in the generation of the Swedish population living between 1901 and 1936 was assembled by Mörner. He had found a total of thirty-six cases by 1936. At that time Renander surveyed all the hospitals in the country and succeeded in finding only one additional case (70). This gives an incidence of stone-forming cystinurics in a population of six million of roughly 1:200,000.

The usual interest was shown in the unusual case. Southam (76) removed a cystine stone from a woman who, fourteen years post-operatively, still excreted cystine. The mother of this patient had been operated on twenty-four years earlier by Southam's father, also for cystine stone, and she continued to excrete cystine for eight years. The chronicity of the disease was further documented by a metabolic study in 1923 (55) of a patient studied seventeen years earlier by Alsberg and Folin (3). The metabolism of the patient had not altered. But the myth persisted in clinical circles that removal of a cystine stone might cure the cystinuria.

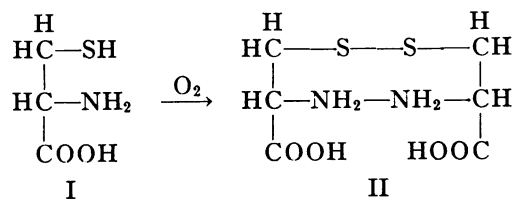
A dignified rivalry to report the largest stone can be discerned in the clinical literature, but chaos governed the contest. The participants, who frequently confused weights in *grams* with *grains* (1 g., gm. or gr.[am] = 15.4 gr.[ains]), always to enlarge a stone about ten-fold (see 70 for references), are fortunately no longer our

family physicians. The record, attested by an independent weighing twenty-seven years later when the stone reposed in the Museum of the Royal College of Surgeons in England, was long held by a vesical calculus weighing 68.04 g. and described by Harrison in 1879. Link found a stone of 86 g. in 1912, but all records toppled when Ancherson in 1914 removed from the bladder of a Norwegian man a cystine stone weighing 102.5 g.

A most notable confusion about the clinical nature of cystinuria was introduced by Abderhalden's description in 1903 of another disease of cystine metabolism. Although Abderhalden called the new disease *familial cystine diathesis* (1), not *cystinurie*, the majority of medical writers up to the present time have considered it to be a manifestation of the same hereditary disturbance as cystinuria. After the next cases were described, in 1926 by Lignac-Leiden, it was also called cystinosis, cystine storage disease and Lignac-de Toni-Fanconi syndrome. The first patient was seen only after death had occurred at the age of twenty-one months following symptoms of inanition. The internal organs were spotted with crystalline deposits of cystine. Abderhalden demonstrated abnormal amounts of cystine in urine of two siblings, the father (9.2 mg/100 ml) and the grandfather (14.0 mg/100 ml) of his patient. A reason for additional confusion was that Lignac's first case had ureteral and renal cystine stones (53), a unique finding in any condition except cystinuria. The typical findings of cystinosis, all of which distinguish it from cystinuria, are the retarded growth and development, the rickets and osteomalacia, the low serum phosphorus, the glucosuria, the generalized aminoaciduria of ten or more amino acids among which cystine is usually not prominent, the very rare formation of cystine stones, the presence of cystine crystals in the tissues of children but not adults with the disease (25), and its frequently fatal termination.

IV. THE METABOLIC MAZE

A more precise definition of cystinuria was dependent upon knowledge of the chemical nature of cystine and of its metabolism. It would seem that both these aspects were well on the way to solution by 1905. In 1900 Simon (74) had complained that little was known of the mode of formation of cystine in the body because it had not been synthesized and investigators were dependent upon the supply of cystine stones. This diffiuclty had actually been overcome one year earlier. K. A. H. Mörner (60) had discovered cystine in protein, and found hydrolyzed hair to be an especially rich source. The correct chemical formula of cystine (II), and its reduced form, cysteine (I) was established in 1902 by Friedmann (31):



I II

Chemical methods to measure urinary "neutral sulfur" (largely cystine) and inorganic sulfate were developed, and the first balance studies on the sulfur metabolism of a cystinuric were reported in 1905 by Alsberg and Folin (3).

But the chemical and the biochemical results produced mystification, not enlightenment. Loewy and Neuberg (54) reported that hair and stone cystine were metabolized differently and were different chemical substances. Alsberg and Folin promptly reported their own and others' reexaminations of the evidence for this conclusion (3): "After having read the above papers, one of us (F.) immediately examined a small stone recently passed by our patient." He could not verify such a difference (which hinged on a different crystal form). But as late as 1927, as a result of analysis of the 78 g. of stones from Tennant's case (79), this error found confirmation (37). The problem of determining the chemical identity of samples of cystine was difficult, but the full explanation of these conflicting results must consider the psychological difficulties produced in the minds of investigators by the curious results from metabolism studies on the cystinuric patients.

The metabolic studies firmly established that an increase in food protein produced an increase of the cystine excreted. But when the food protein was hydrolyzed outside the body and the isolated cystine was given to the patient, that cystine was oxidized to sulfate. Cystine itself, even cystine isolated from the patient's own urine, (80) caused no extra cystine to be excreted. The psychological effect of this paradoxical result, which was amply verified, was compounded by the results of Loewy and Neuberg. They confirmed the above results with stone cystine, which was oxidized to sulfate, but cystine isolated from hair was excreted as cystine! Garrod expressed the pessimism of the time (33): "The study of the metabolic peculiarities of cystinurics has yielded results which are very difficult of explanation, and the more the problem is investigated the more remote seem to be the chances of its satisfactory elucidation. Thus, in every case tested, except that of Loewy and Neuberg, in which cystin has been given by mouth, it has been completely destroyed, although the patients were all the time excreting cystin as such."

During the same period other observations were made from which our modern understanding of cystinuria could have been deduced, had another Niemann collated the findings. But like a hank of string with the key loop in sight, several pulls at other loops resulted in a snarl which took nearly fifty years to untangle.

