Alpha-1-antitrypsin deficiency: a biological enigma

A. G. BEARN

From the New York Hospital—Cornell Medical Center, New York, USA

SUMMARY The association of certain forms of liver disease and a deficiency of alpha-1-antitrypsin is an observation which raises the possibility that other forms of liver disease ultimately will be found to have as their proximate cause a defined metabolic aberration, which may in turn be inherited. Although alpha-1-antitrypsin deficiency is a genetically determined error of protein synthesis, environmental factors, unrecognised at present, are required for the disease to become overt. Thus, this interesting association may herald an increasing number of clinical diseases in which the interaction of environmental stimuli and single genetically determined aberrations are crucially important. The diseases to which we succumb may be largely determined by a genetically determined susceptibility, a point of view which was stated so well by Archibald Garrod in his essay *Inborn Factors in Disease* published nearly half a century ago.

Mendel began his experiments with garden peas two years before the Darwinian revolution. The revolution which was to provide a foundation for the entire structure of modern biology was slow to erupt, although the day of its dawning can be identified precisely. On 1 July 1858, at a meeting of the Linnaean Society of London, C. Darwin, with A. R. Wallace as co-author, read a paper drily entitled 'On the Tendancy of Species to form Varieties and Species; and on the Perpetuation of Varieties and Species by Natural Means of Selection'. There were few guests at the meeting; one who would come to appreciate the profound significance of the paper was too young to attend. Archibald Garrod, future Regius Professor of Medicine at Oxford, and author of *Inborn Errors of Metabolism*, had barely turned his first birthday. Darwin delivered his lecture and returned to work. Darwin, like Garrod, never stopped working. 'When I am obliged to give up observation and experiment', Darwin said, 'I shall die'. He was working on 17 April 1882; he died two days later.

Two years later, in 1884, Gregor Mendel, his research brought to a premature halt, victimised by a meddlesome bureaucracy and buried in paper work of the most inane sort, died in Czechoslovakia. Meanwhile, in London, Archibald Garrod, who was to understand the relevance of both Darwin and Mendel's work to man, and who was to become the father of modern biochemical genetics, was 8 years old. He would be 34 years old before Mendel's work was rediscovered by the Dutch hybridist Hugo de Vries, the German

botanist Carl Correns, and the Austrian botanist Erich von Tschernak. Although Mendel's paper had reached many countries before 1900, the scientific community was too concerned with the fruitless Darwinian-Lamarckian debate to take much notice. But Archibald Garrod, a chemically-minded physician, was quick to appreciate and apply the knowledge derived from Mendel's studies on peas to man and his diseases.

Archibald Garrod was fortunate; he grew up in a family where the collection of scientific data and the employment of scientific method took easy precedence over meandering philosophical discourse. He graduated from St. Bartholomew's Hospital in 1884 and was intrigued by the possible relevance of hereditary influences on human disease. He probably learned about Mendelism through William Bateson, the botanist, and in 1902 Garrod wrote what was perhaps the first definitive paper on human biochemical genetics (Garrod, 1902). Since that time the importance of genetic influences has become slowly but increasingly appreciated, and there are now well over 1000 well-recognised genetic diseases.

Garrod, who was broadly interested in clinical medicine, was also an expert on urinary pigments, particularly urobilin, an interest he developed while working with Gowland Hopkins (Garrod and Hopkins, 1896; Hopkins and Garrod, 1897).

In addition to his chemical studies he wrote on cirrhosis of the liver (Duckworth and Garrod, 1895) and liver tumours (Garrod, 1904). Liver disease is as old as antiquity, and Sherlock (1975)

quotes Erisistratus as having been the first to recognise the association of liver disease and dropsy in 300 BC, but the role of inherited influences in liver disease has not been seriously considered until the last half century. The disorders described in Garrod's own paper on cystic fibrosis (Garrod and Hurlley, 1912) in the *Quarterly Journal of Medicine*, Sheldon's classic book on haemochromatosis (Sheldon, 1935), and the demonstration that Wilson's disease is a clear-cut Mendelian disease (Bearn, 1957) were, until 20 years ago, almost the only diseases of the liver which could be ascribed to genetic causes.

