Wilson's Disease

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WHY does a disease as rare as Wilson's disease attract sufficient interest to warrant its inclusion in this program? I believe the chief reason is that despite its complexity and clinical variation there is a growing conviction that the disease must be caused by a fairly simple primary defect and that many, if not all, the clinical vagaries will be explicable when the nature of the primary defect is recognized.

If I can summarize, and necessarily oversimplify the clinical aspects of the disease, it will perhaps enable the biochemistry and genetics to be put in perspective. In 1911 Kinnier Wilson[1] described a disease which he called "progressive lenticular degeneration, a familial nervous disease associated with cirrhosis of the liver." The disease is extremely variable; in some instances after an early onset, rigidity and muscle spasms, dysphagia and dysarthria are associated with a progressively downhill course and death ensues within a few months. In others, a slow insidious tremor in the early twenties is the only sign of the affliction. Other patients show a gradual deterioration over the years which may still leave the patient ambulant and enjoying life twenty five years after the onset. Sometimes the disease is heralded by hematemesis or ascites and cirrhosis of the liver. Progressive liver failure may dominate the clinical picture. Of great diagnostic importance is the fact that Kayser-Fleischer rings are nearly always present once the disease is recognizable clinically. Epileptic fits, broken bones, arthritis, azure lunulae and pigmentation are among the strange potpourri of symptoms which are present in the disease. Is it reasonable that these widespread and varying symptoms could stem from a single biochemical lesion?

Wilson postulated that the familial nature of the disease was due to nongenetic causes and for a number of years the mode of inheritance was in doubt. A recessive mode of inheritance was suggested[2] but the data in the literature were hard to disentangle due to insufficient information on the numbers of unaffected sibs and relatedness of the parents. The occasional occurrence of the disease in two generations was incorrectly regarded as strong evidence against a recessive inheritance. Collection of pedigrees in recent years and a greater awareness of genetic disorders has resulted in the disease being established as inherited in an autosomal recessive fashion. A high consanguinity rate in the parents, the best evidence for a rare recessive, could be demonstrated,[3] and genetic calculations supported this assumption.

According to current theory, a mutation can be considered an event which alters the base sequence in DNA and as a direct consequence alters the synthesis of a particular protein. This alteration can so upset the program that no recognizable protein can be synthesized or it may only change the message slightly so that a different protein is made. This concept is somewhat analogous to Muller's system of amorphs (or hypomorphs) and neomorphs in new guise. Once it is decided that the disease is due to a single gene in double dose, the ultimate question is what enzyme or protein is disturbed by the mutation. What is the primary gene product of the normal allele at the Wilson's disease locus and is it the same as the product in persons suffering from the disease? Despite the apparent complexity of the disease, its recognition

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as an autosomal recessive has enabled the formulation of precise questions although precise answers have not yet been forthcoming. It may be useful to attempt to appraise our present knowledge of Wilson's disease in this light.

ROLE OF COPPER

Recognition that copper accumulation was an integral part of Wilson's disease was the undoubted beginning of the resurgence of interest in Wilson's disease during the last two decades. These observations were made primarily by clinicians and pathologists who painstakingly examined the tissues of patients who died from the disease. An increased copper content of the body was recognized and it was also shown that the urinary excretion of copper was elevated compared with several neurologic conditions. At about this time, Holmberg[4,5] in Sweden was shown a sample of pig serum which exhibited a distinct blue color. In association with Laurell, isolation and characterization of this blue protein was carried out with great success both in pig serum, and later in normal human serum. The protein proved to be an α_2 protein with a molecular weight of about 151,000 and to have 8 atoms of copper per molecule. The protein behaved like a laccase having oxidase activity towards a variety of substrates but particularly toward paraphenylene diamine.[4,5] It is noteworthy, however, that compared with other oxidases, the activity is very low and whether or not the protein acts as an oxidase *in vivo* is still much in debate. Scheinberg and his colleagues[6-8] have shown that not all the 8 atoms of copper are equally firmly bound. Half can be removed with relative ease and under the right circumstances the copper could be put back on the molecule and activity almost completely restored.[6] An attractive hypothesis was proposed by these workers[7] who suggested that ceruloplasmin might act as a transport protein for copper *in vivo* similar to transferrin for iron transport. However, more recent observations by the same authors[8] provide no evidence that the exchange of copper atoms occurs *in vivo* and other functions for this unique copper protein must be sought.