V. REWRITING HISTORY

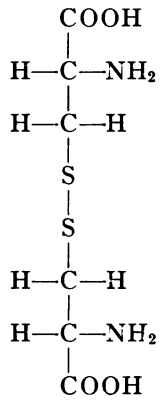
During the first third of the twentieth century what had been sharp differences of opinion about the nature of cystinuria degenerated into an intellectual muddle. Further progress occurred only later, after time had dimmed the memories of facts and irreconcilable conclusions. Then a single group, led by Brand, redid the work. No attempt has been made till now to analyze the muddle left behind. It may be that such a state of affairs was inevitable, given the results of Loewy and Neuberg and the distraction of Abderhalden's new disease. Or the primary fault may not have been the handling of alleged facts, but the inadequacy of the concept of metabolism compartmented into exogenous and endogenous. If so, the scientific method may be expected to fail on occasion. It may help in thinking about this momentous question to review the positive experimental results available in Garrod's time. Through the retrospectroscope, clearly illumined by our present knowledge of cystinuria, certain pivotal findings from the turn of the century stand out.

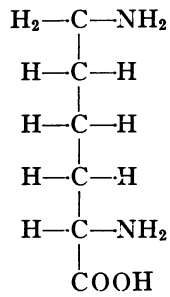
The enzymic apparatus for cystine degradation was obviously intact, since cys-

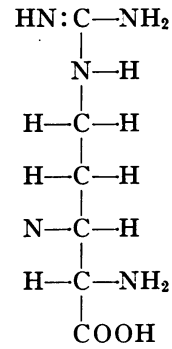
tine given as such to a cystinuric was excreted as sulfate. Any amount given orally could be oxidized by the liver, but if cystine or cysteine was given parenterally, to dogs or rabbits (7) or to a cystinuric patient (85), it was excreted by the kidney as cystine. Failure of tubular resorption, or in the language of that day, a low renal threshold for cystine, was later implicated quite directly by the fact that the level of cystine in the blood of cystinurics was not elevated (15). This eliminated the possibility of a renal overflow mechanism, although it was apparently not appreciated by the authors at the time. The meaning was perhaps not lost to all, however, for in 1934 a clinical case report cites a personal communication from "Dr. [I. M.] Rabinowitch of Montreal" that the mechanism of cystinuria is "analogous to the glycosuria in renal glycosuria . . . as a result of an undue permeability of the kidney . . ." (66). There was left unexplained then, and now (23), only the reason why the kidneys were presented with a greater load of cystine for excretion when protein was fed than when cystine was fed as such.

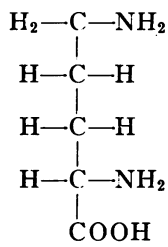
An even earlier series of experiments would seem to lead to the recognition of the other amino acids (lysine, arginine and ornithine) besides cystine that were present in abnormal amounts in cystinuric urine. This discovery in recent times immediately caused the realization that it was the kidney function and not cystine metabolism that was deranged. Ellinger (27) in 1898 found that bacteria would decarboxylate lysine and ornithine to form the foul smelling "ptomaines" or diamines, cadaverine and putrescine. These substances had been isolated from the urine of many (3, 74), but not all, cystinurics. The original patient studied in 1889 (82) had cystitis, and successive investigators over several years found the amounts of diamines increasing in pace with her urinary infection (36). These findings gave rise to the belief that intestinal infection leading to putrefaction was the basic abnormality in cystinuria (82, 83), supplanting the theory that it was a metabolic anomaly like diabetes and gout (74). When Moreigne in 1899 cast doubt on the possibility of a life-long intestinal infection and found that the cystine excretion was not altered by antiseptic treatment of the intestine, the theory was altered to call for a generalized disturbance of protein hydrolysis in the gut. Before even this theory could lead to the recognition of the other amino acids in the urine and their decomposition by bacteria to the diamines, Loewy and Neuberg supported it with evidence of quantitative excretion of the wrong amino acids, tyrosine and aspartic acid (54) (their third result in a single paper which has remained unique among the studies of cystinuria). It is interesting to compare the above results with those from the patient studied more recently by Harris, *et al.* (40) This cystinuric was exceptional among their patients in excreting no lysine. This led them to make a closer examination of the patient, which disclosed the presence of a urinary tract infection, and further analysis of the urine revealed the missing lysine present as cadaverine.

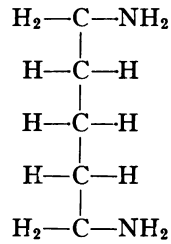
The particular findings of Loewy and Neuberg were tested immediately. Alsberg and Folin reported in the following year (1905) that hair cystine was not excreted as such, but as sulfate; cadaverine and putrescine were not excreted (there was no infection); aspartic acid was completely metabolized. The refutation was complete: "The more we have studied the experimental data presented in the paper of Neuberg and Loewi the less reason have we had to question the accuracy of the work except

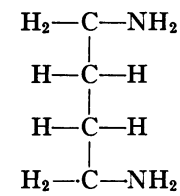

Cystine


Lysine


Arginine


Ornithine


Cadaverine


Putrescine

in one or two minor particulars. Our own experiments to be described in this paper have, however, entirely failed to corroborate their findings." (3).

It is now almost inconceivable that the work of Alsberg and Folin in 1905, quoted above, and that of Wolf and Shaffer in 1908, did not quickly solve the problem of cystinuria. The former were justifiably confident in their newly developed chemical methods, especially the chemical determination of cystine, based on the assumption that it comprised the neutral sulfur fraction. They also tested the theory of defective hydrolysis of dietary protein by measurement of the "undetermined nitrogen" fraction of urine, that part of the total nitrogen left after subtraction of that in urea, ammonia, creatinine, uric acid and cystine. In their patient there was an abnormal excretion of the undetermined nitrogenous substances and of cystine in starvation, and therefore these were produced by the endogenous metabolism. But both abnormal excretory products also increased in proportion to the protein in the diet, and therefore were produced by the exogenous metabolism. The concept of two separate metabolisms nevertheless survived for another generation, although Folin did not work again on cystinuria himself.