There is one genetic disease of man affecting the liver that would have delighted Garrod, would have pleased Mendel, and would have diverted Darwin's monocular genius, if only momentarily. I refer to the association of a deficiency of alpha-1-antitrypsin and certain forms of liver disease. Here is a disease of interest to geneticists, evolutionists and physicians; all indeed who ponder the 'inborn factors in disease' (Garrod, 1931).

History

Fifteen years ago Laurell and Eriksson (1963) observed an association between alpha-1-antitrypsin deficiency and chronic obstructive lung disease. The observation of a family in 1964 with chronic obstructive lung disease in New York City (Kueppers *et al.*, 1964) indicated that this association was not confined to Sweden and that the 'disease' was inherited in a simple autosomal recessive fashion. With the electrophoretic recognition of the allelic products of the alpha-1-antitrypsin locus, it became clear that the products of the variant alleles are inherited in a codominant fashion. In 1969, the first association of liver disease and alpha-1-antitrypsin deficiency was reported (Sharp *et al.*, 1969). This has been followed by numerous reports of neonatal hepatitis, and 'infantile' cirrhosis in which a deficiency of this serum protein can be demonstrated. The association, however, remains puzzling, and a number of self-evident key questions remain unanswered, the most important of which is: how does the deficiency of the alpha-1-antitrypsin protein 'cause' the cirrhosis? I shall use the term 'cause' in the usual meaning of 'an antecedent on which a phenomenon is invariably and unconditionally consequent'. This definition promptly eliminates, *sensu strictu*, alpha-1-antitrypsin deficiency as a cause for neonatal hepatitis, since no more than 1 in 10 of those individuals who have a deficiency of the protein develop cirrhosis and indicates that causes and effects are not simple or atonic. Grammatical habits generated by simple

subject-predicate construction tend to blind us to the actual complexity of causal relationships. The 'cause' of neonatal hepatitis is clearly not ascribable to a particular genetic deficiency but to a complex nexus of perturbed physiological states which issues another complex nexus, similarly concealed behind the term 'effect'. However, the recognition that alpha-1-antitrypsin deficiency is neither a necessary nor a sufficient condition for the disease adds rather than detracts from its innate biological interest.

Alpha-1-antitrypsin locus

From a genetic viewpoint this locus achieves special importance that derives from its extraordinary allelic diversity. Twenty-five alleles are known whose products can be distinguished using standard electrophoretic techniques (Fagerhol, 1974; Kueppers and Christopherson, 1978). The very diversity is a biological challenge. Many alleles exist in frequencies that demand a biological explanation. Frequencies above 1% are hard to maintain without embracing an unacceptably high mutation rate. Thus convention would state that a balanced polymorphism clearly exists, though the neutralists, supported by mathematical arguments beyond common comprehension, would say otherwise. The alleles of greatest medical interest are those clearly conferring a disadvantage. The frequency of the PiZ allele in Sweden is approximately 0.026 (Laurell and Sveger, 1975); in the United States the frequency is lower at 0.01. The frequency of Z/Z homozygotes in any population is simply the square of the gene frequency in that population. Thus, the variability in gene frequency in different population groups makes treacherous conclusions that are based on studies on genetically inhomogeneous patients. Thus, in one study, Fisher and her colleagues (1976) found that 1% of all the patients with liver disease attending a world renowned liver clinic (mainly adult) were homozygous ZZ. As Fisher and her colleagues clearly recognise, the geographical scatter of patients attending this clinic makes generalisation impossible. Thus, the geographic variation in the frequency of various alleles does not permit unequivocal conclusions regarding the pathological significance of the alleles P and S which are also associated with low alpha-1-antitrypsin activity. The rare null phenotype, Pi-, associated with profound deficiency of the protein, may also be associated with cirrhosis. It is hardly surprising that controversy exists in ascribing increased susceptibility to liver disease in individuals heterozygous for one of the alleles associated with decreased activity when it is remembered that no more than

10% (roughly) of ZZ individuals develop liver pathology. Genetically, however, the situation is of great interest. The very existence of allelic products differing from the norm must affect physiological homeostasis. Insight will be gained when the allelic products are identified and characterised and their effects examined at the molecular level.