CERULOPLASMIN

With the recognition that there was excess copper in the body of patients with Wilson's disease, it was a short step to determine the serum copper and ceruloplasmin level in this condition. Surprisingly, despite the excess of copper in the body, the serum copper and ceruloplasmin levels were markedly depressed.[9] Fortunately the disease was becoming of interest to a variety of workers in the field and confirmatory reports of depressed copper and ceruloplasmin quickly appeared.[10,11] A simple hypothesis promptly emerged that perhaps the disease was due to an inability to synthesize the protein ceruloplasmin. If this protein regulated the absorption and/or excretion of copper, a decreased level would cause a net increase in the copper content of the body. With the passage of time this hypothesis has been increasingly hard to accept in its simplest form.

There is no doubt that accumulation of copper in the liver can occur very early. In an asymptomatic three and a half year old sib of a patient with Wilson's disease, Scheinberg[12] has demonstrated a grossly increased copper content of the liver which, apart from the presence of large glycogen nuclei in the liver, did not reveal any gross histologic changes. If this patient is homozygous for the Wilson's disease gene, it has been demonstrated that copper accumulation precedes the development of the cirrhosis. Serial biopsies will be of great interest. The demonstration of large quantities of copper in all parts of the brain correlates with the widespread neurologic symptoms: the disease is a good deal more than pure lenticular degeneration. Copper can be shown to accumulate in the renal tubules and that the nature and extent of the renal lesion is related to the duration of the disease. At first the brunt of the damage falls on the proximal renal tubule and glycosuria, phosphaturia, aminoaciduria and uricosuria ensue. These substances are presumably reabsorbed through mechanisms which are relatively easily poisoned by copper. Later distal tubular functions fail and the circulatory dynamics of the kidney—the glomerular filtration rate and the effective renal plasma flow—become disturbed

The renal lesion has attracted considerable interest since this disturbance of metabolism may be of primary importance. Beta amino isobutyricaciduria, cystinuria and glycinuria are all inherited conditions in which the primary effect of the gene is a disturbance of amino acid metabolism transport. The belief that Wilson's disease might represent a form of inherited aminoaciduria was strengthened by the finding that increased amino acid excretion occurred in some relatives. However, this was not revealed in the majority of relatives of patients with Wilson's disease, and most workers are inclined to regard the amino acid disturbances as secondary phenomena.

There is no doubt that in patients with Wilson's disease there is an increase in the total body copper, although whether or not this is due to increased absorption or decreased excretion is uncertain. Recent evidence of Gitlin and his co-workers[13] suggests that more attention should be paid to the possibility of decreased copper excretion in this disease. The copper cannot be incorporated into ceruloplasmin, resulting in an increased quantity of copper perfusing the tissues loosely bound to serum albumin. Whenever there are substances in the tissues, polypeptides or proteins, which have an affinity for copper greater than albumin, the copper will be deposited and disease will result.

A number of observations at variance with this hypothesis should be mentioned. There is no correlation between the depression of the concentration of ceruloplasmin and the severity of the symptoms. Indeed there are a number of patients with unequivocal Wilson's disease who show normal levels of ceruloplasmin. Moreover, a depression of ceruloplasmin can occur in conditions other than Wilson's disease, although admittedly the duration of the depression is usually considerably shorter. Such conditions include nephrosis, generalized hypoproteinemia and perhaps sprue. Some heterozygotes, as will be discussed, may also have severely depressed ceruloplasmin levels without overt symptoms of disease.

If we wish to continue to regard ceruloplasmin deficiency as a primary disturbance, we are forced to the hypothesis that the disease may be present in at least two allelic forms. In the first and more common, the ceruloplasmin is synthesized at a decreased rate; in the second, the rate of production is almost the same but the product differs. The first corresponds to Muller's hypomorph and a nonsense mutation in the code; the second relates to a mutational event which results in the formation of a neomorph (missense mutation). However, there is no proof, of which I am aware, that the decreased ceruloplasmin in patients with Wilson's disease is structurally normal. Additional studies are urgently needed.

CLINICAL STUDIES

Our series of cases collected in New York were analyzed genetically. The patients could be divided into two main groups: those who came from Eastern Europe and Poland and those who came from Italy. Clearly the disease has occurred all over the world, but the concentration of cases in these two populations appeared worth investigating in an effort to gain further insight into the possibility of heterogeneity. The advantages of looking at genetic data in this way should be emphasized for it may give evidence of the existence of modifying genes present in certain populations which may or may not have important selective advantages. The two groups appear to be slightly different statistically, particularly if several aspects are combined. By and large it appears that in those from Eastern Europe the age of onset is slightly later, the disease milder and the disturbance in Darwinian fitness less than in those from Sicily and Southern Italy. Biochemically there is little to differentiate them. The possibility that the ceruloplasmin may, on the average, be higher in those from Eastern Europe, is not yet established. Thus, there is suggestive genetic and chemical evidence that in some patients the disease may be caused by a different allele.[2] However environmental factors may play a role in the differences between the two groups.