Four years later Wolf and Shaffer (85) confirmed the results of Alsberg and Folin in detail, and went on to build on the new discovery made by Alsberg and Folin: that there was an abnormal excretion of the "undetermined nitrogen" fraction. The results were clear-cut and the conclusions sound, even though they were not

effectively developed until forty years later: "The high undetermined nitrogen is in part due to cystine, and is in part due to other amino acids; for the ratio of amino-acid nitrogen to neutral sulfur is much above that found in normal subjects. Cystinuria is probably never a simple anomaly in which the cystine complex is the only part of the protein molecule which is affected. Owing to the difficulty of their separation, it is impossible to say what are the other fractions which are concerned in the increase in the undetermined nitrogen (85)." Somehow, this clear statement was forgotten. Perhaps it was due to lack of good methods to extend it. Later isolations of crystalline lysine derivatives from cystinuric urine by Ackermann and Kutscher in 1912 (2) and by Hoppe-Seyler in 1933 (43) passed almost unnoticed and perished for lack of corroboration. The latter obtained amounts equivalent to 0.1 g. lysine per liter of original urine, which without allowing for losses, was still grossly more than could be present in normal urine. The discovery of three other amino acids besides cystine in cystinuric urine forty years later surprised that part of the medical world who heard of it.

This abstraction of pertinent findings and clues out of the context of the times makes it natural to wonder, given the observations repeatedly made, why early investigators did not deduce the real nature of the dysfunction in cystinuria. But the eradication of all the wrong results and of inadequate theories would not necessarily have advanced the field in the long run. Such action, for example, would have eliminated Garrod's concept of an inactive enzyme: in many ways an inadequate theory based on wrong facts, but one which was responsible for greatly furthering the understanding of cystinuria and hereditary disease in general.

VI. GARROD'S "INBORN ERRORS OF METABOLISM"

The brilliant concept of the inborn errors of metabolism was enunciated by Garrod at the height of the confusion about cystine metabolism (34). According to this thesis, an hereditary condition characterized by the accumulation of an abnormal metabolite resulted from the hereditary lack of an enzyme that normally removed that metabolite. Here was an illuminating way to regard the accumulation of cystine, which in a normal individual was oxidized to sulfate. Cystinuria was included as one of the inborn errors of metabolism, and lent its weight to this important concept. For a generation the fact was obscured that the supposedly missing enzyme reaction in cystinuric patients was in fact not missing. The reaction had been demonstrated equally well in both normal and cystinuric subjects. In either kind of subject cystine administered as such orally was oxidized and excreted as sulfate. The defect in cystinuria was manifested only if some precursor of cystine, such as protein, was fed. Garrod was not unaware of this paradox, and his original lectures and later revision of them are valuable summaries of the research on cystinuria (32, 34), but he contented himself with the explanation that the metabolic error was partial and incomplete, and that the contradiction would be resolved when more was known of the intermediary steps of sulfur metabolism.

The major contribution of Garrod's thesis specifically to the study of cystinuria was to supply a mechanism accounting for the familial distribution of a highly uniform and subtle disorder. In addition to the occurrences of cystinuria in siblings

mentioned by Niemann, and omitting the cases of familial cystinosis described by Abderhalden, there was ample evidence of its hereditary nature. Pfeiffer (67) had reported four cystinuric children of parents who were first cousins. Cohn described a family with a cystinuric mother, a normal father and ten children. Six of the children were cystinurics, two definitely not, and two untested (17). Two of the children were twins, both cystinurics. Identical twins, both with the disease, who also developed stones almost simultaneously were later described by Kretschmer (47) and in another instance by Harris and Warren (41). Garrod saw that this pattern was meaningful: homozygotes for a rare recessive gene would usually occur among siblings, rarely in other generations of the same family, and more commonly in families where a consanguinous marriage improved the chances that both parents would possess the rare gene. He did note, however, that there was a greater frequency of direct transmission of cystinuria from parent to child than was met in connection with the other metabolic errors he described (i.e. albinism, alkaptonuria and pentosuria). Fifty years later (40) the recognition of a "dominant" form, manifesting cystinuria in the heterozygote, confirmed his impression.

It was fortunate that certain substances giving rise to cystine in the body, such as dietary protein, caused excretion of extra cystine, just as other precursors caused increased formation of the blocked metabolite in the other errors of metabolism where degradation was blocked by enzyme deficiencies. For this reason the concept of Garrod had pragmatic consequences for biochemistry in connection with the intermediary steps of sulfur metabolism, in addition to its genetic implications. The next advance in the study of cystinuria exploited this excretion of cystine formed from precursors *as if it were the result of an enzyme deficiency*. In the same way that the defect in alkaptonuria was used to determine the pathway of the tyrosine precursors of homogentisate, the non-existent enzyme defect in cystinuria was used to reveal the complex pathway of sulfur-containing amino acids.

VII. DOWN THE METABOLIC PATH

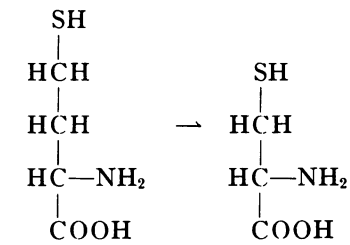
In 1935 the biochemist Brand and the urologist Cahill, with two young cystinuric patients, began to elucidate the metabolic pathway of the sulfur-containing amino acids by feeding experiments. The erroneous assumption of a block in cystine metabolism was pragmatically useful. The identification of cystine precursors was equally well served by the renal leakage of the extra cystine as it would have been by the supposed metabolic block. Cysteine and the new sulfur-containing amino acid, methionine, were promptly identified as cystine precursors (12). Proteins containing more methionine gave rise to higher cystine excretion than proteins with low methionine content. The cystine contained in the protein was oxidized to sulfate. This identification of methionine, and not cystine, as the principal dietary source of urinary cystine was confirmed by Lewis (52).