Although liver disease is common the ZZ phenotype is sufficiently rare that no great risk is taken in assuming a relationship between the deficiency of the protein and patients with neonatal liver disease; the situation with adult liver disease, chronic active hepatitis, and primary biliary cirrhosis is far less clear. The situation regarding hepatoma appears slightly more compelling. Eriksson and Hägerstrand (1974) have reported malignant hepatomas in six of nine patients with alpha-1-antitrypsin deficiency and cirrhosis of the liver. Attribution, however, of a specific role for the Z allele should proceed with caution. Emphysema and cirrhosis in the same individual has been reported but is clearly rare. For overt disease to be present it seems likely that the antitrypsin level should be depressed by 60% or more.

PHYSIOLOGICAL SIGNIFICANCE OF ALPHA-1-ANTITRYPSIN

Knowledge of the existence of biological proteases extends back into the last century. A Cold Spring Harbor Symposium on proteases and biological control (Reich *et al.*, 1975) is eloquent testimony to the renewed interest in these important enzymes. Biological insight has lagged far behind the elegant biochemistry displayed in the pages of the symposium. Alpha-1-antitrypsin does not want for biochemical attention by acknowledged experts; it is on the physiology that we fall down. Alpha-1-antitrypsin accounts for about 90% of the trypsin inhibitory capacity of serum. It is a protease with a wide spectrum of activity; elastin, collagen, chymotrypsin, and thrombin are all effectively neutralised by the anti-enzyme, and much is known about the stoichiometry of the protein-protein interactions. But when all is said and done, the simple paradigm of emphysema and neonatal cirrhosis being due to deficiency of the protein simply will not do. There are too many individuals who are fit and well with an alarmingly low level of alpha-1-antitrypsin—alarming, that is to say, to the physician not the patient. Yet the association is there for all to see; the explanation unknown.

Hepatic manifestations in alpha-1-antitrypsin deficiency

The liver in these patients has been extensively

studied. Globules of alpha-1-antitrypsin, of similar molecular weight have been observed. The alpha-1-antitrypsin may lack sialic acid, and other sugars also appear decreased; the protein may contain more arginine residues than normal. Although defective glycosylation is possible, the reported serum sialyl transferase deficiency appears to be a secondary phenomenon and due to the existing liver disease. The situation remains unclear; the material is scarce and the protein not the easiest to work with. Moreover, it is far from clear why an accumulation of a protein normally synthesised in the liver should give rise to liver damage (Sharp, 1976). Persuasive pathophysiological explanations for the increased incidence of hepatoma are so unconvincing and tortuous that for the moment it must be sufficient to accept its Baconian validity without further mental exertion.

BIOLOGICAL SIGNIFICANCE OF RELATIONSHIP BETWEEN ALPHA-1-ANTITRYPSIN DEFICIENCY AND LIVER DISEASE

The association between antitrypsin deficiency and liver injury may have a general significance for medicine in the future that far exceeds its contemporary hepatic interest. The nature/nurture controversy in the early part of this century was, at its best, a polemical over-simplification of the nature of the forces that combine to shape the development of an individual as well as the diseases with which he may become afflicted; at its worst it was a quasi-scientific justification of mischievous political goals. The publication of *Inborn Factors in Disease* by Garrod in 1931, when Garrod was 74 years old, placed the controversy on a higher plane. The chemical individuality of an individual is beyond calculation. There are probably at least 100 000 genes in man; at the one locus we are considering, the alpha-1-antitrypsin locus, there are a minimum of 25 alternate alleles. At least two of these, the Z allele and the null allele (the latter very rare), are frequently disadvantageous—those homozygous are likely to have malfunctioning of the liver and/or lung. Yet abnormal genes do not of themselves cause disease. If the biological world were without proteases it is unlikely that a deficiency of an inhibitor would be damaging. (The gene for phenylketonuria would lie forever hidden if phenylalanine were not one of our normal dietary amino acids.) It is the subtle interaction of a genetic deficiency with unknown environmental and, possibly, other genetic forces that gives rise to overt disease. The genetic deficiency should, paradoxically, focus our attention on the environment. Each of the alleles gives rise to a different specific gene product. In what way do these products

affect the functioning of the normal cell? What is their precise physiological function *in vivo*? Our biochemical individuality determines the diseases that lie in wait for us. They do not operate by some mysterious mechanism—so long designated a diathesis—but by influencing molecular biological interactions between a myriad of enzyme systems and the ever-changing environment in which they are bathed.