It would be of great importance to know the frequency of the gene in the population. Unfortunately all calculations are very approxi-

mate. Moreover the sample is small. Theoretically the gene frequency might differ in those from Eastern Europe, from Italy and in the general population. Methods for calculating the gene frequency are well established. One method utilizes the relationship between gene frequency and marriages between cousins. However, the incidence of marriages between first cousins in the population from which the sample is drawn must be known. The calculation can be performed with first cousin, second cousin and unrelated parents and q should be the same in the three calculations. However, between groups the value for q might vary widely.

Calculation of the mutation rate is virtually impossible with a rare recessive. The formula of Morton and Chung,[14] in this instance, is of dubious value. The formula is usually expressed $M = q(\alpha^1 + q + h) S$ where α_1 is the long-term inbreeding coefficient and h is the selective coefficient against heterozygotes. α_1 is about 0.006 and h is presumably 0. The formula assumes an equilibrium between selection and mutation. The important point, however, is that we do not have an accurate value for q or S, and it seems unlikely that in the present state of ignorance it is worth calculating the mutation rate for the Wilson's disease gene. A very small advantage of the heterozygote is needed to balance even a lethal homozygote. The advantage has to be roughly proportional to the gene frequency.) Thus, although there may be a heterozygous advantage in Wilson's disease, it will not be detectable under ordinary circumstances.

HETEROZYGOTES

Let us return now to the question of the detection of carriers of the Wilson's disease gene. Only a decade ago, apart from the blood groups, there was no way of detecting heterozygotes with confidence. In the last few years it has become very important to devise methods for detecting the heterozygote. This is not primarily for so-called eugenic reasons but to enable us to understand more about the possible origins and spread of the disease. As a general rule, the ease with which heterozygotes can be detected will depend on how close one is to the primary gene product. Theoretically, if one were in a position to catch the substance as it comes off the ribosomes then in principal the two products of the heterozygote could be detected. The further away from the gene product, the more likely will genetic modifiers and environmental factors influence the detection of the heterozygote. In Wilson's disease a significant proportion of the parents show a decreased quantity of ceruloplasmin. However, the quantity is by no means invariably depressed. Some recent observations from Scheinberg's group have revealed that the use of radioactive copper will disclose a larger percentage of carriers than the determination of ceruloplasmin alone.[15] However, even with this method there is considerable overlap between the groups. This is not unexpected and moreover, the degree of overlap is an important genetic parameter to define. One can be certain that measurement of the ceruloplasmin concentration in the serum ultimately will not prove to be the best way to detect the presence of the Wilson's disease allele.

How can we explain the patients with normal ceruloplasmin? It seemed possible that although the ceruloplasmin was normal in quantity perhaps it was abnormal in quality. In other terms a missense mutation has resulted in the formation of an abnormal ceruloplasmin with different properties. When Broman[16] had shown that ceruloplasmin could be fractionated into two peaks on hydroxyapatite columns, it became of interest to separate the serum of patients with Wilson's disease under identical conditions. It was found that patients with normal and low values of ceruloplasmin had similar ratios of activity in the two peaks, although of course each peak was greatly decreased in those with low levels. In one experiment the ceruloplasmin level was raised by the administration of estrogens and the ratio of activity in the two peaks remained the same at widely differing ceruloplasmin values. This evidence, as far as it goes, shows no difference in the ceruloplasmin. Clearly more work is necessary for it is by no means conclusively proved that the reported heterogeneity of ceruloplasmin is genuine heterogeneity, and not

artifacts produced by the experimental conditions.[17]

SUMMARY

Whatever the cause of Wilson's disease there is one clear cut biochemical abnormality: an increased total body copper. It seems therapeutically plausible to remove the excess copper as quickly and completely as possible. This can be achieved by oral administration of penicillamine. With judicious but humane advice concerning the avoidance of foods containing a high copper content and the regular administration of penicillamine, the copper content of the body returns toward normal and in some cases is associated with undoubted clinical improvement. Replacement of a low ceruloplasmin level either by estrogen administration or by infusion of ceruloplasmin seems of little value. The regular infusion of apoceruloplasmin, to prevent absorption of copper and penicillamine to eliminate the copper already present, would be logical therapy for this particular inborn error of metabolism and already the therapeutic nihilism epitomized in the statement that inherited diseases are cured only by the grave is happily mistaken.

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