During the two years of experimentation the subjects of Brand's experiments (who were thanked in each paper) formed extra cystine from homocysteine, but not homocystine (10) (cf. cysteine but not cystine), and not from a number of other possible sulfur-containing compounds. Brand postulated that cysteine was a product of the catabolism of methionine and that the error in cystinuria was a failure of the

proper utilization of cysteine, not a failure of cystine metabolism. The final results clearly indicated a pathway of metabolism of sulfur-containing amino acids with the surprising sequence of reactions:—(12)

Methionine → Homocysteine → Cysteine → Cystine → Sulfate
↓ Urinary Cystine (in Cystinuria)

The reaction difficult to believe was the shortening of the carbon chain in the conversion of the γ-thio compound, homocysteine, to the β-thio compound, cysteine:



Homocysteine Cysteine

Lewis confirmed the essential findings for this scheme, that methionine and cysteine gave rise to extra cystine, but he refused to accept the same conclusion. His attitude was more cautious, but it was still reminiscent of Baumann's scepticism that an alkaptonuric patient would be able to convert a 4-hydroxyphenyl- to a 2,5-dihydroxyphenyl compound (because this was a difficult chemical change, Baumann said that homogentisate must be formed from tyrosine by intestinal bacteria (46)). Lewis rejected Brand's scheme involving the shortening of a carbon chain because: "It is difficult to picture such a transformation of homocysteine to cysteine and to cystine in the light of any known facts concerning the degradation of the biologically important sulfur-containing compounds either *in vitro* or *in vivo*. Until further evidence is available we are unwilling to accept such a theory of the origin of the urinary cystine, although we are not prepared to suggest an alternative theory by which the increase in cystine excretion, which results from methionine feeding, may be explained." (52).

Brand's scheme (9) was confirmed in detail, however, and with it the validity of the use of a patient with cystinuria for these purposes, by the studies from du Vigneaud's laboratory. S^{35} labelled methionine gave rise to labelled cystine, but C^{13} labelled methionine did not (24). The shortening of the carbon chain referred to above occurred by transfer of the sulfur from the 4-carbon compound, homocysteine, to a different 3-carbon compound, serine, to form cysteine made up of the original sulfur but with new carbon atoms. The intermediate of this interchange was cystathionine, a combination of both homocysteine and serine in one molecule. This compound, too, was later shown to give rise to cystine (68).

After the pathway of sulfur amino acid metabolism in man was delineated by

Methionine ⇄ Homocysteine ⇄ Cystathionine ⇄ Cysteine + Homoserine
↓↑ ↓↑
Homocystine Cystine

Brand through his studies of a patient with the hereditary condition of cystinuria, a very similar pathway (in the reverse direction leading to methionine) was proved for *Neurospora* by the use of several mutants of this species (44). The sequence of these discoveries provides another instance to illustrate that what is now called biochemical genetics was effectively begun by observations on human material by the physician Garrod.

Cystinuria in Animals: Of the "inborn errors of metabolism" enumerated by Garrod, only two, albinism and cystinuria, have definitely been recognized in animals. This promising field of research is still largely unexploited, but what has been done was started in connection with Brand's work just described.

The sporadic occurrence of urinary cystine stones in dogs had been reported earlier, first from Paris in 1823 by Lassaigne (48), again in 1861 (38), and in a male Dachshund in 1921 (49). But always the stone was recognized after the death of the animal and no metabolic studies had been made. The tendency to form cystine stones was not associated with a single breed, as was the frequent development of urate stones in the Dalmatian coach hound. Then in 1935, Morris, a veterinarian, found an Irish terrier suffering from urinary retention to have cystine crystalluria. At operation several vesicular and urethral calculi of cystine were successfully removed (61). Brand and Cahill joined the study, and undertook the necessary chemical studies to identify the disease and arranged a breeding program to investigate the genetics of the condition.

The project apparently languished and the dogs were dispersed sometime before Brand's death in 1953—just before the new concept of the renal tubular mechanism in human cystinuria could be tested on cystinuric dogs. Only fragmentary reports of this important study were ever published. Some old-style metabolic studies of the dogs were recorded, and these nearly duplicated the findings from contemporaneous human studies. Cystine accounted for 13 to 17 per cent of the total urinary sulfur in the cystinuric dogs (13, 35), compared with about 20 to 30 per cent in the human cystinuric (52). Brand's metabolic scheme for sulfur-containing amino acids was supported by the excess cystine excreted when the dogs were fed extra protein, methionine, or cysteine. The feeding of cystine did not result in extra cystine excretion (42).

Through the American Kennel Club registrations of the sire and dam of the first cystinuric dog, four related dogs were found to begin a breeding program. One, a cousin of the proband, also had cystinuria (11). Twenty-five unrelated animals of the same breed were not cystinuric. Three hundred dogs of the cystinuric strain were raised, and twelve cystinuric animals were identified. All affected animals were males (8, 13). The original cystinuric male produced litters with an unrelated female and with two half-sisters. None of the 15 pups was cystinuric (35). No genetic analysis of the results was ever made, but the few published pedigrees resembled, or at least did not exclude, a sex-linked mode of inheritance (13, 42), one quite different from the recessive mode seen in man.

It remains to be shown, when cystinuric dogs are found again, that a similar discrete renal transport system under hereditary control is affected in the cystinuria of both men and dogs. It is important to establish whether or not other amino acids

besides cystine are excreted in abnormal amounts by the cystinuric dogs. It was in normal dogs that the competition between the several different amino acids for renal tubular excretion was first demonstrated (6), and on this was initially based the belief that the excretion of the several amino acids in man was due to a deficiency of a single renal transport system. But the apparently different mode of inheritance in dogs, coupled with the existence in another species (see below) of cystinuria without other aminoaciduria, suggests that canine cystinuria may only be a phenocopy of human cystinuria.

The second species of animal with cystinuria is an obscure creature, the blotched genet, a kind of wild cat from Kenya. In a survey by paper chromatography of the amino acids excreted by different animals at a zoo, Datta and Harris (18) found every blotched genet examined to be cystinuric This condition was present in a number of unrelated animals, so it was not just a familial condition *within* the species, but a species characteristic. It was perhaps fortuitous that Harris, like Brand, was also working on cystinuria in man before making this discovery.