Medicine in the next several decades will explore and define the functional relationships between genetic and epigenetic influences in the causation of disease. Manipulation of this interaction, almost invariably at the environmental level, will enable us to decrease the load of individual human misery, as well as explaining why some patients with a deficiency of alpha-1-antitrypsin are healthy and others are not.

References

- Bearn, A. G. (1957). Wilson's disease. An inborn error of metabolism with multiple manifestations. *American Journal of Medicine*, **22**, 747-757.
- Duckworth, D., and Garrod, A. E. (1895). A case of hepatic cirrhosis, with obstruction in the superior vena cava. *St. Bartholomew's Hospital Reports*, **32**, 71-77.
- Eriksson, S., and Hägerstrand, J. (1974). Cirrhosis and malignant hepatoma in alpha-1-antitrypsin deficiency. *Acta Medica Scandinavica*, **195**, 451-458.
- Fagerhol, M. K. (1974). Pi typing techniques. In *Protides of the Biological Fluids*, pp. 493-495. Edited by H. Peters. Elsevier: London.
- Fisher, R. L., Taylor, L., and Sherlock, S. (1976). Alpha-1-antitrypsin deficiency in liver disease: the extent of the problem. *Gastroenterology*, **71**, 646-651.
- Garrod, A. E. (1902). The incidence of alkaptonuria: a study in chemical individuality. *Lancet*, **2**, 1616-1620.
- Garrod, A. E. (1904). Tumour of the liver in a boy aet. 10 years. *Transactions of the Clinical Society of London*, **37**, 222-223.
- Garrod, A. E. (1909). *Inborn Errors of Metabolism*. Oxford University Press: London.
- Garrod, A. E. (1931). *The Inborn Factors in Disease: an Essay*. Clarendon Press: Oxford.
- Garrod, A. E., and Hopkins, F. G. (1896). On urobilin. Part 1. The unity of urobilin. *Journal of Physiology*, **20**, 112-144.
- Garrod, A. E., and Hurtley, W. H. (1912). Congenital family steatorrhea. *Quarterly Journal of Medicine*, **6**, 242-258.
- Hopkins, F. G., and Garrod, A. E. (1897). On urobilin. Part II. The per-centage composition of urobilin. *Journal of Physiology*, **22**, 451-464.
- Jeppsson, J.-O., Laurell, C.-B., and Fagerhol, M. (1978). Properties of isolated human alpha-1-antitrypsins of Pi types M, A and Z. *European Journal of Biochemistry*, **83**, 143-153.
- Kueppers, F., Briscoe, W. A., and Bearn, A. G. (1964). Hereditary deficiency of serum alpha-1-antitrypsin. *Science*, **146**, 1678-1679.
- Kueppers, F., and Christopherson, M. J. (1978). Alpha-1-antitrypsin: further genetic heterogeneity revealed by isoelectric focusing. *American Journal of Human Genetics*, **30** (July 1978).
- Laurell, C. B., and Eriksson, S. (1963). The electrophoretic alpha-1-globulin pattern of serum in alpha-1-antitrypsin deficiency. *Scandinavian Journal of Clinical and Laboratory Investigation*, **15**, 132-140.
- Laurell, C. B., and Sveger, T. (1975). Mass screening of newborn Swedish infants for alpha-1-antitrypsin deficiency. *American Journal of Human Genetics*, **27**, 213-217.
- Reich, E., Rifkin, D. B., and Shaw, E. (eds.) (1975). *Proteases and Biological Control*. Cold Spring Harbor Conferences on Cell Proliferation, Volume 2. Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y.
- Sharp, H. L. (1976). The current status of alpha-1-antitrypsin, a protease inhibitor, in gastrointestinal disease. *Gastroenterology*, **70**, 611-621.
- Sharp, H. L., Bridges, R. A., Krivit, W., and Freier, E. F. (1969). Cirrhosis associated with alpha-1-antitrypsin deficiency, a previously unrecognised inherited disorder. *Journal of Laboratory and Clinical Medicine*, **73**, 934-939.
- Sheldon, J. H. (1935). *Haemachromatosis*. Oxford University Press: London.
- Sherlock, S. (1975). *Diseases of the Liver and Biliary System*. Blackwell Scientific: Oxford.

Postscript

Since this manuscript was completed the amino acid structure of alpha-1-antitrypsin and its variants has been studied in greater detail and suggests that amino acid substitutions in the protein influence its secretion (Jeppsson *et al.*, 1978).