The cystinuria of the Kenya genet differs from that in man in that there is no excretion of high concentrations of other amino acids. Additional studies cited in brief (22) established that the cystinuria occurred by a renal mechanism, i.e. a deficiency of tubular reabsorption. Strangely enough the concentration of cystine in the genet urine was said to be about 1 to 2 mg/ml *in true solution*, that is, about four times its solubility in human urine. Yet the genets do not form urinary cystine stones. The failure of cystine to precipitate from these urines raises again the possibility of a cystine-containing complex existing in human (14, 5, 12) and dog (42) cystinuric urines, although the existence of such a complex has been emphatically denied (52). The early belief in such a cystine complex may have arisen from a theory that when cystine was absorbed as a peptide, because of faulty digestion, it escaped oxidation in the tissues and produced cystinuria (72). No stable complex of cystine was noted in genet urine, at any rate. The unusually high urea concentration of 10 to 20 g. per cent in genet urine, and the absence of other amino acids (16) might affect the solubility of cystine. Many questions of physical chemistry and physiology, and perhaps even therapeutic possibilities for cystinuria in man, are raised by these high urinary levels of cystine, but all must await further studies of the condition in the Kenyan genet.

Very recently cystine urinary stones have been discovered in another animal species, the mink (65). The economic importance of this animal should guarantee that the academically important questions about an analogue of a human disease will be answered. Already other mink with the disease have been found among those related to the initial case (personal communication).

VIII. THE BREAK-THROUGH: PATTERN OF AMINOACIDURIA

For a decade after Brand's work on cystinuria no further advances were made in the understanding of why cystine was excreted by the patients with this disease. Then, from a most unexpected quarter, came decisive and revolutionary findings which altered the very definition of the disease. In 1947 microbiological determinations of the individual amino acids in the urine of a cystinuric girl (compared with

the results of seven female controls) showed a high cystine excretion, but also arginine and lysine excretions about ten times the normal level (87)! These abnormalities in the excretion of other amino acids at last freed investigators from the bias imposed by the clinical nature of the disease. The unique insolubility of cystine caused its crystallization in nearly pure form in the urinary tract, as if it were the only substance present in abnormal amounts. The earlier methods which had given suggestions that other substances were present had not been sufficiently reliable to destroy this bias. But the specificity and quantitative accuracy of the microbiological methods now used at once established the new findings as fundamental characteristics of the disease. Equally powerful and even more convenient methods of amino acid analysis soon confirmed these new abnormalities.

The first explanation of the large amounts of two new amino acids found in the urine of cystinuria was progressive renal damage, producing first the leakage of these few amino acids and later, perhaps, the generalized aminoaciduria seen in Fanconi's syndrome (i.e. Abderhalden's disease, *v. supra*) (87). However, long clinical experience belied this. A non-specific renal damage would not show such specific leakages. Column chromatography of the urinary amino acids in six cystinuric patients, all with histories of cystine stone formation, showed that a definite pattern of aminoaciduria was characteristic of this disease (77). In addition to the cystine, lysine and arginine already identified, ornithine, a relative and associate of arginine in the urea cycle, was also found in abnormal amounts. The remaining amino acids were not different from normal. Moreover, in each of five patients, the abnormal amino acids were excreted in similar relative amounts, approximating the molar ratio of 1:1.3:2:5 for ornithine-cystine-arginine-lysine. While the constancy of these ratios was to some extent fortuitous and determined by the particular patients chosen (40), it emphasized the specific nature of the lesion in cystinuria. Stein suggested that instead of a renal tubule defect (77), a metabolic defect, enzymic in nature but probably in the kidney, simultaneously affected the reactions of all these different amino acids.

Renal Physiology: The possibility that a discrete renal tubular transport mechanism with enzyme-like specificity could be defective in cystinuria and give rise to the specific aminoaciduria was first appreciated in 1951 by Dent and Rose (21). It was known that arginine and lysine competed for the same tubular reabsorptive mechanism in the kidney of the dog, mutually lowering their Tm's (6), and it was suggested that this mechanism was missing in cystinuria. The first evidence to substantiate this was an elaboration of an old finding, by Brown and Lewis in 1937 (15), that the level of cystine in blood of a cystinuric was not elevated. In ten normal and nine cystinuric individuals the level of cystine in the plasma averaged 0.82 and 0.69 mg. per cent, respectively (29). It was argued that if cystinuria was the result of a renal defect, the plasma level would be normal or low as it appeared to be, while if it was the result of a blocked cystine metabolism, the plasma level should have been elevated for excretion to occur. This logic omitted from consideration the most recent hypothesis (Brand), that defective utilization of cysteine, not cystine, was the cause of cystinuria. That theory died unattended during the rush of the new advance. Evidence was presented that the levels in blood were normal

for the other amino acids also excreted in cystinuria (20). It was unlikely anyway that the several amino acids would share a common metabolic reaction, and the data on blood levels made it increasingly unlikely that any single metabolic block could be responsible for the disease.

Still, the crucial evidence for a specific renal mechanism required that the renal clearance of the amino acids be measured, and that the normally low clearances be found elevated, nearly to the glomerular filtration rate, by the loss of the ability of the tubules to reabsorb the affected amino acids. Exactly these results were found for cystine in a classical study by Dent, Senior and Walshe (23). Cystine clearance in normal individuals was of the order of 4 ml/min. In two cystinuric patients (both with considerable renal damage from stone formation and therefore probably with reduced glomerular filtration rates) the cystine clearances were about 100 ml/min., i.e. they approached the normal glomerular filtration rate. Moreover, in a person known to be heterozygous for the cystinuria gene, a definitely elevated cystine clearance (14 ml/min.) was found. It remained to be shown, only for completeness, that similarly elevated clearances existed for the other amino acids excreted in abnormal amounts.

Elevated renal clearances in cystinurics of cystine, arginine, ornithine and lysine were then demonstrated by Robson and Rose (71). The clearances of cystine, arginine and ornithine were not further increased by the rapid intravenous infusion of 5 g. of L-lysine, because the clearances were already near the glomerular filtration rate and there was little or no tubular reabsorption to be inhibited. In normal individuals and heterozygotes of cystinuria there was an active reabsorption system to be inhibited by the load of lysine. The lysine, because it normally shared a common stage in reabsorption with the three other amino acids, caused in the heterozygotes transient increases in the clearances of the three other amino acids, and only of the three.

The general nature of renal tubular transport systems of the kind at fault in cystinuria had been defined in experiments similar to those given above by earlier workers in the field of renal physiology. Shannon had shown that xylose, for example, with one less carbon than glucose but with the same configuration as in the first five carbons of glucose, was reabsorbed by the same tubular mechanism as was glucose. This transport system had not only enzyme-like substrate specificity, but related substrates would competitively inhibit it. Other substances were known to share still different transport mechanisms. The total action of an undetermined number of such discrete mechanisms accounted for tubular function. At some one step in tubular reabsorption, a specific reversible combination between an element of the transport system and the substance being transferred must occur (73).

Cystinuria could then be defined as an hereditary inactivity of a specific renal transport system which normally combined with and reabsorbed the several related dibasic amino acids. The inactivity would result from the absence or defect of a specific enzyme-like combining substance in the tubular cells. More or less complete inactivity of the system caused clinical signs, and occurred in individuals with two genes for the condition. But even the heterozygote, the carrier of a single gene who had no clinical signs, showed the stigma of this gene, in the one individual

tested, by a moderately reduced cystine reabsorptive ability because his tubule cells were partially deficient in the specific transporting substance under hereditary control.

IX. AN HEREDITARY BIOCHEMICAL DEFECT

While the group led by Dent at University College, London, was developing the renal explanation to replace Garrod's theory of an hereditary metabolic block in cystinuria, another group under Harris at the same institution used the methods of genetics to define the lesion of cystinuria in considerable detail. From these combined studies emerged at last the picture of an hereditary biochemical defect with the specificity envisaged by Garrod, but affecting an enzyme-like transport system instead of a metabolic enzyme. Thus the solution of this problem forced an expansion of Garrod's original concept to include hereditary defects in other functional systems besides enzymes.

When the diagnosis of cystinuria had depended almost solely on the appearance of stones, the familial incidence of the disease among siblings was such that Garrod correctly suggested its inheritance through a rare recessive gene. Even then, however, too many instances of direct transmission from parent to child were known, and Garrod entertained the possibility that the condition might be dominant. An alternative explanation, that the gene was very common instead of rare, would also account for the presence of the disease in two successive generations. An individual with the disease would then not uncommonly mate with a heterozygote (carrier) and the disease would reappear among the offspring of the mating. This explanation was possible only if cystinuria was much more common than had appeared from the incidence of stone formation. Indeed, chemical tests for urinary cystine revealed a much greater incidence of cystinuria (1:600) than had been suspected on the basis of stone formation or crystalluria (1:10,000 or 20,000).

Incidence of Cystinuria: Between 1929 and 1931 H. B. Lewis examined the urine of 10,534 Michigan University students with the cyanide-nitroprusside test, and when that was positive, by the more specific Sullivan test for cystine (51). The normal excretion of cystine is in the range of 40 to 80 mg. per day (69) and about the same amount per gram of urinary creatinine (41). It has been estimated that Lewis' cyanide-nitroprusside test would be distinctly positive with urines containing 200 mg. cystine per day (19) and positive with at least 100–125 mg. cystine per gram of creatinine (41). In the large population tested Lewis discovered eighteen individuals, or 1 in 600, who consistently excreted cystine. Eleven of these were studied in more detail and all showed an abnormally elevated urinary organic sulfur fraction. Four of them showed repeated cystine crystalluria (4 in 10,000). Earlier estimates of the incidence of cystine crystalluria were less than one-fourth this high, but these estimates were based on single examinations for a condition known to occur only intermittently. None of the individuals found by Lewis had ever had symptoms suggestive of renal stone or colic and only one gave a family history of such disease. A sister of one of the individuals had formed a cystine stone, but his parents and five other siblings did not excrete cystine. The urine of twenty-two other individuals in the population studied gave weakly positive tests consistently

or occasionally, but all had nearly normal sulfur partitions, and were considered normal. Malleson (cited in 41) confirmed the high incidence of chemical cystinuria, with the finding of four positive urines from about 1,000 students at University College, London. These urines contained 106 to 161 mg. cystine per gram of creatinine, and paper chromatography demonstrated in all of them an increased output of lysine as well as cystine.

It was immediately apparent that only a small fraction of the cystinurics that could be diagnosed chemically would form stones. But prior to this time, the best evidence was consistent with Niemann's belief that almost all cystinurics (diagnosed by crystalluria) ultimately formed stones. Since stone formation occurred randomly at all ages, it was reasonable to suppose that about half of all the cystinurics in the population at a given time had already formed stones and the other half eventually would in the future, an incidence of stones in cystinuria of about 50 per cent at any given time, although this estimate was never explicitly stated. On the other hand, a curiously different estimate, that only "2½ per cent of cystinurics develop stones" (26, 28), crept into the medical literature about 1923 and was widely repeated without question (62, 84) (see (70) for other references to this myth). It was probably derived from a misinterpretation of some statistics giving the percentage of cystine stones found among all urinary calculi (see (58)). This figure, though pulled out of the air, does happen to be the approximate frequency of stones among chemically detectable cystine excretors, but was cited before Lewis had discovered how relatively common chemical cystinuria was. Lewis commented wryly on this recurrent error that anticipated his discovery, that since cystinuria "was diagnosed almost invariably in connection with the presence of calculi, the basis of such an estimate is not clear to the present author" (51). Lewis' new evidence, that chemical cystinuria was relatively common, while cystine stones were rare, demanded that an additional criterion, apart from stone formation, be developed to identify the specific disease in stoneless people and distinguish it from other conditions where cystine excretion might occur. Twenty years later the specific aminoaciduria of four amino acids provided the necessary criterion for recognizing cystinuria in the absence of stone formation.

The indicated investigation was promptly undertaken by Dent and Harris (19). No further purpose will be served by using the term cystinuria in a general sense, as they did, to describe abnormal amounts of cystine in the urine from whatever cause. The result of their study was simply to redefine cystinuria as the specific condition characterized by the presence of the four amino acids in abnormal amounts, and to prove it was distinct from the Fanconi syndrome and from Wilson's disease. In the latter diseases cystine excretion might occur as part of a general aminoaciduria. The families of three individuals with the latter two diseases contained three other individuals with a similar general aminoaciduria and none with the specific cystine-arginine-lysine type of aminoaciduria. The families of seven classical cystinurics, six of whom had stones, contained seven more individuals of the same specific type and none with the generalized aminoaciduria. These findings directly disproved the idea that cystinuria was a stage in the development of the Fanconi syndrome

(87), which had subsequently been taken up by other authors without further proof. The findings proved each of these diseases to be genetically independent.

The Pattern of Inheritance: Questions and Answers: The incidence of cystinuria found in this study, which included seven new cases from seven propositi, was not different from that expected on the basis given above, that about equal numbers with and without stones would be found at a given time. But even twice the number of total cases as were known by stone formation could greatly alter the apparent pattern of inheritance. From a consideration of the published pedigrees of cystinuria, complete except for those given by Niemann (64), Kretschmer (47) and Mörner (59), Dent and Harris concluded that the available data was of little genetic value since only five families had been examined with chemical methods. The diagnoses could all be questioned because none of these family studies gave information about the excretion of other amino acids. The chance of this diagnostic error would seem to be very small, given the presence of cystine urinary calculi in a family. But the inclusion of Abderhalden's cases of familial cystine diathesis among the cystinuric pedigrees reviewed by Dent and Harris themselves stood in mute testimony of this danger. The data from the earlier studies simply re-emphasized the mutually contradictory points made by Garrod: the frequency of parental consanguinity and of cases among siblings indicated that cystinuria was a homozygous recessive disease; but the presence of affected individuals in three successive generations of the same family (the example of Andrews and Brooks, a cystinuric family tree of twenty-five, seven of whom were cystinurics (4), can replace the dubious one of Abderhalden) almost certainly indicated that some patients in this family were heterozygotes, i.e. the condition was sometimes dominant.

Analysis of the new family data collected by Dent and Harris did not resolve this problem. In two instances the parents of cystinurics were normal, so the condition was not regularly manifested in heterozygotes. The occurrence of affected parent-child pairs in two of the seven families could be explained on the recessive hypothesis in view of the postulated high frequency of the gene in the population (one individual in twelve would be a heterozygote if Lewis' incidence of 1 in 600 represented the homozygous form). But if the gene were so common, consanguinity would not be important for its expression. Yet even in this small series there was one consanguinous marriage. It was apparent that the genetics of cystinuria, half-known for so long on the basis of the qualitative excretion of cystine, would yield fully only to a detailed quantitative study.

Quantitative studies by Harris and coworkers of the amino acid excretion in cystinuric families provided the answer in two years' time. Initially, the quantitative measurements of cystine excretion in twenty-one families (41) showed only in some of the families the expected clear separation into cystinuric and normal individuals. In the other families, there was a continuous gradation from the normal to the extremely abnormal amounts of cystine excretion in the different individuals. It was evident that the disease appeared in two forms, separately inherited. Type I, the more common of the two, appeared in families where all individuals excreted either normal or highly abnormal amounts of urinary cystine, and no individuals

had intermediary values. There was a sharp segregation for the property of cystine excretion, and the family distribution was of the type expected if the cystinuria occurred in individuals homozygous for a rare recessive gene. The parents, who were apparently normal, were necessarily heterozygotes. But the character of the one recessive gene which they possessed was not strong enough to create any noticeable effect on cystine excretion. Thus, the defect appeared only among the homozygous offspring. Four instances of consanguinity in these families further supported this view.

In families of Type II, normal, intermediate and high values of cystine excretion were all found. The family distributions suggested that the individuals with moderately raised values of cystine excretion were heterozygous and those with high values, like the individuals in Type I families, were homozygous for a single rare gene. Because the character of the "incompletely recessive" gene in Type II families was strong enough to produce intermediary values in the heterozygotes, it was assumed that the individuals with normal cystine values did not possess the gene for cystinuria. Whether two distinct diseases or two variants of the same disease had been discovered could only be answered by further quantitative studies of the other amino acids excreted, but it was clear that the earlier contradictory results had found their explanation in two different modes of inheritance of what appeared to be the same renal defect.

Two Variant Forms of Cystinuria: The variant forms of the disease originated in inheritance of different degrees of the renal defect in cystinuria, as was clear from study of the lysine and arginine excretions (39). The number studied included twenty-eight stone-forming cystinurics and 121 of their relatives, undoubtedly the largest collection of cystinuric families ever observed by a single group of investigators. As before all degrees of cystinuria from 20 to 800 mg. of cystine per gram creatinine were found. Only in individuals excreting 250 mg. cystine per gram creatinine or more had stone formation occurred. This was the level at which a saturating concentration of cystine in urine, about 300 mg per L, could be reached and precipitation would be likely to occur. The lysine excretion was regularly about twice that of cystine and paralleled the cystine excretion throughout all its gradations. Arginine and ornithine, however, were preferentially reabsorbed by the tubular mechanism in question. These amino acids were not excreted in abnormal amounts until the cystine and lysine reabsorptions were seriously impaired. Thus a genetic study revealed the hierarchy of affinities of substances for a renal physiologic mechanism. At a cystine excretion of 250 mg. per gram creatinine, where stone formation became likely, arginine and ornithine also appeared in the urine in abnormal amounts and increased proportionally with the excretion of higher amounts of cystine and lysine. The genetically determined degrees of the renal defect, if partial, permitted the preferential reabsorption of arginine and ornithine, and if more complete, resulted in the renal loss of all four amino acids, including cystine in sufficient concentration for it to precipitate and form stones.

The biochemical and genetic data of the preceding studies (39, 41) were finally correlated in a definitive paper on "Phenotypes and Genotypes in Cystinuria" (40). A total of twenty-nine families, each with at least one stone-forming cystinuric

member, had been studied. Two of these families could not be definitely categorized as Types I or II from the data available, but in all families the homozygous individuals were indistinguishable. They excreted approximately the same highly abnormal amounts of the four amino acids, and nearly all formed cystine stones. In nineteen of the families (Type I) the condition was recessive and no degree of abnormality was found except the extreme form in the homozygotes. In eight families (Type II) the condition was "incompletely recessive," and the heterozygotes showed moderately increased excretions of cystine and lysine without increased excretion of arginine or ornithine. Only in rare instances did the latter excrete sufficient cystine to form stones.

Implicit in these figures of the relative frequency of the two types, with about one-third of the families manifesting cystinuria in the heterozygotes, was the explanation of the rarity of cystine stone and the commonness of chemical cystinuria. Accepting a conservative order of magnitude for Garrod's estimate of the incidence of cystinuria with stones or crystalluria as 1 in 20,000 people in the population, it is indeed a rare condition. Only one-third of these, or 1 in 60,000 people, would then represent the still rarer type whose heterozygous relatives would show chemical cystinuria without stones. Yet the astonishing frequency of the heterozygotes of a rare homozygous condition is such that, even with this degree of rarity, it would be easy to account for the observed incidence of chemical cystinuria. The frequency of heterozygotes of the rarer form would be about $2\sqrt{\text{incidence}}$ of the homozygous form, or 1 in 125! The incidences of chemical cystinuria observed by Lewis (1 in 600 consistently and 1 in 260 including the occasional excretors) or by Malleson (1 in 250) were less, and this suggests that cystinuria with stones is less than half as common as Garrod estimated. The "complete ascertainment" of Mörner of the incidence in Sweden would set the minimal incidence at 1 in 200,000.

Alleles?: An unsolved problem concerned the genetic relation between the two types of cystinuria, distinguishable only by whether or not cystine and lysine excretion occurred in the heterozygotes. Since the same renal system was affected in both types, and they differed only in the heterozygotes in regard to the quantitative effect on the renal system of the single gene, it is not likely that separate genes at different loci are responsible for the two types. Other considerations were also against this view (40, 41). A single mutant gene, accompanied in certain families by modifiers at other loci, possibly could give rise to the two observed types. The simplest explanation, however, would attribute the phenomenon to multiple alleles, that is, to at least two mutant conditions of one gene. One would have a mild and the other a severe effect on the tubular reabsorption of the four amino acids in the heterozygous state. Genetic proof of this reasonable possibility, Harris suggested, must await the identification of a homozygous cystinuric born of parents, one the apparently normal Type I heterozygote and the other a Type II heterozygote. Families ostensibly of this kind are on record, with one parent who excreted cystine but did not form stones, the other parent "normal", and a stone-forming offspring. But detailed chemical identification of the type of each individual in such a pedigree has not been done.

Direct chemical identification of the phenotypes of the parents of a stone-forming

cystinuric would now appear to be a possible and a more economical way of establishing the allelism of the two variant genes. Since one gene has a mild, and the other a more severe effect on the tubular reabsorption of cystine and lysine, the measured renal clearance of these amino acids should differentiate the two kinds of heterozygotes from each other, and distinguish both from the normal. At least, such measurements would identify the heterozygotes who should be subjected to more intensive genetic studies to decide this question.

The Moral: It appears that research on cystinuria had little influence on the conceptual thinking of the time, but was captive to the conceptual schemes of the times. The eventual demise of the concepts of "diatheses" of disease, of separate endogenous and exogenous metabolisms, and even of inborn errors of metabolism as exclusively enzyme deficiencies, appears to be unrelated to their unsatisfactory accounting for the facts of cystinuria. The popularity of the theories slowed the learning about cystinuria. If this relationship were true for other diseases, the medicine of the time would stand revealed as not a science, but a para-science, only borrowing and applying what it could, instead of producing its own conceptual schemes to account for its own phenomena. The scheme which today accounts for that rare curiosity, cystinuria, and other biological phenomena as well, could have been engendered by the findings in cystinuria, but was not.

A reluctance to give the facts about a rare disease primacy over the popular theories of the day may account for the remarkable periodicity of advance and stagnation in the history of ideas about cystinuria. The impetus from the initial discoveries in 1810 of the chemical cystine, and the disease, cystinuria, reached a surprisingly sophisticated level of understanding by 1823, when Prout wrote "la cause tient plutôt à l'action dépravée des reins qu'à une lésion générale du système." (70). The clinical definition of the disease, and the first pregnant statement about a block in metabolism had to wait for Niemann in 1876. Then shortly before 1900 began a burst of activity which produced the chemical structure of cystine, the best metabolic balance studies in cystinuria, and the concept of inborn errors of metabolism. A stalemate ensued. The power of Garrod's idea, that specific blockages of metabolism were inherited, prohibited the obvious conclusions from Folin's studies that the cystine metabolism was normal in the hereditary disease of cystinuria. Brand's work in 1936 represented the ultimate attempt to provide experimental supports for Garrod's concept. Some years after this agonal twitch in a dead approach, between the initial discovery by Yeh, *et al.*, in 1947, and the definitive paper of Harris, *et al.*, in 1955, the problem was solved. Cystinuria was an inherited defect of kidney function.

Garrod's concept of inborn errors of metabolism was not vitiated, as has been thought, because a specific renal tubular reabsorption system and not an enzyme under hereditary control was inactive in cystinuria. Any vitiation should more properly be attributed to the earlier recognition that the Dalmatian coach hound was unusual among dogs by excreting uric acid, not because he lacks uricase, which he does not lack (45), but because his renal tubule fails to reabsorb uric acid (30). On the strength of the modern findings in these two conditions in man and dog, conditions previously thought to illustrate the hereditary control of enzymes of

intermediary metabolism, it is clear that Garrod's concept should not be discarded, but must be extended to include the hereditary control of specific functional substances in the body other than enzymes. In the present instance the inheritable substance is an "enzyme-like" transport system in the renal tubule. It could be called a specific "here-to-there 'ase," to emphasize its analogy to the substances thought to be affected in the original statement of the inborn errors of metabolism. Into this enlarged scheme, engendered by the phenomenon of cystinuria, can be fitted immediately a number of other hereditary conditions characterized by "low renal thresholds," such as renal glycosuria.

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